CMLS, Cell. Mol. Life Sci. 59 (2002) 1166–1184
1420-682X/02/071166-19 $ 1.50 + 0.20/0
© Birkhäuser Verlag, Basel, 2002

CMLS Cellular and Molecular Life Sciences

Biomedicine and Diseases: Review

Assembling the human immunodeficiency virus type 1

A. Cimarelli and J.-L. Darlix*

U412, Laborétro, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon (France), Fax + 33 4 7272 8777, e-mail: jldarlix@ens-lyon.fr

Received 12 November 2001; received after revision 2 January 2002; accepted 7 February 2002

Abstract. Retroviral assembly proceeds through a series of concerted events that lead to the formation and release of infectious virion particles from the infected cell. Upon translation, structural proteins are targeted to the plasma membrane where they accumulate. There, the nascent particle forces the plasma membrane to form a bud, which pinches off releasing the virion particle from the cell. In this review we describe the molecular mechanisms now known to be behind the process of virion assembly. In particular, we focus on the human immunodeficiency virus type 1, the prototype member of the lentivirus subfamily of the Retroviridae.

Key words. Retrovirus; HIV-1; Gag virion assembly; raft.

Introduction

In the following review, we will describe the molecular mechanisms by which infectious virion particles are formed and released from an infected cell, the process of virion assembly. We will focus on what is now known about the assembly of the human immunodeficiency virus type 1 (HIV-1), the prototype member of the lentivirus subfamily of the Retroviridae. Throughout, we shall keep in mind that most of the major steps described here for HIV-1 are also shared by other retroviruses. For the sake of clarity, we have chosen to introduce the HIV-1 life cycle and genomic structure first and then to describe in detail the formation of virion-like particles, those formed by a single viral structural polyprotein, Gag. This discussion will illustrate the major problems in virion assembly. Finally, and for simplicity, we will add to the picture only a few of the known components that contribute to determine the infectivity of the virion particle.

* Corresponding author.

HIV-1 life cycle, genomic structure and structural genes

The life cycle of HIV-1

The life cycle of HIV-1 begins when an infectious particle encounters a cell bearing the appropriate receptor (CD4) and coreceptor (CCR5 or CXCR4) [see ref. 1 for review and citations] (fig. 1). Following recognition between viral envelope glycoproteins and cell receptors, membrane fusion is triggered and the viral core carrying the dimeric RNA genome penetrates into the cytoplasm of the cell. The single-stranded genomic RNA is then converted into double-stranded proviral DNA by reverse transcriptase (RT) with the aid of the nucleocapsid protein (NC). Subsequently, proviral DNA is transported into the nucleus and integrated into the host genome, via the viral enzyme integrase (IN). As their name suggests, reverse transcription of RNA into DNA is a characteristic and distinctive feature of retroviruses. Once proviral DNA is integrated, transcription of viral mRNA occurs from the viral long terminal repeat (LTR) via the host cell RNA polymerase II [see ref. 2 for a comprehensive review of the life cycle of retroviruses]. Provirus integration is a hallmark of successful replication of a retrovirus.

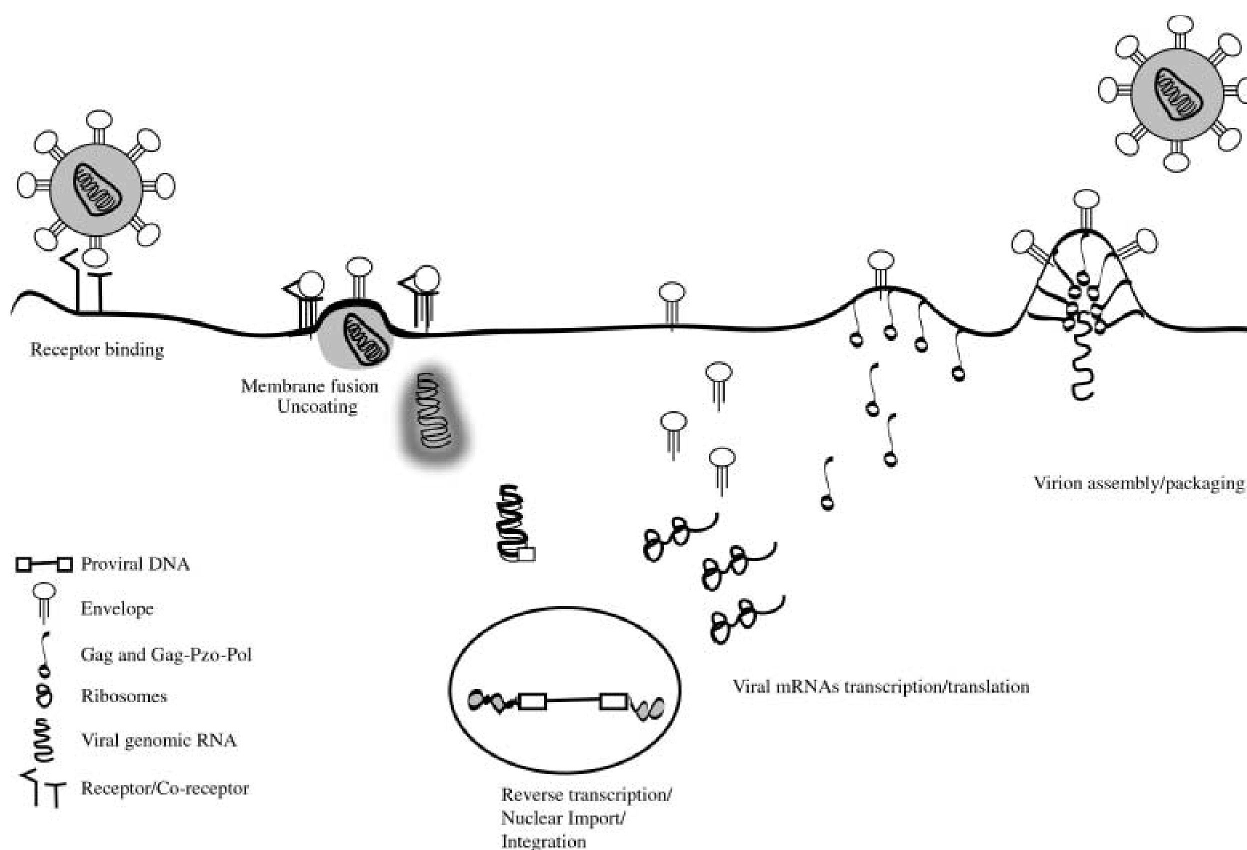

Figure 1. Schematic representation of the retroviral life cycle. Early events in the viral life cycle begin with attachment of the virus to the surface of a cell bearing the appropriate receptor/s. Reverse transcription of viral genomic RNA begins in the cytoplasm, although some indications suggest it may have already started in the virion particle, and is completed in the nucleus. Early events in the viral life cycle culminate in the integration of the proviral DNA into the host genome. Late events in the viral life cycle lead to the formation of infectious virion particles that are released from the cell. In this scheme, accumulation of structural proteins occurrs directly on the plasma membrane, as is the case for type C retroviruses, like HIV-1.

Its life cycle can be thus divided into events that precede or follow integration, defined as early and late steps of the viral life cycle, respectively.

The HIV-1 genome

The HIV-provizal genome is flanked by two LTRs and encodes the structural genes *gag*, *pro*, *pol* and *env* and the non-structural genes *tat* (for trans-activator), *rev* (for regulator of expression of virion protein), *nef* (for negative factor), *vif* (for virion infectivity), *vpr* and *vpu* (for viral proteins R and U, respectively). The presence of such a large number of non-structural proteins sets lentiviruses apart from other classes of retroviruses (fig. 2). In addition, the viral genome contains a number of cis-acting sequences, such as the Tat-acting region (TAR), the primer-binding site (PBS), the dimer initiation site (DIS), the packaging sequence (psi), the polypurine tract (PPT located at the 3′ end of the genome and a second cPPT present at the center of the genome) and the Rev-responsive element (RRE). These sequences participate in a large number of events during the viral life cycle, such as transcription (TAR), translation and nuclear export (RRE), packaging (psi), dimerization of viral genomic RNA (DIS) and reverse transcription (PBS, PPT and cPPT) [reviewed in ref. 2].

Viral transcripts originate from a single promoter in the U3 region of the 5′ LTR. Basal transcription activity from the viral promoter is modulated by the provirus site of integration and by the activation state of the cell. Thus, the steady-state level of viral RNAs in the cell can be low or high depending on the availability of specific host cell factors acting on the LTR [reviewed in refs 3–7]. The first transcripts to appear in the cytoplasm of infected cells are multiply spliced mRNAs from which the non-structural proteins Tat, Rev and Nef are translated (the so-called 2-kb mRNAs). Upon translation, Tat reaches the nucleus and trans-activates viral RNA transcription. This is accomplished not by binding to the viral promoter but rather by allowing elongation of nascent RNA molecules [the mechanism of Tat action is reviewed in refs 8, 9]. However, it is Rev that allows the regulation of viral gene

Figure 2. (*A*) Schematic representation of the viral genome and the genetic structure of the HIV-1 provirus. Cis elements important for various steps in the viral life cycle are indicated as color coded. Structural and non-structural genes are represented as white and gray-filled boxes, respectively. (*B*) Viral mRNA transcripts and polyprotein precursors of HIV-1, Gag, Gag-Pro-Pol and Env. The first transcripts originating from the viral LTR are multiply spliced and result in the synthesis of the Tat, Rev and Nef proteins. Following the action of Rev, which promotes viral RNA export from the nucleus, single and unspliced mRNAs accumulate which give rise to the remaining proteins of HIV-1. The single domains contained within HIV-1 polyproteins (white box) are indicated. These are the mature proteins that, upon processing by the viral protease, are found inside virion particles. The sites of protease cleavage are shown with a black bar in both Gag and Gag-Pro-Pol, and are further indicated by arrows in the case of Gag-Pro-Pol. Note that the PR processing sites are not equivalent and are recognized with different affinity. The additional protease processing site within RT is shown by a dotted line and indicated by a small-headed arrow. The Env site processed by furin is shown with a black bar. The cytoplasmic tail of gp41 is black filled.

expression. Rev acts on an RNA element present in *env*, RRE, and promotes the export of RRE-containing mRNAs from the nucleus [the reader is invited to consult refs 10 and 11, for reviews and further references]. As a result of Rev action, unspliced viral genomic RNA and single-spliced mRNAs are exported from the nucleus into the cytoplasm where structural genes and the remaining non-structural genes are translated.

Structural proteins of HIV-1

Gag, Pro, Pol and Env are translated in the form of polyprotein precursors that, with the exception of Env, are processed by the viral-encoded protease. Processing involves the specific recognition by the viral protease of cleavage sites present in the polyproteins and results in the accumulation of the mature proteins found in infectious virion particles. Thus, Gag is initially expressed from cytoplasmic viral genomic RNA into a polyprotein precursor of 55 kDa (p55) that upon processing yields, from the N terminus, the following proteins: matrix (MA, p17), capsid (CA, p24), nucleocapsid (NC, p7) and p6. The NC is flanked by two spacer peptides SP1 and SP2, otherwise called p2 and p1, respectively [see refs 12 and 13 for reviews]. As we shall describe in detail later, these proteins as part of the Gag precursor carry out necessary and specific functions during viral assembly and are often referred to as domains of Gag.

In HIV-1, the gene encoding the viral protease, *pro*, and the gene expressing the polymerase and integrase, *pol*, are translated as part of a single Gag-Pro-Pol polyprotein precursor of 160 kDa (p160). Translation of Pro-Pol requires a –1 frameshift that allows reading through the *gag* stop codon. This event is imposed in cis by a slippery sequence present on the genomic RNA and occurs at a frequency of about 1 in 20 translation events, which accounts for the relative ratio between Gag and Gag-Pro-Pol [see ref. 2 for a comprehensive overview]. As a result of viral protease processing, the protease itself (PR), the reverse transcriptase heterodimer (RT, composed of a 66-

and a 51-kDa subunit) and the integrase (IN) are released from the precursor.

While the genomic RNA is translated by cytoplasmic ribosomes, *env* mRNA is translated on rough endoplasmic reticulum (RER)-associated ribosomes. Thus, the Env precursor enters the endoplasmic reticulum (ER) where it becomes glycosylated (hence its name gp160) and cleaved by cellular proteases such as furin into its subunits gp120 (SU, for surface) and gp41 (TM, for transmembrane). The two glycosylated protein products remain associated, and through the Golgi reach the plasma membrane where they are incorporated on the virion surface.

Overview of the retroviral assembly process

Electron microscopy studies have revealed the existence of two morphologically distinct mechanisms of assembly for retroviruses [14–18]. Virion particles can be preformed in the cytoplasm and transported to the plasma membrane (morphogenesis of type B and D), or they can assemble directly on the plasma membrane (morphogenesis of type C). HIV-1 virion particles follow the second modality of assembly. Viral structural proteins accumulate under the plasma membrane, multimerize and ultimately force the membrane into a curvature that protrudes from the cell and eventually pinches off, releasing the virion particle [for reviews see refs 12 and 13]. Given these events, which are easily observed by electron microscopy, the aim of those studying virion assembly is the dissection of the molecular mechanisms behind the process. Thus, we study the mechanism responsible for targeting particle assembly to the plasma membrane rather than intracellular membranes and try to understand how viral proteins come together to form higher-order multimeric structures that are finally released from the cell. As Gag is the main structural constituent of virions and is able to form virion-like particles in the absence of other viral products, we will mainly focus our discussion by describing the assembly of Gag.

The assembly of Gag

Expression of the retroviral Gag polyprotein by itself is sufficient for the formation and release of virion-like particles [19–24]. Thus, Gag contains all the information and the domains required to assemble into virion-like – albeit not infectious – particles, and can truly be considered the major player in the entire virion assembly process. To schematize the series of events that occurs during virion assembly, one can think of newly synthesized Gag molecules present in the cytoplasm as facing three major problems: where to go (targeting), when and how to interact with other similar molecules (multimerization) and how to exit the cell (budding).

Targeting to the plasma membrane

Early studies on HIV-1 demonstrated that mutations that abolished plasma membrane targeting of Gag prevented the formation of virion particles [17, 25–31]. These results showed that targeting of Gag to the plasma membrane is a leading event during virion assembly. They also revealed the presence at the N terminus of retroviral Gag polyproteins of a conserved region, the function of which is to target Gag to the plasma membrane; this region has been termed the M domain [32].

The M domain

The M domain of HIV-1 has been precisely mapped to the first 31 amino acids of Gag [31]. This domain is sufficient to target heterologous proteins to the plasma membrane and has counterparts in similar domains present on cellular proteins [reviewed in ref. 33]. The M domain of HIV-1 is a bipartite membrane-targeting domain that relies on acylation and basic residues, both being required for its function [17, 25–31]. As the first methionine of Gag is removed cotranslationally, Gag is modified by the covalent addition of a fatty acid, myristate, to its first glycine residue [27, 34, 35]. Myristoylation is accomplished by cellular enzymes which recognize the consensus sequence Met-Gly-x-x-x-Ser/Thr [interestingly, present also in Nef, reviewed in ref. 33]. Basic amino acids are clustered in a region between residues 17 and 31 of HIV-1 MA. Although the features of the HIV-1 M domain are shared by other retroviruses, several exceptions exist. For example, in the Rous sarcoma virus, the M domain is modified by addition of an acetyl group to the first methionine rather than by myristoylation on glycine and basic residues are dispersed in as much as 80 amino acids [36, 37]. This evidence raises the possibility that M domains of certain retroviruses may be regulated differently from that of HIV-1.

The biophysical features of the two-signal plasma membrane-binding motif (myristate plus basic residues) have been determined [31, 38–40]. The myristate inserts hydrophobically into the lipid bilayer and the basic amino acids form electrostatic interactions with the headgroups of acid phospholipids on the inner leaflet of the membrane bilayer. Both interactions are needed to provide enough binding energy to anchor Gag into the membrane. Nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography of HIV-1 MA support this model by showing that basic residues form an amphipatic β sheet and are exposed on the top surface of the molecule where they can interact with membrane phospholipids [41, 42].

But not all membranes are equivalent, and Gag has recently been shown to accumulate in specific micro-

domains of the plasma membrane, the rafts. Since rafts are becoming an area of increasing interest for virologists, immunologists and cell biologists, we will introduce them below.

Rafts

Rafts are discrete domains within the plasma membrane enriched in sphingomyelin, glycosphingolipids, cholesterol and glycosyl-phosphatidylinositol (GPI)-anchored proteins. Due to their composition, rafts are resistant to extraction with cold non-ionic detergents such as Triton X-100 or NP-40 and can thus be isolated from soluble and non-raft membrane proteins. Their unique lipid composition makes rafts a docking site for a number of cellular proteins that are thus enriched at these sites. As a consequence, the raft becomes the site of choice in which specific cellular processes take place, as best shown in the case of T and B cell receptor engagement [43–49]. Interestingly, protein access to rafts seems regulated by acylation; myristoylation, as in the case of HIV-1 Gag and Src, palmitoylation, or both, as in Ras [reviewed in ref. 33].

Recognition of the liaison between viruses and rafts is relatively new but is becoming a common theme in virology. Viruses as diverse as influenza, measles, Ebola and Sendai seem to utilize rafts to assemble virion particles [50–54]. Retroviruses seem no exception, since MoMuLV, HTLV-I and HIV-1 structural proteins (and HIV-1 Nef) are enriched in rafts [55–57]. In support of the hypothesis that assembly may occur preferentially in rafts, virion particles have high cholesterol and sphingomyelin content [58–60] and incorporate a number of proteins present in rafts [55, 61–63].

Mechanism of M domain function

The mechanism of raft association of Gag is still unclear. As cellular proteins modified similarly to Gag are recruited into rafts, the lipid composition of rafts in itself may be responsible for the specific targeting of Gag to the plasma membrane, acting as a sort of intracellular receptor on the membrane. However, Gag itself, once on the plasma membrane, may contribute to the formation of the raft de novo.

In addition, the dynamics of the association between Gag and rafts need further investigation. Gag associates within minutes of its synthesis with the plasma membrane [64], but in any given experiment, only 20–30% of the total Gag protein is present in rafts, while the majority is present on detergent-soluble plasma membrane [55–57, 65–67]. Clearly, a comprehensive kinetic study of the association between Gag and membrane may help clarify the picture.

The M domain of HIV-1 is sufficient to target heterologous proteins to the plasma membrane, but is insufficient for their targeting into rafts [65]. This suggests that other domains of Gag may be needed to promote raft association and/or organization. Recent evidence suggests that the latter is promoted by the multimerization of Gag [65, 66], which by itself does not influence the ability of Gag to bind the plasma membrane [31, 68, 69]. If this were true, we could hypothesize a dynamic scenario in which the interaction between Gag and the plasma membrane occurs first, followed by recruitment of Gag into rafts, as recently suggested [66]. Recruitment may occur by lateral clustering of membrane-bound Gag molecules and may be influenced by Gag-Gag interactions [65, 66]. This hypothesis is very attractive, because lateral clustering is thought to be a major mechanism by which rafts are formed [reviewed in ref. 44). To complicate the issue, provocative evidence is emerging suggesting that rafts are a heterogeneous population characterized by distinct sensitivity to detergent and even containing distinct GPI proteins [70, 71]. Thus Gag may in the future be found on one raft type rather than another.

The second issue to consider is suggested by studies on HIV replication in murine cells. HIV-1 replication in murine cells is hindered at multiple steps: at virus entry, in proviral expression and in virion assembly. When the first two defects are corrected for, HIV-1 Gag is produced and myristoylated, but virion particles form on the plasma cell membrane very inefficiently [72]. Thus, there is in murine cells a plasma membrane-targeting defect of HIV-1 Gag, which can be rescued by exchanging the MA of HIV-1 for that of MLV [73, 74]. Because of this targeting defect and similar to defects in other M domain mutants, Gag processing, which reflects the proper activation of the viral protease, does not occur. Recent heterokaryon studies have suggested that the membrane-targeting domain of HIV-1 Gag may need a cellular factor to function properly [75]. In view of recent data on raft localization, this factor may be a component of the raft itself that is either missing or expressed in low amounts in murine cells or it may be a factor that chaperones Gag specifically to the plasma membrane. Taken together, these data suggest that selection of the site of assembly is a complex event and is likely to be influenced not only by the M domain but also by other domains of Gag and/or possibly by specific cellular factors.

Gag multimerization

Multimerization of retroviral Gag molecules is at the basis of the virion particle structure and, provided that nucleic acid is present, multimerization is an intrinsic property of the Gag molecule, since purified Gag can form virion-like particles in vitro [23, 76–82]. Particles formed in vitro are similar to those formed in cells in that they contain approximatively 1200 molecules of Gag per virion and 5–8% of their mass is nucleic acid [83]. As the virion is a complex and dynamic structure, multimeriza-

tion is the result of multiple contacts among Gag molecules and involves different domains of Gag.

The interaction domains

Several domains responsible for Gag-Gag interaction have been identified following a number of biochemical assays including in vitro binding assays [84], yeast two-hybrid systems [85], and copackaging of mutant Gag proteins into wild-type virions in vivo [68, 84]. Finally, although the structure of the entire Gag polyprotein precursor is not yet available, that of its single domains, corresponding to the mature viral proteins, has shed light on how each among the MA, CA and NC domains contributes to Gag multimerization. As we will discuss below, the major contribution to Gag-Gag interaction lies in the NC domain, so that NC is often referred to as the I domain [86]. However, since multiple domains contribute to Gag-Gag interaction, we prefer to refer to them collectively as interaction domains.

A role for MA in Gag multimerization has been recently reported [84]. In support of this hypothesis, the crystal structure of MA is trimeric [41, 87] and Gag trimers dependent on MA can be found in solution [88]. Similar results are obtained when SIVmac MA is examined, indicating that trimerization may be conserved among lentiviruses [89]. The role of MA trimerization in virion assembly has not been clearly determined. The entire MA can be substituted by a heterologous membrane-targeting domain suggesting that the main function of MA is to target Gag to the plasma membrane and that trimerization of MA might be dispensable for assembly [29, 90, 91]. On the other hand however, certain mutations in MA that span the proposed trimerization interface affect virion assembly, so, although minor, MA-MA interactions may truly influence Gag multimerization [26, 92].

A second domain involved in Gag multimerization is the capsid protein. CA is the major determinant of the viral core, as purified CA or CA-NC fusion proteins can form core-like structures in vitro [93–99]. The crystal structure of the individual N and C termini of CA is available [100–102], as is the structure of the entire CA complexed with a Fab [103]. These studies have allowed for image reconstruction of viral cores and have revealed that the N-terminal part of CA shapes core structure by organizing into hexameric rings, while the C-terminal part dimerizes, connecting the different rings [104]. Consistent with these results, mutations within the N terminus of CA generally affect virion morphology, while mutations at the C terminus affect virion assembly [105]. Interestingly, even though all the different domains of Gag can be fairly well substituted with heterologous proteins or portions thereof, the C-terminal domain of CA with SP1 is the only portion so far absolutely required for the proper assembly of virion particles [90, 91]. This most probably reflects the important role played by the C terminus of CA in shaping the structure of the virion particle itself. In proximity to its dimerization region, but not contributing to it, CA contains a stretch of 20 amino acids conserved in all retroviruses, called the major homology region (MHR). Although the functions of the MHR are unclear at the moment, this region is certainly important, as evidenced by its conservation and by the phenotype of mutations in the MHR that affect core structure and assembly [105–109].

The third domain involved in Gag-Gag interactions maps in NC. Deletions of NC strongly reduce Gag-Gag interaction in vitro and in vivo [68, 84, 85] and impair virion assembly and production, although this defect can be mitigated in high-level expression systems [20, 110–113]. Mutations in NC have, in addition to effects on assembly, the most diverse effects on virion structure [114–119], viral RNA packaging [115, 120, 121] reverse transcription [114, 115, 118, 122, 123] and possibly integration [118, 122, 124, 125], reflecting the multiple roles of NC during the viral life cycle. We shall deal with some of these roles later in the review.

Retroviral NC proteins are characterized by the presence of Cys-His boxes (either one or two as in HIV-1, with the sole exception of spumaviruses which have none) and of a large number of basic residues distributed throughout the protein [for reviews see refs 13, 126, 127]. NC is the major viral RNA-binding protein and its interaction with the RNA has been shown to be highly complex. As part of Gag, the NC domain plays the major role in packaging of the viral genomic RNA through specific interactions between the Cys-His boxes and a packaging sequence present in *cis* on the genomic RNA (termed ψ), but it also extensively coats the viral genome once it is incorporated into the particle. Thus, NC can associate with RNA specifically as in the case of packaging but also in a rather non-specific manner all along the genomic RNA. Structural studies of NC alone or complexed with small portions of the HIV packaging sequence, ψ, have provided structural explanations for these findings and have revealed both specific interactions of Cys-His boxes with guanosine residues of the RNA and non-specific electrostatic interaction of certain basic residues of NC with the phosphate groups of the RNA molecule [128–135].

Mechanism of Gag multimerization

Given the properties of the domains involved in multimerization, what mechanism is responsible for Gag-Gag interactions? Examples from other viruses show that nucleocapsid assembly may rely uniquely on protein-protein interactions [136–139] or may be strictly driven by protein-RNA interactions, as best shown for alphaviruses and plant viruses [140–144]. In HIV-1, both protein-protein and protein-RNA interactions seem to be important

for assembly. Protein-protein interactions are suggested by structural studies and clearly involve both MA-MA and CA-CA interactions and may also involve hydrophobic NC-NC interactions, as NC multimers are observed in solution once NC is bound to large RNAs [132, 135, 145], although not in NMR studies conducted so far with small RNAs [128–130, 133, 134]. Furthermore, in the Gag-Pol precursor, additional IN-IN, PR-PR and RT-RT interactions do occur [146–153].

Protein-RNA interactions mediated by NC are implied by the requirement for nucleic acid in the assembly of purified Gag or CA-NC proteins in vitro [23, 77, 78, 81, 94, 154]. In addition they are suggested by the strict correlation existing between the ability of NC to associate with RNA and the ability of the virus to assemble in cells [114, 155]. This suggests that one of the major functions of NC during the assembly process is to bind RNA and, indeed, NC can be functionally substituted by heterologous RNA-binding domains [112, 119, 156]. While this evidence shows that NC-RNA interactions are indispensable for virion assembly, we must remember that purified NC bound to nucleic acid will form aggregates but not virion particles in vitro [131, 135, 145]. Thus, protein-protein interactions are also important and most likely shape the virion structure itself.

Given all this information, we may hypothesize that by binding to RNA, NC concentrates Gag molecules onto one or more RNA molecules, a process known as nucleation. By increasing the local concentration of Gag, this nucleation event can favor protein-protein interactions among the different domains of Gag, which then shape the structure of the particle. In support of this hypothesis, the nucleation event can be achieved by substitution of NC with leucine zippers, that is by protein-protein interaction modules that effectively increase the local concentration of Gag [90, 119]. This model of virion assembly argues that RNA molecules or rather NC-RNA complexes act as a recruitment platform, a scaffold, onto which Gag molecules accumulate. Under this assumption, RNA may be considered a structural element of the virion particle, as recently suggested [157]. The RNA involved need not be of viral origin given that virion assembly occurs in the absence of either Cys-His boxes or ψ [118, 158–162]. Thus, whether the various ribosomal and cellular RNAs found in retroviral particles are incorporated via NC because they serve a structural role during assembly, or non-specifically, remains to be determined. Examination of the RNA content of a minimal Gag mutant, in which NC has been substituted by a leucine zipper (dimeric or trimeric), may help clarify the issue, as this mutant should theoretically achieve nucleation without RNA [90, 119].

Another important point to consider is at what point during the assembly process, Gag multimerization occurs. Myristoylation-deficient Gag proteins (which are thus cytosolic) can be incorporated into wild-type virions, but this rescue does not discriminate when the wild-type and mutant molecules interact. More specifically, this assay of copackaging does not distinguish whether the two molecules associate while both are in the cytosol or when the wild-type Gag is already bound to the plasma membrane. The existence of cytosolic Gag complexes resistant to detergent extraction has been suggested [163, 164]. Interestingly, these complexes were also obtained with a myr– mutant of Gag, indicating that they may represent true cytosolic complexes. If these are true assembly intermediates that reach the plasma membrane, or if they represent dead ends of the assembly process, is not yet known.

Late stages of assembly

The last event to take place during the assembly process is budding. Mutants blocked at this stage accumulate Gag into virion-like particles that do not detach from the plasma membrane and remain tethered to each other [20, 90, 165–169]. As this event occurs very late during virion assembly [166, 167], the region responsible for this defect has been named the L domain [32].

L domain

In HIV-1 Gag, the L domain maps to a conserved P(T/S)AP motif in p6 [166, 167]. Slightly different conserved motifs are present in other retroviruses such as a YxxL domain in equine infectious anemia virus (EIAV) and a PPxY motif in Mason-Pfizer monkey virus, murine leukemia virus and Rous sarcoma virus [reviewed recently in ref. 170]. Retroviral L domains can functionally replace each other regardless of their location in the Gag molecule, indicating that retroviruses use a common pathway for budding and suggesting that the L domain is not providing structural information [171].

The mechanism of budding

Clues to the mechanism of budding came very recently from the identification of cellular partners that interact with the L domain of different retroviruses. These cellular partners include the AP-2 complex involved in clathrin-mediated endocytosis for EIAV [172], a member of the family of E3 protein ubiquitin ligases for RSV [the neuronal precursor cell-expressed developmentally down-regulated 4, Nedd4; ref. 173] and an homologue of the E2 ubiquitin-conjugating enzymes for HIV-1 [tumor susceptibility gene 101, Tsg101; refs 116, 174]. Although the exact mechanism of budding remains to be elucidated, strong evidence suggests that the function of the L domain may indeed be to recruit the endocytosis machinery to the site of budding. In support of this hypothesis, all the L domain-interacting proteins belong to the endocytic pathway and, more importantly, expression of Gag causes a redistribution of Tsg101 to the site of assembly [175]. Among

the L domain-interacting proteins, Tsg101 might provide a particularly instructive example to elucidate how budding occurs. Tsg101 is part of the vacuolar protein-sorting pathway which sorts membrane-bound proteins for degradation in the lysosome [for a review see ref. 176]. Proteins can enter this pathway either via endocytosis from the plasma membrane or via vesicular trafficking from the Golgi. Deletion of the Tsg101 orthologue in yeast blocks vacuolar protein sorting from the Golgi, a process that topologically resembles the budding of virion particles from the cell (protrusion from a membrane).

A second player that has come into focus in recent years and that may contribute to our understanding of the mechanism of budding is ubiquitin. Ubiquitin was initially found to be incorporated in avian retrovirus particles [177] and later in HIV-1 virions, both in a free form and as monoconjugate to a fraction of p6 [178]. The link between ubiquitin and virion assembly came when proteasome inhibitors, by preventing recirculation of ubiquitin and by lowering its intracellular concentration, were shown to cause a defect similar to that of L domain mutants, albeit much reduced in magnitude [168, 179].

The role of Gag ubiquitination in assembly is not clear. In particular, whether ubiquitination of p6 is required for recruitment of other cellular factors, as recently proposed for Tsg101 [165], or whether it is the indirect reflection of the presence of a ubiquitin ligase activity [180] has yet to be determined. The fact that the two ubiquitin acceptor lysines in p6 can be modified without apparent defects in virion assembly [181], does not argue against one or the other hypothesis, because the ubiquitin ligase activity can target adjacent lysines in the absence of those in p6. However, the presence of mono-ubiquitinated p6 is particularly interesting in view of the recent findings mentioned above. Mono-ubiquitination is clearly different from poly-ubiquitination, whose role in protein degradation is well known. Indeed, a number of studies have shown that mono-ubiquitination is a signal for receptor endocytosis [recently reviewed in ref. 182], raising the possibility that ubiquitin may truly contribute to the budding process.

Other components needed for the formation of an infectious virion

The steps that lead to the formation of Gag particles describe the virion assembly process, but they do not exhaust the subject, because Gag particles are not infectious. We have chosen to describe here only a few components that contribute to virion infectivity: viral RNA which allows us to introduce the problem of packaging; Env, through which we will deal with the problem of envelope incorporation, and two accessory proteins, Vpu and Vif, that influence virion release and virion infectivity, respectively (fig. 3).

Viral genomic RNA packaging

Viral genomic RNA packaging is mediated by specific binding between the Cys-His boxes of NC and a ψ sequence composed of four stem-loops, named SL1–4, present only on the complete viral genomic RNA [for reviews see refs 126, 127]. As a result of this interaction, two copies of viral genomic RNA are selected from the pool of viral and cellular RNAs and incorporated into virion particles. Viral genomic RNA is found in virion particles in the form of a dimer [183–186]. Dimerization is initiated at a stem-loop structure named the dimerization initiation site (DIS). The DIS of HIV-1 corresponds to a highly conserved structure with a self-complementary loop sequence, which is involved in the formation of a typical loop-loop 'kissing' complex [187–194]. RNA has been suggested to be packaged into nascent virions in a dimeric form that undergoes maturation (a conformational change that makes the RNA dimer more resistant to heat denaturation) after the virus is released from the cell [195, 196]. Dimer maturation is driven by NC [195–199]. A certain number of studies suggest that the dimeric form of the genomic RNA positively influences reverse transcription and recombination among the two molecules of genomic RNA [200, 201], although some disagree [202; reviewed in refs 203, 204].

Although the biochemical features involved in the Gag-genomic RNA interaction have been well characterized in vitro, some of the aspects of the process of viral genomic RNA packaging in vivo remain elusive. In particular, we do not know when this interaction takes place during assembly nor do we know the relationship existing between packaging and Gag multimerization. If viral genomic RNA contributes to the formation of an RNA scaffold for the accumulation of Gag molecules, then packaging should begin with Gag multimerization. According to this hypothesis, since Gag multimers can be found in the cytoplasm of infected cells [163, 164], selection of viral genomic RNA may already occur in the cytoplasm. Alternatively, packaging may proceed independently of Gag multimerization, and genomic RNA may be included later in the virion particle formation, when Gag is already bound to the plasma membrane.

A second issue to consider is the interplay between packaging and translation. A translating mRNA with moving ribosomes may be a problem for incoming Gag molecules that want to associate with it. To circumvent this problem, the existence of two functional pools of RNA was suggested, one competent for translation, the other for packaging. However, evidence for the existence of such pools is lacking in lentiviruses [205]. Alternatively, upon its translation, Gag might associate with the translating genomic RNA, due to its proximity and to its local concentration, as recently suggested for HIV-2 [205]. In the process, Gag may even associate with other RNA molecules present during translation, which are in fact found

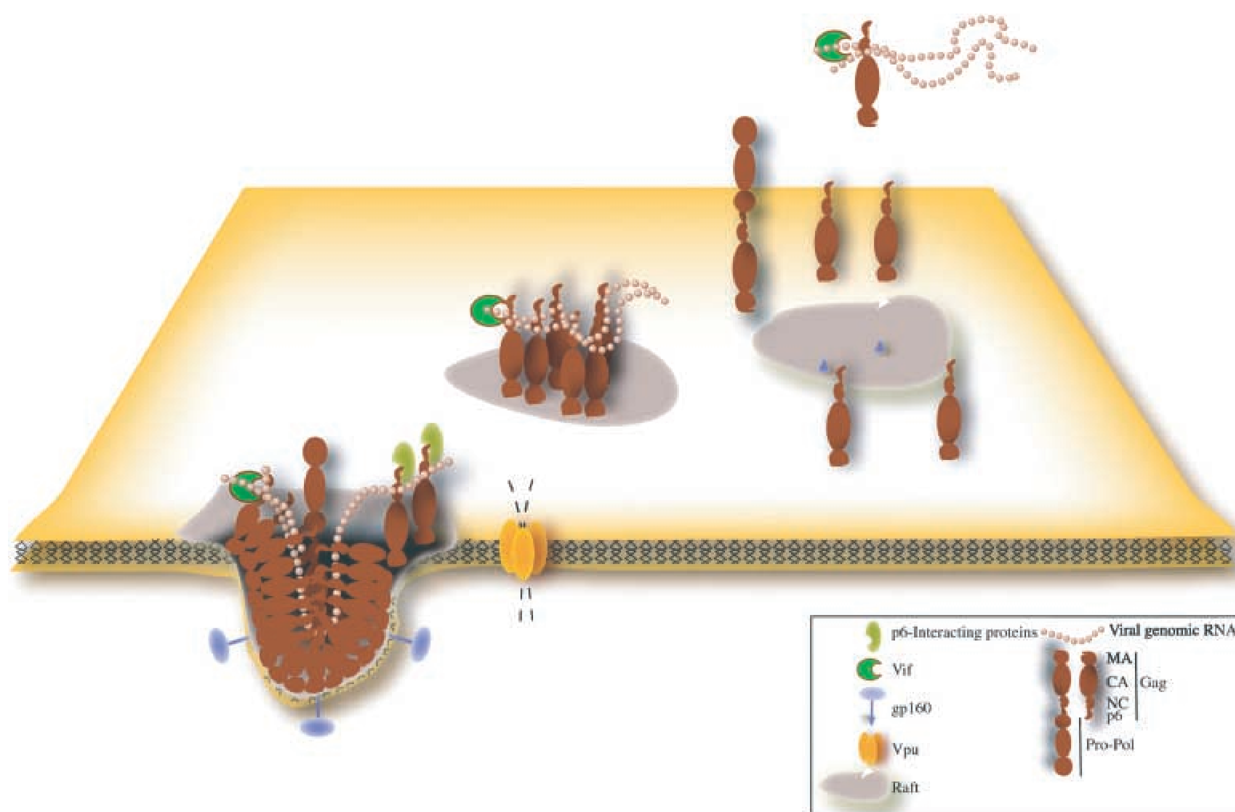

Figure 3. Focus on the assembly process of infectious particles of HIV-1. The figure shows the steps of virion assembly occurring on the cytoplasmic face of the plasma membrane. The viral Gag polyprotein reaches the plasma membrane shortly after translation (right portion of the figure). For simplicity, Gag multimerization is shown to occur directly on rafts (center portion of the figure), although there is evidence for its prior occurrence in the cytoplasm. Accumulation onto rafts is driven by Gag multimerization. Gag-Gag interactions are represented here as being induced by the dimer form of the viral genomic RNA that acts to nucleate Gag molecules. However, cellular RNAs can and do serve similar purposes with respect to Gag nucleation. The presence of Env in rafts (the cytoplasmic tail of gp41 is shown protruding from the rafts inside the cytoplasm, right portion of the figure) may serve to attract Gag molecules onto the site of assembly. On the plasma membrane, Vpu influences virion release by an unknown mechanism that may involve its ability to form ion channels which in turn may influence membrane permeability or even raft formation. Vif is shown here to chaperone viral genomic RNA, together with Gag, protecting it from the action of a yet unknown cellular factor. At a late step during the assembly process, p6 attracts Tsg101, and possibly other cellular factors, for the conclusion of assembly and the release of infectious particles from the cell.

incorporated into virion particles. This would reconcile the RNA-binding properties of the NC domain with the need for an RNA scaffold during Gag assembly. The overlap that exists between sequences involved in packaging (ψ) and the site of translation initiation of Gag further strengthens the hypothesis for a subtle interplay between packaging and translation [206–208].

Envelope

A long-standing question in virion assembly is whether Env is passively incorporated onto virions simply because of its presence on the plasma membrane or if it plays an active role during the assembly process. Interaction between the cytoplasmic tail (CT) of gp41 (TM) and MA was suggested by the proximity of the two proteins on the plasma membrane. Now, evidence indicates that Gag and Env do interact. This includes mutations in MA or gp41 that block Env incorporation [209–214], direct binding between Env and Gag [215, 216], structural studies [41, 42, 87, 88] and, more importantly, the rescue of Env incorporation in CT mutant virion particles by compensatory mutations in MA [217]. Structurally, the cytoplasmic tail of gp41 of primate lentiviruses is unusually long compared with that of other retroviruses (around 150 as opposed to 30–50 residues) and the spaces between MA trimers seen in the crystal structure seem tailored to fit such tails. However, while this is evidence that HIV-1 Env and Gag interact, it does not imply an active role of Env in the assembly process. In fact, Env may be passively incorporated into virions solely because of its presence in rafts [55, 65, 218–219], because other surface molecules present in rafts such as major histocompatibility complex class II molecules, CD59 and even Env of other viruses can also be incorporated onto the virion surface [62, 63]. The latter phenomena, called Env-

pseudotyping, is the strongest evidence for a passive mechanism of Env incorporation, since structurally diverse membrane proteins can become virion associated.

Although virion assembly does occur in the absence of Env, Env may still play a role when present during the assembly process, for example in the selection of the site of virion assembly. This is certainly the case in polarized epithelial cells. These cells are characterized by the presence of two functionally distinct plasma membrane domains, the apical and the basolateral, which are separated by well-defined tight junctions. When Gag is expressed alone in these cells it assembles from both surfaces, but in the presence of Env, assembly occurs only from the basolateral surface, where Env is normally localized [220, 221]. In this system, disruption of the CT of gp41 completely abolished Gag relocalization, strongly suggesting that Gag-Env interactions play an active role during virion assembly. The finding that Env may redirect Gag assembly is all the more interesting in view of the fact that Gag and Env accumulate in rafts. Env targeting to rafts is promoted by palmitoylation at two cysteine residues at position 764 and 837 of the CT of gp41, and mutation of these residues impairs both Env partitioning into rafts and Env incorporation into virion particles [219, 222].

We may hypothesize that accumulation of Env into rafts increases the affinity of Gag for the rafts, possibly due to the presence of palmytate, leading to constant recruitment of Gag into these small membrane domains. Gag recruited into rafts may on the one hand attract new Gag molecules (by lateral clustering due to multimerization, as suggested by Lindwasser et al. [65] and on the other, by interacting with Env, may protect the latter from endocytosis. In this respect, trimerization of MA may play a role in these events by both contributing to Gag-Gag interactions and by protecting the CT of gp41. This may occur either by directly displacing the adaptin complex or by protecting the adaptin-binding site in the CT of gp41 [see the following references for HIV-1 Env endocytosis: 223–227]. In support of this hypothesis, mutations expected to disrupt Gag-Env interaction result in down-regulation of Env from the cell surface [224–228]. A prediction of this model is that once the recruitment reaction starts at a particular location, that location becomes an assembly factory where multiple budding events occur, because accumulated Gag attracts more Gag molecules. The observation that multiple virion particles tethered together can be visualized in L domain mutants seems to suggest the existence of such sites [165, 166, 168, 179].

Viral protease

By processing the Gag and Gag-Pro-Pol polyprotein precursors, the viral protease promotes a profound reorganization of the virion structure that leads to virion maturation. Processing occurs in a relatively ordered manner, dictated mostly by the primary sequence of the processing sites on the polyproteins [see refs 12 and 13 for review]. A long-standing question is whether protease activity and its characteristic kinetics of cleavage are subjected to regulation. Several lines of evidence suggest that the protease itself is a limiting factor for its activation. PR acts as a dimer and is synthesized in HIV-1 as part of the Gag-Pro-Pol precursor, so that PR activation and processing occur only in sites where Gag and Gag-Pro-Pol molecules accumulate. Indeed, the products of PR activity are observed at the late stages of assembly, either at the plasma membrane or early in the released virion particle [150, 153]. If PR is overexpressed as a dimer, intracellular processing of Gag is forced and virion assembly impaired [229–233]. Interestingly however, virions of type B/D morphology assemble immature nucleocapsids in the cytoplasm, and protease activation does not take place until they reach the plasma membrane [234]. This may suggest a second level of control that relies on the overall structural accessibility of Gag to the viral protease. The latter may be triggered by the binding of Gag to the plasma membrane or by Gag multimerization. Protease processing of an NC-p6 substrate is positively influenced by the presence of RNA in vitro [235, 236]. Although, based on these studies, a direct effect of RNA on protease activity has been proposed, the effect of RNA on the viral protease may be indirect. Indeed, RNA may mediate multimerization among NC-p6 molecules, thereby influencing their conformation and/or accessibility to the protease itself.

Vif

Vif is a phosphoprotein of about 23 kDa abundantly expressed in infected cells where it is mainly localized in the cytoplasm and associated with the cytoskeleton [237–239]. A small amount of Vif appears to be incorporated into HIV-1 virion particles [61, 238, 240, 241], where it is resistant to detergent extraction [242, 243]. However, the specificity of Vif packaging has been questioned, since Vif can be incorporated into MLV particles [61] and its levels in virions generally mirror the ones in producing cells [61, 238, 241, 244].

Vif per se does not affect virion assembly but its absence results in the production of virions that are 10- to 100-fold less infectious than wild type [245–248]. This defect is clearly cell type dependent, so that cells can be classified as permissive or non-permissive depending on their requirement for Vif [247, 249–253]. Vif-deficient virions are impaired in their ability to reverse transcribe [61, 253–255] and compromised in the stability of their core [243]. These defects are apparently not irreversible because high levels of dNTPs can, to a certain extent, rescue the defect in reverse transcription of Vif-deficient virions [256].

The mechanism by which Vif influences the reverse transcription process is unclear. Recent evidence suggests that Vif may associate with viral genomic RNA and NC. Indeed, Vif associates with viral RNA as shown by UV-cross-linking in the cytoplasm of infected cells [257]. Although the specificity of this association is not yet clear since Vif can also associate with cellular RNAs [257, 258], that Vif packaging into virions is severely compromised in the absence of viral genomic RNA is intriguing [242]. This suggests that Vif is chaperoning the viral genomic RNA into particles. Recent data using heterokaryon studies suggest that the function of Vif is to overcome an endogenous inhibitor [250, 252]. Such an inhibitor may be part of some, still unspecified, mechanism of cell defense that targets viral genomic RNA.

Vpu

Vpu is an ~16-kDa membrane-spanning protein unique to HIV-1 and to the related chimpanzee lentivirus SIV cpz [259–261]. It is phosphorylated and not incorporated into virion particles [262–265]. Vpu has two known and distinct functions: it binds CD4 molecules in the ER and targets them for degradation through the proteasome-proteolysis pathway [for more appropriate reading see refs 266–268 and references within], and increases the efficiency of virion release [83, 269–273]. Its two properties have been shown to be distinct and mediated by different portions of the molecule.

The mechanism by which Vpu augments virion production is not known but appears not to be specific to HIV-1, as budding of other retroviruses is also increased in the presence of Vpu [269]. In addition, the magnitude of this effect varies greatly depending on the cell type used [274, 275] or on the cell growth rate [276].

Recent studies have revealed that Vpu has an ion channel-forming ability [277–282]. Ion channels are integral membrane proteins that can form pores in membranes through which ion flux occurs. Recently, a number of viral proteins that influence different steps in the viral life cycle by altering membrane permeability have been described, the best studied of which is the influenza virus M2 protein. These proteins have thus been termed viroporins [for a review see ref. 283]. Vpu shares striking structural similarities with previously described viroporins, and by altering membrane potential in producing cells Vpu may modify the structure of the plasma membrane domains where assembly takes place. An interplay between ion flux and rafts has been recently suggested [284, 285], and determining if Vpu associates with rafts would be valuable. Interestingly, Vpu functions may be provided by the cytoplasmic tail of gp41 in HIV-2 and SIV mac, which lack *vpu* [286, 287].

Concluding remarks and future prospects

As a brief conclusion to our review, we can say that a number of events leading to the assembly of virion particles have now been identified. As questions become more focused, we hope that the study of the viral life cycle will give us new insights, not only into the viral life cycle, but into conserved cellular pathways as well. We do believe this will be the case for raft biology as well as for the mechanism of endocytosis, both of which are called upon by assembling Gag molecules.

Deeper characterization of the molecular aspects of virion assembly will certainly benefit from determination of the structure of the entire Gag polyprotein. This may clarify the importance and modalities by which each domain of Gag contributes to the construction of Gag virion-like particles. This information may potentially lead to the discovery of new anti-viral drugs that inhibit Gag multimerization, budding or binding to the plasma membrane. On the other hand, this wealth of information could be used for the construction of a new class of lentiviral vectors further reduced in their viral genome content to be used in gene therapy. Functional replacement of one domain of Gag with cellular domains of similar functions is now possible. This replacement strategy could be used to effectively diminish the amount of viral component in virion particles and may lead to the construction of lentiviral-derived vectors with much improved biosafety.

Acknowledgements. We do apologize to those whose work could not be cited here due to lack of space. We acknowledge François-Loïc Cosset, Pascal Leblanc and Teophile Ohlmann for critical reading of the manuscript. A. C. is supported by INSERM, and J. L. D. by INSERM, ANRS and Sidaction.

1 Berger E. A., Murphy P. M. and Farber J. M. (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. **17:** 657–700
2 Coffin J. M., Hughes S. H. and Varmus H. E. (1997) Retroviruses, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.
3 Laughlin M. A. and Pomerantz R. J. (1994) Cellular latency in HIV-1 infection. Clin. Lab. Med. **14:** 239–255
4 Li C. J., Dezube B. J., Biswas D. K., Ahlers C. M. and Pardee A. B. (1994) Inhibitors of HIV-1 Transcription. Trends Microbiol. **2:** 164–169
5 Marzio G. and Giacca M. (1999) Chromatin control of HIV-1 gene expression. Genetica **106:** 125–130
6 Mirkovitch J. (1997) The role of hromatin in HIV-1 transcriptional regulation. Immunobiology **198:** 279–290
7 Pereira L. A., Bentley K., Peeters A., Churchill M. J. and Deacon N. J. (2000) A compilation of celluar transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res **28:** 663–668
8 Garber M. E., Wei P. and Jones K. A. (1998). HIV-1 Tat interacts with cyclin T1 to direct the P-TEFb CTD kinase complex to TAR RNA. Cold Spring Harb. Symp. Quant. Biol. **63:** 371–380
9 Karn J. (1999) Tackling Tat. J. Mol. Biol. **293:** 235–254

10 Hope T. J. (1999) The ins and outs of HIV. Rev. Arch. Biochem. Biophys. **365:** 186–191
11 Pollard V. W. and Malim M. H. (1998) the HIV-1 Rev protein. Annu. Rev. Microbiol. **52:** 491–532
12 Krausslich H. G. and Welker R. (1996) Intracellular transport of retroviral capsid components. Curr. Top. Microbiol. Immunol. **214:** 25–63
13 Freed E. O. (1998) HIV-1 gag proteins: diverse functions in the virus life cycle. Virology **251:** 1–15
14 Bolognesi D. P., Montelaro R. C., Frank H. and Schafer W. (1978) Assembly of type C oncornaviruses: a model. Science. **199:** 183–186
15 Heine U. I., Margulies I., Demsey A. E. and Suskind R. G. (1979) Quantitative electron microscopy of intracytoplasmic type A particles at kinetochores of metaphase chromosomes isolated from Chinese hamster and murine cell lines. J. Gen. Virol. **45:** 631–640
16 Luftig R. B., McMillan P. N. and Bolognesi D. P. (1974) An ultrastructural study of C-type virion assembly in mouse cells. Cancer Res. **34:** 3303–3310
17 Rhee S. S. and Hunter E. (1987) Myristylation is required for intracellular transport but not for assembly of D-type retrovirus capsids. J. Virol. **61:** 1045–1053
18 Yeger H. and Kalnins V. I. (1976) Electron microscopy of mammalian type-C RNA viruses: use of conditional lethal mutants in studies on virion maturation and assembly. Virology **74:** 459–469
19 Delchambre M., Gheysen D., Thines D., Thiriart C., Jacobs E., Verdin E. et al. (1989) The GAG precursor of simian immunodeficiency virus assembles into virus-like particles. EMBO J. **8:** 2653–2660
20 Gheysen D., Jacobs E., de Foresta F., Thiriart C., Francotte M., Thines D. et al. (1989) Assembly and release of HIV-1 precursor Pr55gag virus-like particles from recombinant baculovirus-infected insect cells. Cell **59:** 103–112
21 Karacostas V., Nagashima K., Gonda M. A. and Moss B. (1989) Human immunodeficiency virus-like particles produced by a vaccinia virus expression vector. Proc. Natl. Acad. Sci. USA **86:** 8964–8967
22 Morikawa S., Booth T. F. and Bishop D. H. (1991) Analyses of the requirements for the synthesis of virus-like particles by feline immunodeficiency virus gag using baculovirus vectors. Virology **183:** 288–297
23 Morikawa Y., Goto T. and Sano K. (1999) In vitro assembly of human immunodeficiency virus type 1 Gag protein. J. Biol. Chem. **274:** 27997–28002
24 Royer M., Hong S. S., Gay B., Cerutti M. and Boulanger P. (1992) Expression and extracellular release of human immunodeficiency virus type 1 Gag precursors by recombinant baculovirus-infected cells. J. Virol. **66:** 3230–3235
25 Bryant M. L., Heuckeroth R. O., Kimata J. T., Ratner L. and Gordon J. I. (1989) Replication of human immunodeficiency virus 1 and Moloney murine leukemia virus is inhibited by different heteroatom-containing analogs of myristic acid. Proc. Natl. Acad. Sci. USA **86:** 8655–8659
26 Chazal N., Gay B., Carriere C., Tournier J. and Boulanger P. (1995) Human immunodeficiency virus type 1 MA deletion mutants expressed in baculovirus-infected cells: cis and trans effects on the Gag precursor assembly pathway. J. Virol. **69:** 365–375
27 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. Acadl. Sci. USA **86:** 5781–5785
28 Jorgensen E. C., Pedersen F. S. and Jorgensen P. (1992) Matrix protein of Akv murine leukemia virus: genetic mapping of regions essential for particle formation. J. Virol. **66:** 4479–4487
29 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
30 Spearman P., Wang J. J., Vanter 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
31 Zhou W., Parent L. J., Wills J. W. and Resh M. D. (1995) 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
32 Wills J. W. and Craven R. C. (1991) Form, function and use of retroviral Gag proteins. AIDS **5:** 639–654
33 Resh M. D. (1999) Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim. Biophys. Acta **1451:** 1–16
34 Bathurst I. C., Chester N., Gibson H. L., Dennis A. F., Steimer K. S. and Barr P. J. (1989) N myristylation of the human immunodeficiency virus type 1 gag polyprotein precursor in *Saccharomyces cerevisiae*. J. Virol. **63:** 3176–3179
35 Goddard C., Aquino A., Glazer R. I. and Felsted R. L. (1989) Chemical characterization of p17gag from human immunodeficiency virus as an N-terminally myristoylated protein. Eur. J. Biochem. **182:** 323–326
36 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
37 Verderame M. F., Nelle T. D. and Wills J. W. (1996) The membrane-binding domain of the Rous sarcoma virus Gag protein. J. Virol. **70:** 2664–2668
38 Murray D., Ben-Tal N., Honig B. and McLaughlin S. (1997) Electrostatic interaction of myristoylated proteins with membranes: simple physics, complicated biology. Structure **5:** 985–989
39 Murray D., Hermida-Matsumoto L., Buser C. A., Tsang J., Sigal C. T., Ben-Tal N. et al. (1998) Electrostatics and the membrane association of Src: theory and experiment. Biochemistry **37:** 2145–2159
40 Sigal C. T., Zhou W., Buser C. A., McLaughlin S. and Resh M. D. (1994) Amino-terminal basic residues of Src mediate membrane binding through electrostatic interation with acidic phospholipids. Proc. Natl. Acad. Sci. USA **91:** 12253–12257
41 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. USA **93:** 3099–3104
42 Massiah M. A., Worthylake D., Christensen A. M., Sundquist W. I., Hill C. P. and Summers M. F. (1996) Comparison of the NMR and X-ray structures of the HIV-1 matrix protein: evidence for conformational changes during viral assembly. Protein Sci. **5:** 2391–2398
43 Bi K. and Altman A. (2001) Membrane lipid microdomains and the role of PKCtheta in T cell activation. Semin. Immunol. **13:** 139–146
44 Cheng P. C., Cherukuri A., Dykstra M., Malapati S., Sproul T., Chen M. R. et al. (2001) Floating the raft hypothesis: the roles of lipid rafts in B cell antigen receptor function. Semin. Immunol. **13:** 107–114
45 Cherukuri A., Dykstra M. and Pierce S. K. (2001) Floating the raft hypothesis: lipid rafts play a role in immune cell activation. Immunity **14:** 657–660
46 Matsuuchi L., Gold M. R. (2001) New views of BCR structure and organization. Curr. Opin. Immunol. **13:** 270–277
47 Miceli M. C., Moran M., Chung C. D., Patel V. P., Low T. and Zinnanti W. (2001) Co-stimulation and counter-stimulation: lipid raft clustering controls TCR signaling and functional outcomes. Semin Immunol. **13:** 115–128

48 Simons K. and Toomre D. (2000) Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. **1:** 31–39
49 Viola A. (2001) The amplification of TCR signaling by dynamic membrane microdomains. Trends Immunol. **22:** 322–327
50 Ali A., Avalos R. T., Ponimaskin E. and Nayak D. P. (2000) Influenza virus assembly: effect of influenza virus glycoproteins on the membrane association of M1 protein. J. Virol. **74:** 8709–8719
51 Ali A. and Nayak D. P. (2000) Assembly of Sendai virus: M protein interacts with F and HN proteins and with the cytoplasmic tail and transmembrane domain of F protein. Virology **276:** 289–303
52 Barman S., Ali A., Hui E. K., Adhikary L. and Nayak D. P. (2001) Transport of viral proteins to the apical membranes and interaction of matrix protein with glycoproteins in the assembly of influenza viruses. Virus Res. **77:** 61–69
53 Manie S. N., Debreyne S., Vincent S. and Gerlier D. (2000) Measles virus structural components are enriched into lipid raft microdomains: a potential cellular location for virus assembly. J. Virol. **74:** 305–311
54 Vincent S., Gerlier D. and Manie S. N. (2000) Measles virus assembly within membrane rafts. J. Virol. **74:** 9911–9915
55 Pickl W. F., Pimentel-Muinos F. X. and Seed B. (2001) Lipid rafts and pseudotyping. J. Virol. **75:** 7175–7183
56 Wang J. K., Kiyokawa E., Verdin E. and Trono D. (2000) The Nef protein of HIV-1 associates with rafts and primes T cells for activation. Proc. Natl. Acad. Sci. USA **97:** 394–399
57 Zheng Y., Plemenitas A., Linnemann T., Fackler O. T. and Peterlin B. M. (2001) Nef increases infectivity of HIV via lipid rafts. Curr. Biol. **11:** 875–879
58 Aloia R. C., Jensen F. C., Curtain C. C., Mobley P. W. and Gordon L. M. (1988) Lipid composition and fluidity of the human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **85:** 900–904
59 Pessin J. E. and Glaser M. (1980) Budding of Rous sarcoma virus and vesicular stomatitis virus from localized lipid regions in the plasma membrane of chicken embryo fibroblasts. J. Biol. Chem. **255:** 9044–9050
60 Quigley J. P., Rifkin D. B. and Reich E. (1971) Phospholipid composition of Rous sarcoma virus, host cell membranes and other enveloped RNA viruses. Virology **46:** 106–116
61 Camaur D. and Trono D. (1996) Characterization of human immunodeficiency virus type 1 Vif particle incorporation. J. Virol. **70:** 6106–6111
62 Ott D. E. (1997) Cellular proteins in HIV virions. Rev. Med. Virol. **7:** 167–180
63 Poon D. T., Coren L. V. and Ott D. E. (2000) Efficient incorporation of HLA class II onto human immunodeficiency virus type 1 requires envelope glycoprotein packaging. J. Virol. **74:** 3918–3923
64 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
65 Lindwasser O. W. and Resh M. D. (2001) Multimerization of human immunodeficiency cirus type 1 Gag promotes its localization to barges, raft-like membrane microdomains. J. Virol. **75:** 7913–7924
66 Ono A. and Freed E. O. (2001) Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc. Natl. Acad. Sci. USA **98:** 13925–13930
67 Zhou W. and Resh M. D. (1996) Differential membrane binding of the human immunodeficiency virus type 1 matrix protein. J. Virol. **70:** 8540–8548
68 Ono A., Demirov D. and Freed E. O. (2000) Relationship between human immunodeficiency virus type 1 Gag multimerization and membrane binding. J. Virol. **74:** 5142–5150
69 Platt E. J. and Haffar O. K. (1994) Characterization of human immunodeficiency virus type 1 Pr55gag membrane association in a cell-free system: requirement for a C-terminal domain. Proc. Natl. Acad. Sci. USA **91:** 4594–4598
70 Gomez-Mouton C., Abad J. L., Mirra E., Lacalle R. A., Gallardo E., Jimenez-Baranda S. et al. (2001) Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc. Natl. Acad. Sci. USA **98:** 9642–9647
71 Prior I. A. and Hancock J. F. (2001) Compartmentalization of Ras proteins. J. Cell Sci. **114:** 1603–1608
72 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
73 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. USA **11:** 11
74 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:** 636–443
75 Mariani R., Rasala B. A., Rutter G., Wiegers K., Brandt S. M., Krausslich H. G. (2001) Mouse-human heterokaryons support efficient human immunodeficiency virus type 1 assembly. J. Virol. **75:** 3141–3151
76 Campbell S., Fisher R. J., Towler E. M., Fox S., Issaq H. J., Wolfe T. et al. (2001) Modulation of HIV-like particle assembly in vitro by inositol phosphates. Proc. Natl. Acad. Sci. USA. **98:** 10875–10879
77 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
78 Campbell S. and Vogt V. M. (1997) In vitro assembly of virus-like particles with Rous sarcoma virus Gag delition mutants: identification of the p10 domain as a morphological determinant in the formation of spherical particles. J. Virol. **71:** 4425–4435
79 Klikova M., Rhee S. S., Hunter E. and Ruml T. (1995) Efficient in vivo and in vitro assembly of retroviral capsids from Gag precursor proteins expressed in bacteria. J. Virol. **69:** 1093–1098
80 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
81 Sakalian M., Parker S. D., Weldon R. A. Jr. and Hunter E. (1996) Synthesis and assembly of retrovirus Gag precursors into immature capsids in vitro. J. Virol. **70:** 3706–3715
82 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
83 Terwilliger E. F., Cohen E. A., Lu Y. C., Sodroski J. G. and Haseltine W. A. (1989) Functional role of human immunodeficiency virus type 1 vpu. Proc. Natl. Acad. Sci. USA **86:** 5163–5167
84 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
85 Franke E. K., Yuan H. E., Bossolt K. L., Goff S. P. and Luban J. (1994) Specificity and sequence requirements for interactions between various retroviral Gag proteins. J. Virol. **68:** 5300–5305
86 Bennett R. P., Nelle T. D. and Wills J. W. (1993) Functional chimeras of the Rous sarcoma virus and human immunodeficiency virus gag proteins. J. Virol. **67:** 6487–6498
87 Forster M. J., Mulloy B. and Nermut M. V. (2000) Molecular modelling study of HIV p17gag (MA) protein shell utilising data from electron microscopy and X-ray crystallography. J. Mol. Biol. **298:** 841–857

88 Morikawa Y., Zhang W. H., Hockley D. J., Nermut M. V. and Jones I. M. (1998) Detection of a trimeric human immunodeficiency virus type 1 Gag intermediate is dependent on sequences in the matrix protein, p17. J. Virol. **72:** 7659–7663
89 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
90 Accola M. A., Strack B. and Gottlinger H. G. (2000) 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
91 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
92 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
93 Barklis E., McDermott J., Wilkens S., Fuller S. and Thompson D. (1998) Organization of HIV-1 capsid proteins on a lipid monolayer. J. Biol. Chem. **273:** 7177–7180
94 Campbell S. and Vogt V. M. (1995) Self-assembly in vitro of purified CA-NC proteins from Rous sarvoma virus and human immunodeficiency virus type 1. J. Virol. **69:** 6487–6497
95 Ehrlich L. S., Agresta B. E. and Carter C. A. (1992) Assembly of recombinant human immunodeficiency virus type 1 capsid protein in vitro. J. Virol. **66:** 4874–4883
96 Ehrlich L. S., Liu T., Scarlata S., Chu B. and Carter C. A. (2001) HIV-1 capsid protein forms spherical (immature-like) and tubular (mature-like) particles in vitro: structure switching by pH-induced conformational changes. Biophys. J. **81:** 586–594
97 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
98 Gross I., Hohenberg H., Wilk T., Wiegers K., Grattinger M., Muller B. et al. (2000) A conformational switch controlling HIV-1 morphogenesis. EMBO J. **19:** 103–113
99 von Schwedler U. K., Stemmler T. L., Klishko V. Y., Li S., Albertine K. H., Davis D. R. et al. (1998) Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. EMBO J. **17:** 1555–1568
100 Gamble T. R., Yoo S., Vajdos F. F., von Schwedler U. K., Worthylake D. K., Wang H. et al. (1997) Structure of the carboxyl-terminal dimerization domain of the HIV-1 capsid protein. Science **278:** 849–853
101 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
102 Momany C., Kovari L. C., Prongay A. J., Keller W., Gitti R. K., Lee B. M. et al. (1996) Crystal structure of dimeric HIV-1 capsid protein. Nat. Struct. Biol. **3:** 763–770
103 Berthet-Colominas C., Monaco S., Novelli A., Sibai G., Mallet F. and Cusack S. (1999) Head-to-tail dimers and interdomain flexibility revealed by the crystal structure of HIV-1 capsid protein (p24) complexed with a monoclonal antibody Fab. EMBO J. **18:** 1124–1136
104 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
105 Dorfman T., Bukovsky A., Ohagen A., Hoglund S. and Gottlinger H. G. (1994) Functional domains of the capsid protein of human immunodeficiency virus type 1. J. Virol. **68:** 8180–8187
106 Mammano F., Ohagen A., Hoglund S. and Gottlinger H. G. (1994) Role of the major homolgy region of human immunodeficiency virus type 1 in virion morphogenesis. J. Virol. **68:** 4927–4936
107 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
108 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
109 von Poblotzki A., Wagner R., Niedrig M., Wanner G., Wolf H. and Modrow S. (1993) Identification of a region in the Pr55gag-polyprotein essential for HIV-1 particle formation. Virology **193:** 981–985
110 Carriere C., Gay B., Chazal N., Morin N. and Boulanger P. (1995) Sequence requirements for encapsidation of deletion mutants and chimeras of human immunodeficiency virus type 1 Gag precursor into retrovirus-like particles. J. Virol. **69:** 2366–2377
111 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
112 Dawson L. and Yu X. F. (1998) The role of nucleocapsid of HIV-1 in virus assembly. Virology **251:** 141–157
113 Jowett J. B., Hockley D. J., Nermut M. V. and Jones I. M. (1992) Distinct signals in human immunodeficiency virus type 1 Pr55 necessary for RNA binding and particle formation. J. Gen. Virol. **73:** 3079–3086
114 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
115 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
116 Gay B., Tournier J., Chazal N., Carriere C. and Boulanger P. (1998) Morphopoietic determinants of HIV-1 Gag particles assembled in baculovirus-infected cells. Virology **247:** 160–169
117 Ottmann M., Gabus C. and Darlix J. L. (1995) The central globular domain of the nucleocapsid protein of human immunodeficiency virus type 1 is critical for virion structure and infectivity. J. Virol. **69:** 1778–1784
118 Tanchou V., Decimo D., Pechoux C., Lener D., Rogemond V., Berthoux L. et al. (1998) Role of the N-terminal zinc finger of human immunodeficiency virus type 1 nucleocapsid protein in virus structure and replication. J. Virol. **72:** 4442–4447
119 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
120 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
121 Sakaguchi K., Zambrano N., Baldwin E. T., Shapiro B. A., Erickson J. W., Omichinski J. G. et al. (1993) Identification of a binding site for the human immunodeficiency virus type 1 nucleocapsid protein. Proc. Natl. Acad. Sci. USA **90:** 5219–5223
122 Cimarelli A., Sandin S., Hoglund S. and Luban J. (2000) Rescue of multiple viral functions by a second-site suppressor of a human immunodeficiency virus type 1 nucleocapsid mutation. J. Virol. **74:** 4273–4283
123 Lapadat-Tapolsky M., Gabus C., Rau M. and Darlix J. L. (1997) Possible roles of HIV-1 nucleocapsid protein in the specificity of proviral DNA synthesis and in its variability. J. Mol. Biol. **268:** 250–260

124 Carteau S., Batson S. C., Poljak L., Mouscadet J. F., de Rocquigny H. and Darlix J. L. (1997) Human immunodeficiency virus type 1 nucleocapsid protein specifically stimulates Mg^{2+}-dependent DNA integration in vitro. J. Virol. **71:** 6225–6229.

125 Carteau S., Gorelick J. and Bushman F. D. (1999) Coupled integration of human immunodeficiency virus type 1 cDNA ends by purified integrase in vitro: stimulation by the viral nucleocapsid protein. J. Virol. **73:** 6670–6679

126 Berkowitz R., Fisher J. and Goff S. P. (1996) RNA packaging. Curr. Top. Micorbiol. Immunol. **214:** 177–218

127 Darlix J. L., Lapadat-Tapolsky M., de Rocquigny H. and Roques B. P. (1995) First glimpses at structure-function relationships of the nucleocapsid protein of retroviruses. J. Mol. Biol. **254:** 523–537

128 Amarasinghe G. K., De Guzman R. N., Turner R. B., Chancellor K. J., Wu Z. R. and Summers M. F. (2000) NMR structure of the HIV-1 nucleocapsid protein bound to stem-loop SL2 of the psi-RNA packaging signal: implications for genome recognition. J. Mol. Biol. **301:** 491–511

129 De Guzman R. N., Wu Z. R., Stalling C. C., Pappalardo L., Borer P. N. and Summers M. F. (1998) Structure of the HIV-1 nucleocapsid protein bound to the SL3 psi-RNA recognition element. Science **279:** 384–388

130 Demene H., Dong C. Z., Ottmann M., Rouyez M. C., Jullian N., Morellet N. et al. (1994) ^{1}H NMR structure and biological studies of the His23→Cys mutant nucleocapsid protein of HIV-1 indicate that the conformation of the first zinc finger is critical for virus infectivity. Biochemistry **33:** 11707–11716

131 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

132 Mely Y., Jullian N., Morellet N., de Rocquigny H., Dong C. Z., Piemont E. et al. (1994) Spatial proximity of the HIV-1 nucleocapsid protein zinc fingers investigated by time-resolved fluorescence and fluorescence resonance energy transfer. Biochemistry **33:** 12085–12091

133 Morellet N., de Rocquigny H., Mely Y., Jullian N., Demene H., Ottmann M. et al. (1994) Conformational behaviour of the active and inactive forms of the nucleocapsid NCp7 of HIV-1 studied by ^{1}H NMR. J. Mol. Biol. **235:** 287–301

134 Morellet N., Jullian N., de Rocquigny H., Maigret B., Darlix J. L. and Roques B. P. (1992) Determination of the structure of the nucleocapsid protein NCp7 from the human immunodeficiency virus type 1 by ^{1}H NMR EMBO J. **11:** 3059–3065

135 Mely Y., de Rocquigny H., Piemont E., Demene H., Jullian N., Fournie-Zaluski M. C. et al. (1993) Influence of the N- and C-terminal chains on the zinc-binding and conformational properties of the central zinc-finger structure of Moloney murine leukaemia virus nucleocapsid protein: a steady-state and time-resolved fluorescence study. Biochim. Biophys. Acta **1161:** 6–18

136 Homa F. L. and Brown J. C. (1997) Capsid assembly and DNA packaging in herpes simplex virus. Rev. Med. Virol. **7:** 107–122

137 Rose R. C., Bonnez W., Reichman R. C. and Garcea R. L. (1993) Expression of human papillomavirus type 11 L1 protein in insect cells: in vivo and in vitro assembly of viruslike particles. J. Virol. **67:** 1936–1944

138 Salunke D. M., Caspar D. L. and Garcea R. L. (1986) Self-assembly of purified polyomavirus capsid protein VP1. Cell. **46:** 895–904

139 Zlotnick Al., Johnson J. M., Wingfield P. W., Stahl S. J. and Endres D. (1999) A theoretical model successfully identifies features of hepatitis B virus capsid assembly. Biochemistry. **38:** 14644–14652

140 Abouhaidar M. and Bancroft J. B. (1978) The initiation of papaya mosaic virus assembly. Virology **90:** 54–59

141 Bancroft J. B. (1970) The self-assembly of spherical plant viruses. Adv. Virus Res. **16:** 99–134

142 Sorger P. K., Stockley P. G. and Harrison S. C. (1986) Structure and assembly of turnip crinkle virus. II. Mechanism of reassembly in vitro. J. Mol. Biol. **191:** 639–658

143 Tellinghuisen T. L., Hamburger A. E., Fisher B. R., Ostendorp R. and Kuhn R. J. (1999) In vitro assembly of alphavirus cores by using nucleocapsid protein expressed in *Escherichia coli.* J. Virol. **73:** 5309–5319

144 Wengler G., Boege U., Bischoff H. and Wahn K. (1982) The core protein of the alphavirus Sindbis virus assembles into core-like nucleoproteins with the viral genome RNA and with other single-stranded nucleic acids in vitro. Virology **118:** 401–410

145 Tanchou V., Gabus C., Rogemond V. and Darlix J. L. (1995) Formation of stable and functional HIV-1 nucleoprotein complexes in vitro. J. Mol. Biol. **252:** 563–571

146 Eijkelenboom A. P., van den Ent F. M., Wechselberger R., Plasterk R. H., Kaptein R. and Boelens R. (2000) Refined solution structure of the dimeric N-terminal HHCC domain of HIV-2 integrase. J. Biomol. NMR **18:** 119–128

147 Esposito D. and Craigie R. (1999) HIV integrase structure and function. Adv. Virus Res. **52:** 319–333

148 Kohlstaedt L. A., Wang J., Friedman J. M., Rice P. A. and Steitz T. A. (1992) Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science **256:** 1783–1790

149 Lodi P. J., Ernst J. A., Kuszewski J., Hickman A. B., Engelman A., Craigie R. et al. (1995) Solution structure of the DNA binding domain of HIV-1 integrase. Biochemistry **34:** 9826–9833

150 Navia M. A., Fitzgerald P. M., McKeever B. M., Leu C. T., Heimbach J. C., Herber W. K. et al. (1989) Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. Nature **337:** 615–620

151 Unge T., Ahola H., Bhikhabhai R., Backbro K., Lovgren S., Fenyo E. M. et al. (1990) Expression, purification, and crystallization of the HIV-1 reverse transcriptase (RT). AIDS Res. Hum. Retroviruses **6:** 1297–1303

152 Unge T., Knight S., Bhikhabhai R., Lovgren S., Dauter Z., Wilson K. et al. (1994) 2.2 A resolution structure of the amino-terminal half of HIV-1 reverse transcriptase (fingers and palm subdomains). Structure **2:** 953–961

153 Wlodawer A., Miller M., Jaskolski M., Sathyanarayana B. K., Baldwin E., Weber I. T. et al. (1989) Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. Science **245:** 616–621

154 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

155 Schmalzbauer E., Strack B., Dannull J., Guehmann S. and Moelling K. (1996) Mutations of basic amino acids of NCp7 of human immunodeficiency virus type 1 affect RNA binding in vitro. J. Virol. **70:** 771–777

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

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

158 Clavel F. and Orenstein J. M. (1990) A mutant of human immunodeficiency virus with reduced RNA packaging and abnormal particle morphology. J. Virol. **64:** 5230–5234

159 Darlix J. L., Gabus C., Nugeyre M. T., Clavel F. and Barre-Sinoussi F. (1990) Cis elements and trans-acting factors involved in the RNA dimerization of the human immunodeficiency virus HIV-1. J. Mol. Biol. **216:** 689–699

160 Harrison G. P., Miele G., Hunter E. and Lever A. M. (1998) Functional analysis of the core human immunodeficiency

virus type 1 packaging signal in a permissive cell line. J. Virol. **72:** 5886–5896

161 Luban J. and Goff S. P. (1994) Mutational analysis of cis-acting packaging signals in human immunodeficiency virus type 1 RNA. J. Virol. **68:** 3784–3793

162 Zeffman A., Hassard S., Varani G. and Lever A. (2000) The major HIV-1 packaging signal is an extended bulged stem loop whose structure is altered on interaction with the Gag polyprotein. J. Mol. Biol. **297:** 877–893

163 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

164 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

165 Garrus J. W., von Schwedler U. K., Pornillos O. W., Morham S. G., Zavitz K. H., Wang H. E. et al. (2001) Tsg101 and the vacuolar protein sorting pathway are exxential for HIV-1 budding. Cell **107:** 55–65

166 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. USA **88:** 3195–3199

167 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

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

169 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

170 Vogt V. M. (2000) Ubiquitin in retrovirus assembly: actor or bystander? Proc. Natl. Acad. Sci. USA **97:** 12945–12947

171 Parent L. J., Bennett R. P., Craven R. C., Nelle T. D., Krishna N. K., Bowzard J. B. et al. (1995) Positionally independet and exchangeable late budding functions of the Rous sarcoma virus and human immunodeficiency virus Gag proteins. J. Virol. **69:** 5455–5460

172 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

173 Kikonyogo A., Bouamr F., Vana M. L., Xiang Y, Aiyar A., Carter C. et al. (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. USA **98:** 11199–11204

174 VerPlank L., Bouamr F., LaGrassa T. J., Agresta B., Kikonyogo A., Leis J. et al. (2001) Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). Proc. Natl. Acad. Sci. USA **98:** 7724–7729

175 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

176 Lemmon S. K. and Traub L. M. (2000) Sorting in the endosomal system in yeast and animal cells. Curr. Opin. Cell Biol. **12:** 457–466

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

178 Ott D. E., Coren L. V., Copeland T. D., Kane B. P., Johnson D. G., Sowder R. C., 2nd et al. (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

179 Schubert U., Ott D. E., Chertova E. N., Welker R., Tessmer U., Princiotta M. F. et al. (2000) Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. Proc. Natl. Acad. Sci. USA **97:** 13057–13062

180 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. USA **97:** 13063–13068

181 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

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

183 Bender W. and Davidson N. (1976) Mapping of poly(A) sequences in the electron microscope reveals unusual structure of type C oncornavirus RNA molecules. Cell **7:** 595–607

184 Canaani E., Helm K. V. and Duesberg P. (1973) Evidence for 30–40S RNA as precursor of the 60–70S RNA of Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **70:** 401–405

185 Duesberg P., Canaani E. and Von der Helm K. (1973) Evidence for 30–40S RNA as precursor of the 60–70S RNA of Rous sarcoma virus. Am. J. Clin. Pathol. **60:** 57–64

186 Korb J., Travnicek M. and Riman J. (1976) The oncornavirus maturation process: quantitative correlation between morphological changes and conversion of genomic virion RNA. Intervirology **7:** 211–224

187 Dardel F., Marquet R., Ehresmann C., Ehresmann B. and Blanquet S. (1998) Solution studies of the dimerization initiation site of HIV-1 genomic RNA. Nucleic Acids Res. **26:** 3567–3571

188 Girard F., Barbault F., Gouyette C., Huynh-Dinh T., Paoletti J. and Lancelot G. (1999) Dimer initiation sequence of HIV-1 Lai genomic RNA: NMR solution structure of the extended duplex. J. Biomol. Struct. Dyn. **16:** 1145–1157

189 Laughrea M. and Jette L. (1996) Kissing-loop model of HIV-1 genome dimerization: HIV-1 RNAs can assume alternative dimeric forms, and all sequences upstream or downstream of hairpin 248–271 are dispensable for dimer formation. Biochemistry **35:** 1589–1598

190 Ly H., Nierlich D. P., Olsen J. C. and Kaplan A. H. (1999) Moloney murine sarcoma virus genomic RNAs dimerize via a two-step process: a concentration-dependent kissing-loop interaction is driven by initial contact between consecutive guanines. J. Virol. **73:** 7255–7261

191 Paillart J. C., Marquet R., Skripkin E., Ehresmann C. and Ehresmann B. (1996) Dimerization of retroviral genomic RNAs: structural and functional implications. Biochimie **78:** 639–653

192 Skripkin E., Paillart J. C., Marquet R., Ehresmann B. and Ehresmann C. (1994) Identification of the primary site of the human immunodeficiency virus type 1 RNA dimerization in vitro. Proc. Natl. Acad. Sci. USA **91:** 4945–4949

193 Sundquist W. I. and Heaphy S. (1993) Evidence for interstrand quadruplex formation in the dimerization of human immunodeficiency virus 1 genomic RNA. Proc. Natl. Acad. Sci. USA **90:** 3393–3397

194 Takahashi K, Baba S., Hayashi Y., Koyanagi Y., Yamamoto N., Takaku H. et al. (2000) NMR analysis of intra- and inter-molecular stems in the dimerization initiation site of the HIV-1 genome. J. Biochem. (Tokyo) **127:** 681–686

195 Feng Y. X., Copeland T. D., Henderson L. E., Gorelick R. J., Bosche W. J., Levin J. G. et al. (1996) HIV-1 nucleocapsid protein induces 'maturation' of dimeric retroviral RNA in vitro. Proc. Natl. Acad. Sci. USA **93:** 7577–7581

196 Fu W., Gorelick R. J. and Rein A. (1994) Characterization of human immunodeficiency virus type 1 dimeric RNA from wild-type and protease-defective virions. J. Virol. **68:** 5013–5018

197 Bonnet-Mathoniere B., Girard P. M., Muriaux D. and Paoletti J. (1996) Nucleocapsid protein 10 activates dimerization of the RNA of Moloney murine leukaemia virus in vitro. Eur. J. Biochem. **238:** 129–135

198 Fu W. and Rein A. (1993) Maturation of dimeric viral RNA of Moloney murine leukemia virus. J. Virol. **67:** 5443–5449

199 Shehu-Xhilaga M., Kraeusslich H. G., Pettit S., Swanstrom R., Lee J. Y., Marshall J. A. et al. (2001) Proteolytic processing of the p2/nucleocapsid cleavate site is critical for human immunodeficiency virus type 1 RNA dimer maturation. J. Virol. **75:** 9156–9164

200 Balakrishnan M., Fay P. J. and Bambara R. A. (2001) The kissing hairpin sequence promotes recombination within the HIV-I 5′ leader region. J. Biol. Chem. **276:** 36482–36492

201 Weiss S., Hausl G., Famulok M. and Konig B. (1993) The multimerization state of retroviral RNA is modulated by ammonium ions and affects HIV-1 full-length cDNA synthesis in vitro. Nucleic Acids Res. **21:** 4879–4885

202 Zhang J. and Temin H. M. (1996) The recombination rate is not increased when retroviral RNA is missing an encapsidation sequence. J. Virol. **70:** 2019–2021

203 Darlix J. L., Cristofari G., Rau M., Pechoux C., Berthoux L. and Roques B. (2000) Nucleocapsid protein of human immunodeficiency virus as a model protein with chaperoning functions and as a target for antiviral drugs. Adv. Pharmacol. **48:** 345–372

204 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

205 Dorman N. and Lever A. (2000) Comparison of viral genomic RNA sorting mechanisms in human immunodeficiency virus type 1 (HIV-1), HIV-2, and Moloney murine leukemia virus. J. Virol. **74:** 11413–11417

206 Buck C. B., Shen X., Egan M. A., Pierson T. C., Walker C. M. and Siliciano R. F. (2001) The human immunodeficiency virus type 1 gag gene encodes an internal ribosome entry site. J. Virol. **75:** 181–191

207 Deffaud C. and Darlix J. L. (2000) Rous sarcoma virus translation revisited: characterization of an internal ribosome entry segment in the 5′ leader of the genomic RNA. J. Virol. **74:** 11581–11588

208 Ohlmann T., Lopez-Lastra M. and Darlix J. L. (2000) An internal ribosome entry segment promotes translation of the simian immunodeficiency virus genomic RNA. J. Biol. Chem. **275:** 11899–11906

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

210 Freed E. O. and Martin M. A. (1996) Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. J. Virol. **70:** 341–351

211 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

212 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

213 Ono A., Huang M. and Freed E. O. (1997) Characterization of human immunodeficiency virus type 1 matrix revertants: effects on virus assembly, Gag processing, and Env incorporation into virions. J. Virol. **71:** 4409–4418

214 Yu X., Yuan X., Matsuda Z., Lee T. H. and Essex M. (1992) 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

215 Cosson P. (1996) Direct interaction between the envelope and matrix proteins of HIV-1. EMBO J. **15:** 5783–5788

216 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

217 Murakami T. and Freed E. O. (2000) Genetic evidence for an interaction between human immunodeficiency virus type 1 matrix and alpha-helix 2 of the gp41 cytoplasmic tail. J. Virol. **74:** 3548–3554

218 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

219 Rousso I., Mixon M. B., Chen B. K. and Kim P. S. (2000) Palmitoylation of the HIV-1 envelope glycoprotein is critical for viral infectivity. Proc. Natl. Acad. Sci. USA **97:** 13523–13525

220 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

221 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. USA **88:** 3987–3991

222 Yang C., Spies C. P. and Compans R. W. (1995) The human and simian immunodeficiency virus envelope glycoprotein transmembrane subunits are palmitoylated. Proc. Natl. Acad. Sci. USA **92:** 9871–9875

223 Berlioz-Torrent C., Shacklett B. L., Erdtmann L., Delamarre L., Bouchaert I., Sonigo P. et al. (1999) Interactions of the cytoplasmic domains of human and simian retroviral transmembrane proteins with components of the clathrin adaptor complexes modulate intracellular and cell surface expression of envelope glycoproteins. J. Virol. **73:** 1350–1361

224 Egan M. A., Carruth 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

225 Ohno H., Aguilar R. C., Fournier M. C., Hennecke S., Cosson P. and Bonifacino J. S. (1997) Interaction of endocytic signals from the HIV-1 envelope glycoprotein complex with members of the adaptor medium chain family. Virology **238:** 305–315

226 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

227 Wyss S., Berlioz-Torrent C., Boge M., Blot G., Honing S., Benarous R. et al. (2001) The highly conserved C-terminal dileucine motif in the cytosolic domain of the human immunodeficiency virus type 1 envelope glycoprotein is critical for its association with the AP-1 clathrin adapter. J. Virol. **75:** 2982–2992

228 Lee Y. M., Tang X. B., Cimakasky L. M., Hildreth J. E. and Yu X. F. (1997) Mutations in the matrix protein of human immunodeficiency virus type 1 inhibit surface expression and virion incorporation of viral envelope glycoproteins in CD4+ T lymphocytes. J. Virol. **71:** 143–1452

229 Bennett R. P., Rhee S., Craven R. C., Hunter E. and Wills J. W. (1991) Amino acids encoded downstream of gag are not required by Rous sarcoma virus protease during gag-mediated assembly. J. Virol. **65:** 272–280

230 Burstein H., Bizub D and Skalka A. M. (1991) Assembly and processing of avian retroviral gag polyproteins containing linked protease dimers. J. Virol. **65:** 6165–6172

231 Karacostas V., Wolffe E. J., Nagashima K., Gonda M. A. and Moss B. (1993) Overexpression of the HIV-1 gag-pol polyprotein results in intracellular activation of HIV-1 protease and inhibition of assembly and budding of virus-like particles. Virology **193:** 661–671

232 Luukkonen B. G., Fenyo E. M. and Schwartz S. (1995) Overexpression of human immunodeficiency virus type 1 protease increases intracellular cleavage of Gag and reduces virus infectivity. Virology **206:** 854–865

233 Xiang Y., Ridky T. W., Krishna N. K. and Leis J. (1997) Altered Rous sarcoma virus Gag polyprotein processing and its effects on particle formation. J. Virol. **71:** 2083–2091

234 Sommerfelt M. A., Petteway S. R. Jr., Dreyer G. B. and Hunter E. (1992) Effect of retroviral proteinase inhibitors on Mason-Pfizer monkey virus maturation and transmembrane glycoprotein cleavage. J. Virol. **66:** 4220–4227

235 Sheng N. and Erickson-Viitanen S. (1994) Cleavage of p15 protein in vitro by human immunodeficiency virus type 1 protease is RNA dependent. J. Virol. **68:** 6207–6214

236 Sheng N., Pettit S. C., Tritch R. J., Ozturk D. H., Rayner M. M., Swanstrom R. et al. (1997) Determinants of the human immunodeficiency virus type 1 p15NC-RNA interaction that affect enhanced cleavage by the viral protease. J. Virol. **71:** 5723–5732

237 Goncalves J., Jallepalli P. and Gabuzda D. H. (1994) Subcellular localization of the Vif protein of human immunodeficiency virus type 1. J. Virol. **68:** 704–712

238 Karczewski M. K. and Strebel K (1996) Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. J. Virol. **70:** 494–507

239 Simon J. H., Fouchier R. A., Southerling T. E., Guerra C. B., Grant C. K. and Malim M. H. (1997) The Vif and Gag proteins of human immunodeficiency virus type 1 colocalize in infected human T cells. J. Virol. **71:** 5259–5267

240 Liu H., Wu X., Newman M., Shaw G. M., Hahn B. H. and Kappes J. C. (1995) The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. J. Virol. **69:** 7630–7638

241 Sova P., Volsky D. J., Wang L. and Chao W. (2001) Vif is largely absent from human immunodeficiency virus type 1 mature virions and associates mainly with viral particles containing unprocessed gag. J. Virol. **75:** 5504–5517

242 Khan M. A., Aberham C., Kao S., Akari H., Gorelick R., Bour S. et al. (2001) Human immunodeficiency virus type 1 Vif protein is packaged into the nucleoprotein complex through an interaction with viral genomic RNA. J. Virol. **75:** 7252–7265

243 Ohagen A. and Gabuzda D. (2000) Role of Vif in stability of the human immunodeficiency virus type 1 core. J. Virol. **74:** 11055–11066

244 Dettenhofer M. and Yu X. F. (1999) Highly purified human immunodeficiency virus type 1 reveals a virtual absence of Vif in virions. J. Virol. **73:** 1460–1467

245 Bouyac M., Rey F., Nascimbeni M., Courcoul M., Sire J., Blanc D. et al. (1997) Phenotypically Vif– human immunodeficiency virus type 1 is produced by chronically infected restrictive cells. J. Virol. **71:** 2473–2477

246 Fouchier R. A., Simon J. H., Jaffe A. B. and Malim M. H. (1996) Human immunodeficiency virus type 1 Vif does not influence expression or virion incorporation of gag-, pol-, and env-encoded proteins. J. Virol. **70:** 8263–8269

247 Gabuzda D. H., Lawrence K., Langhoff E., Terwilliger E., Dorfman T., Haseltine W. A. et al. (1992) Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. J. Virol. **66:** 6489–6495

248 Sakai H., Shibata R., Sakuragi J., Sakuragi S., Kawamura M. and Adachi A. (1993) Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. J. Virol. **67:** 1663–1666

249 Ma X. Y., Sova P., Chao W. and Volsky D. J. (1994) Cysteine residues in the Vif protein of human immunodeficiency virus type 1 are essential for viral infectivity. J. Virol. **68:** 1714–1720

250 Madani N. and Kabat D. (1998) An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. J. Virol. **72:** 10251–10255

251 Nascimbeni M., Bouyac M., Rey F., Spire B. and Clavel F. (1998) The replicative impairment of Vif– mutants of human immunodeficiency virus type 1 correlates with an overall defect in viral DNA synthesis. J. Gen. Virol. **79:** 1945–1950

252 Simon J. H., Gaddis N. C., Fouchier R. A. and Malim M. H. (1998) Evidence for a newly discovered cellular anti-HIV-1 phenotype. Nat. Med. **4:** 1397–1400

253 Sova P. and Volsky D. J. (1993) Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with vif-negative human immunodeficiency virus type 1. J. Virol. **67:** 6322–6326

254 Courcoul M., Patience C., Rey F., Blanc D., Harmache A., Sire J. et al. (1995) Peripheral blood mononuclear cells produce normal amounts of defective Vif– human immunodeficiency virus type 1 particles which are restricted for the preretrotranscription steps. J. Virol. **69:** 2068–2074

255 von Schwedler U., Song J., Aiken C. and Trono D. (1993) Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:** 4945–4955

256 Dornadula G., Yang S., Pomerantz R. J. and Zhang H. (2000) Partial rescue of the Vif-negative phenotype of mutant human immunodeficiency virus type 1 strains from nonpermissive cells by intravirion reverse transcription. J. Virol. **74:** 2594–2602

257 Dettenhofer M., Cen S., Carlson B. A., Kleiman L. and Yu X. F. (2000) Association of human immunodeficiency virus type 1 Vif with RNA and its role in reverse transcription. J. Virol. **74:** 8938–8945

258 Zhang H., Pomerantz R. J., Dornadula G. and Sun Y. (2000) Human immunodeficiency virus type 1 Vif protein is an integral component of an mRNP complex of viral RNA and could be involved in the viral RNA folding an packaging process. J. Virol. **74:** 8252–8261

259 Cohen E. A., Terwilliger E. F., Sodroski J. G. and Haseltine W. A. (1988) Identification of a protein encoded by the vpu gene of HIV-1. Nature **334:** 532–534

260 Maldarelli F., Chen M. Y., Willey R. L. and Strebel K. (1993) Human immunodeficiency virus type 1 Vpu protein is an oligometric type I integral membrane protein. J. Virol. **67:** 5056–5061

261 Myers G., Korber B., Pavlakis G. N., Jeang K. T., Henderson L. E. and Wain-Hobson S. (1994) A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences, Los Alamos National Laboratory, Los Alamos, N. M.

262 Friborg J., Ladha A., Gottlinger H., Haseltine W. A. and Cohen E. A. (1995) Functional analysis of the phosphorylation sites on the human immunodeficiency virus type 1 Vpu protein. J. Acquir. Immun. Defic. Syndr. Hum. Retrovirol. **8:** 10–22

263 Paul M. and Jabbar M. A. (1997) Phosphorylation of both phosphoacceptor sites in the HIV-1 Vpu cytoplasmic domain is essential for Vpu-mediated ER degradation of CD4. Virology **232:** 207–216

264 Paul M., Mazumder S., Raja N. and Jabbar M. A. (1998) Mutational analysis of the human immunodeficiency virus type 1 Vpu transmembrane domain that promotes the enhanced release of virus-like particles from the plasma membrane of mammalian cells. J. Virol. **72:** 1270–1279

265 Tiganos E., Friborg J., Allain B., Daniel N. G., Yao X. J. and Cohen E. A. (1998) Structural and functional analysis of the membrane-spanning domain of the human immunodeficiency virus type 1 Vpu protein. Virology **251:** 96–107

266 Emerman M. and Malim M. H. (1998). HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science **280:** 1880–1884

267 Piguet V., Schwartz O., Le Gall S. and Trono D. (1999) The downregulation of CD4 and MHC-I by primate lentiviruses: a paradigm for the modulation fo cell surface receptors. Immunol. Rev. **168:** 51–63
268 Speth C. and Dierich M. P. (1999) Modulation of cell surface protein expression by infection with HIV-1. Leukemia **13(suppl. 1):** S99–S105
269 Gottlinger H. G., Dorfman T., Cohen E. A. and Haseltine W. A. (1993) Vpu protein of human immunodeficiency virus type 1 enhances the release of capsids produced by gag gene constructs of widely divergent retroviruses. Proc. Natl. Acad. Sci. USA **90:** 7381–7385
270 Matsuda Z., Chou M. J., Matsuda M., Huang J. H., Chen Y. M., Redfield R. et al. (1988) Human immunodeficiency virus type 1 has an additional coding sequence in the central region of the genome. Proc. Natl. Acad. Sci. USA **85:** 6968– 6972
271 Schubert U., Clouse K. A. and Strebel K. (1995) Augmentation of virus secretion by the human immunodeficiency virus type 1 Vpu protein is cell type independent and occurs in cultured human primary macrophages and lymphocytes. J. Virol. **69:** 7699–7711
272 Strebel K., Klimkait T., Maldarelli F. and Martin M. A. (1989) Molecular and biochemical analyses of human immunodeficiency virus type 1 vpu protein. J. Virol. **63:** 3784–3791
273 Strebel K., Klimkait T. and Martin M. A. (1988) A novel gene of HIV-1, vpu, and its 16-kilodalton product. Science **241:** 1221–1223
274 Geraghty R. J., Talbot K. J., Callahan M., Harper W. and Panganiban A. T. (1994). Cell type-dependence for Vpu function. J. Med. Primatol. **23:** 146–150
275 Sakai H., Tokunaga K., Kawamura M. and Adachi A. (1995) Function of human immunodeficiency virus type 1 Vpu protein in various cell types. J. Gen. Virol. **76:** 2717–2722
276 Deora A. and Ratner L. (2001) Viral protein U (Vpu)-mediated enhancement of human immunodeficiency virus type 1 particle release depends on the rate of cellular proliferation. J. Virol. **75:** 6714–6718
277 Coady M. J., Daniel N. G., Tiganos E., Allain B., Friborg J., Lapointe J. Y. et al. (1998) Effects of Vpu expression on *Xenopus* oocyte membrane conductance. Virology **244:** 39–49
278 Cordes F. S., Kukol A., Forrest L. R., Arkin I. T., Sansom M. S. and Fischer W. B. (2001) The structure of the HIV-1 Vpu ion channel: modelling and simultation studies. Biochim. Biophys. Acta **1512:** 291–298
279 Ewart G. D., Sutherland T., Gage P. W. and Cox G. B. (1996) The Vpu protein of human immunodeficiency virus type 1 forms cation-selective ion channels. J. Virol. **70:** 7108–7115
280 Gonzalez M. E. and Carrasco L. (1998) The human immunodeficiency virus type 1 Vpu protein enhances membrane permeability. Biochemistry **37:** 13710–13719
281 Grice A. L., Kerr I. D. and Sansom M. S. (1997) Ion channels formed by HIV-1 Vpu: a modelling and simulation study. FEBS Lett. **405:** 299–304
282 Schubert U., Ferrer-Montiel A. V., Oblatt-Montal M., Henklein P., Strebel K. et al. (1996) Identification of an ion channel acitivity of the Vpu transmembrane domain and its involvement in the regulation of virus release from HIV-1-infected cells. FEBS Lett. **398:** 12–18
283 Sansom M. S., Forrest L. R. and Bull R. (1998) Viral ion channels: molecular modeling and simulation. Bioessays **20:** 992–1000
284 Babiychuk E. B. and Draeger A. (2000) Annexins in cell membrane dynamics: Ca(2+)-regulated association of lipid microdomains. J. Cell Biol. **150:** 1113–1124
285 Martens J. R., Sakamoto N., Sullivan S. A., Grobaski T. D. and Tamkun M. M. (2001) Isoform-specific localization of voltage-gated K^+ channels to distinct lipid raft populations: targeting of Kv1.5 to caveolae. J. Biol. Chem. **276:** 8409–8414
286 Bour S. and Strebel K. (1996) The human immunodeficiency virus (HIV) type 2 envelope protein is a functional complement to HIV type 1 Vpu that enhances particle release of heterologous retroviruses. J. Virol. **70:** 8285–8300
287 Iida S., Fukumori T, Oshima Y, Akari H., Koyama A. H. and Adachi A. (1999) Compatibility of Vpu-like activity in the four grops of primate immunodeficiency viruses. Virus Genes **18:** 183–187

To access this journal online:
http://www.birkhauser.ch