Host factors involved in retroviral budding and release

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Abstract | The plasma membrane is the final barrier that enveloped viruses must cross during their egress from the infected cell. Here, we review recent insights into the cell biology of retroviral assembly and release; these insights have driven a new understanding of the host proteins, such as the ESCRT machinery, that are used by retroviruses to promote their final separation from the host cell. We also review antiviral host factors such as tetherin, which can directly inhibit the release of retroviral particles. These studies have illuminated the role of the lipid bilayer as the unexpected target for virus restriction by the innate immune response.

Virological synapse
An organized structure that forms at the junction between the infected cell and the uninfected target cell. This structure facilitates retroviral transfer in the absence of cell-to-cell fusion.

Cytonemes
Thin membranous bridges that connect two cells in the absence of a cytoplasmic connection.

ESCRT
(Endosomal sorting complex required for transport). A conserved cellular machinery for the sorting of ubiquitylated cargo proteins into vesicles that bud away from the cytoplasm, and the subsequent scission of the membrane neck. This machinery is also required for abscission, the last step in cell division. The recruitment of ESCRT proteins through retroviral late-budding domains (L-domains) is essential for viral assembly.

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The assembly and egress of enveloped mammalian viruses from their host cell requires a complex orchestration of interactions between the viral genome, its structural proteins and parasitized cellular factors, resulting in a nascent virion budding through a lipid bilayer. This is followed by a fission event that physically separates the viral particle from the cell. The recent study of retroviruses, particularly HIV-1, has provided great insight into the fundamental cell biology of these processes. Technological advances in real-time microscopy have, for the first time, allowed examination of the assembly kinetics of single virions (BOX 1). The study of retroviral cell-to-cell spread has uncovered the formation of complex polarized assembly platforms (the virological synapse and cytonemes), which facilitate the efficient transfer of infectious virions to uninfected cells. The implication of the ESCRT machinery in the envelopment of retroviral particles has revealed that there are common features in the assembly processes of many diverse mammalian viruses, and has led to a better understanding of the fundamental biology of the ESCRT proteins themselves and their roles in endosomal sorting and cytokinesis. Finally, HIV-1 mutants with defects in the accessory protein Vpu exhibit cell type-specific defects in release, and investigations into these defects have shown that, even after the physical separation of viral and cellular membranes, the nascent virion is susceptible to tetherin (also known as BST2 or CD317), an interferon-induced host protein that inhibits a wide range of enveloped viruses.

Here, we review our current understanding of retroviral assembly and release, and of the fascinating cell biology that its study has uncovered.

Overview of HIV-1 assembly

HIV-1 encodes nine proteins that are required for its replication and for manipulation of the host[1]. Gag and the envelope glycoprotein (Env) are the main components of virions. The polyprotein Pol is cleaved into the viral enzymes that are required for infection: protease (PR), reverse transcriptase (RT) and integrase (IN). Tat and Rev regulate gene expression, three of the accessory proteins (Vif, Vpu and Nef) are dedicated to the evasion and modulation of adaptive and innate antiviral immunity, and the role of the fourth accessory protein (Vpr) remains unclear.

Retroviral assembly is driven primarily by Gag (FIG. 1), a polyprotein that contains all the activities that are required for virion assembly and egress[2]. The incorporation of the viral enzymes during assembly is mediated by the interaction between Gag and Gag–Pol, a fusion protein that is produced by ribosomal frameshifting at the carboxyl terminus of Gag. Processing of HIV-1 Gag by PR generates four proteins that are required at different steps in the assembly process: matrix (MA), capsid (CA), nucleocapsid (NC) and p6 (REF. 3). MA provides the contact between the assembling virions and the plasma membrane through the insertion of its amino-terminal myristate into the lipid bilayer and through a patch of basic residues in its N terminus[3–5]. These basic residues determine the specific interaction of Gag with the plasma membrane by binding to phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$)[5,6], a plasma membrane-enriched phospholipid. The globular head of MA also regulates the interaction of monomeric Gag molecules with intracellular membranes by concealing the myristate,

Box 1 | Live imaging of retroviral budding

Our understanding of the dynamics of the HIV-1 assembly process *in vivo* has been dramatically enhanced recently through the use of tools such as conventional fluorescent proteins and modified variants that are either photoactivatable or photoconvertible[163]. Additional methodological advances include total internal reflection fluorescence microscopy (TIRFM) for visualization of events occurring at the plasma membrane, fluorescence resonance energy transfer (FRET) to visualize protein–protein interactions, and fluorescence recovery after photobleaching (FRAP) for quantification of lateral diffusion[164]. The combined use of Gag fusions with photoactivatable GFP and FRAP analysis initially showed that the mobility of Gag in the cytoplasm is dramatically reduced when these Gag molecules are assembled into virus-like particles (VLPs)[165]. However, the limitation of Gag–GFP constructs is that the GFP moiety interferes with the formation of morphologically normal VLPs[166], although co-transfection of unfused Gag eliminates the aberrant budding structures so that assembly resembles authentic viral assembly. The use of TIRFM[167] has allowed the direct observation and quantitative analysis of single virion-budding events, and this has shown that productive assembly events are not associated with endosomal or endocytic vesicles but, instead, the individual Gag molecules that participate in virion assembly are preferentially recruited from a cytosolic pool[167,168]. These studies have determined that the average time for VLP assembly ranges between approximately 5 and 9 minutes; however, the caveat with these approximations is that the actual assembly time is likely to be longer owing to there being sub-detectable numbers of Gag molecules during the early events of assembly[169]. Interestingly, visualization of the incorporation of viral genomic RNA into individual assembly sites in real time has also been possible with TIRFM using HIV-1 RNA tagged with a fluorescent version of the bacteriophage MS2 protein[170]. These studies suggested that the RNA genome arrives at the plasma membrane as a dimer and that its retention at the sites of viral assembly requires Gag anchoring to the membrane[169,171].

an inhibitory mechanism that is relieved by both recognition of PtdIns(4,5)P_2 at the plasma membrane and Gag oligomerization[7,8], thus ensuring an ordered sequence of events during the early stages of viral assembly. Finally, MA is involved in the incorporation of Env into the virion[1].

CA and the spacer SP1 determine the lateral interactions that are required between Gag monomers for the formation of the Gag lattice in immature virions[9]. The subsequent processing of Gag by PR induces dramatic morphological changes that shape the conical capsid, a closed structure formed of approximately 1,500 units of CA enclosing two copies of the unspliced viral genomic RNA as well as the viral replicative enzymes RT and IN[1,3,9]. In addition to its structural role in assembly, CA in the incoming virion is targeted by a subset of host restriction factors[10].

Binding of low-order multimers (dimers or trimers) of Gag to viral genomic RNA occurs in the cytoplasm[3,9,11]. This interaction is followed by the incorporation and coating of the viral genomic RNA by the NC portion of Gag[12], which contains two zinc-finger motifs and several clusters of basic amino acids that preferentially interact with viral genomic RNA. Crucially, viral genomic RNA has a central role in assembly by promoting Gag multimerization, mainly through its interaction with NC, and this function can be bypassed by substituting NC with a heterologous dimerization domain[13,14]. More recent work has demonstrated that there are additional interactions between Gag and viral genomic RNA through MA, suggesting that viral genomic RNA inhibits nonspecific interactions of Gag with cellular membranes[7,15], perhaps increasing the specificity of the highly basic region in MA for lipids that reside at the plasma membrane. The functional coordination between viral genomic RNA and MA is also highlighted by the rescue of defective HIV-1 assembly in mouse cells by an alteration to the route of nuclear export of viral genomic RNA[16]. This alternative export route activates MA-dependent membrane targeting in mouse cells[17], although the underlying mechanism for this link remains unclear.

Last, p6 is a small protein at the C-terminus of Gag that has no structural role in the mature virion but is needed for virion incorporation of the accessory protein Vpr[1]. A more established role for p6 is the so-called late-budding domain (L-domain)[3] activity, which is essential for the resolution of the membrane stalk that connects the nascent virions to the host cell at the end of assembly[18–20]. Recent work using electron tomography and scanning transmission electron microscopy (EM) has proposed that the ESCRT proteins which are recruited by the HIV-1 L-domain are also involved in closing the Gag sphere, or 'vesiculation', of the released virions[21]. The current view is that p6 works mainly as an adaptor protein for the cellular machinery that is engaged by retroviral L-domains to promote budding (discussed below).

The site of retroviral assembly

The subcellular localization of retroviral assembly has received much interest recently, particularly the coupling of particle production with virion transfer to new target cells. Most retroviruses assemble at the plasma membrane, and the nature and distribution of plasma membrane assembly sites may involve the generation of cholesterol-rich membrane assembly platforms in a process that is regulated by tetraspanins[22,23]. By contrast, betaretroviruses and foamy viruses pre-assemble their particles in the cytoplasm in perinuclear areas before budding into the endoplasmic reticulum or recycling compartments through an interaction with Env[24,25].

The cell type that has stirred the most debate regarding HIV-1 assembly and release is the macrophage. In EM images of infected macrophages, viral assembly seemed to occur in late endosomal compartments[26], leading to speculation that HIV-1 assembles in endosomes and is released by exocytosis. However, further studies revealed this hypothesis to be problematic: rational targeting of HIV assembly to late endosomes results in poor particle release, and real-time analysis of Gag localization shows that assembly initiates at the plasma membrane in primary macrophages, but fully assembled particles accumulate in phagosomes[27]. More recent studies have demonstrated that these observations are a reflection of the complex membrane structures in these cells. These 'endosomal' vesicles are in fact mostly non-acidic compartments[28] that are often deeply invaginated surface-accessible regions of the plasma membrane and are marked with the tetraspanins CD9, CD53 and CD81 (REFS 29–31). It is unclear whether these structures are induced by the virus or are pre-existing features of mature-macrophage morphology. Detailed real-time

Vpu
A small integral membrane protein that is encoded by HIV-1. Vpu binds to human tetherin and counteracts its antiviral activity.

Tetherin
A mammalian interferon-induced type II membrane protein that inhibits the release of enveloped viral particles from infected cells.

Gag
A polyprotein encoded by all retroviruses that is processed to form the structural components of the mature viral particle.

Photoactivatable
Pertaining to a fluorescent protein: able to increase in fluorescence when excited by 488 nm light.

Photoconvertible
Pertaining to a fluorescent protein: able to change the emission spectrum on exposure to ultraviolet light.

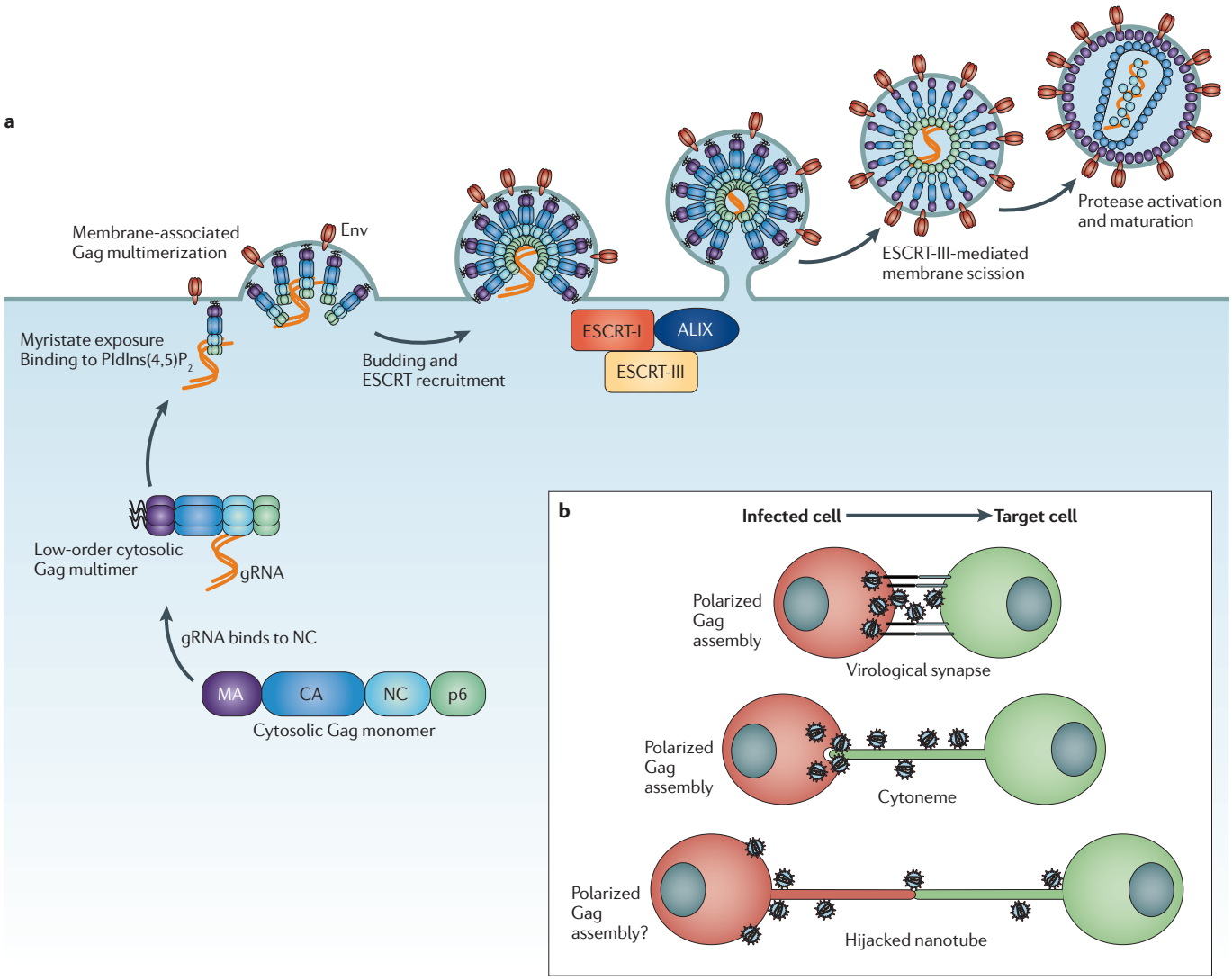

Figure 1 | **Mechanisms of retroviral budding, release and spread. a** | Overview of HIV-1 assembly and release. In HIV-1-infected cells, the Gag polyprotein is synthesized in the cytoplasm, initially with its amino-terminal myristate modification sequestered inside the matrix (MA) subunit. Binding of viral genomic RNA and low-order multimerization of Gag leads to myristate exposure, which results in the Gag–RNA complex being targeted predominantly to the plasma membrane. Further nucleation of Gag and Gag–polymerase (Pol) polyproteins at the plasma membrane initiates the budding process. Envelope glycoproteins (Envs) are recruited into the budding virions through interactions with MA. During this process, ESCRT-I is recruited to the plasma membrane through interactions between viral p6 and host TSG101 and ALIX (also known as PDCD6IP). This in turn recruits ESCRT-III, the membrane scission machinery that resolves the membrane neck between the cell and the immature virion. From the initial Gag–viral RNA membrane association to membrane scission takes approximately 5–10 minutes depending on total cellular Gag content. Gag and Gag–Pol multimerization leads to activation of the viral protease, which then processes Gag into its constituent subunits, and these proteins then undergo the structural rearrangements that give rise to the mature infectious virion. **b** | Different mechanisms of retroviral cell-to-cell spread. Retroviral spread between cells can occur by cell-free and cell-associated mechanisms. Cell-associated spread is accompanied by the generation or co-option of polarized contacts between infected and uninfected cells. In infected $CD4^+$ T cells, HIV-1 and human T-lymphotropic virus 1 (HTLV-1) induce a virological synapse contact that is dependent on Env–receptor interactions. The virological synapse is long-lasting and is stabilized and maintained by the recruitment of adhesion molecules and by actin rearrangements. Viral assembly and release become polarized to the contact zone, and nascent virions are transferred across the intercellular space. Alternatively, membranes containing assembling virions may be transferred to the target cell via endocytic uptake of components of the synapse. An analogous structure, called a cytoneme, has been observed for retroviral spread in adherent cells. Env–receptor interactions cause an actin-dependent filopodium to be extruded from the target cells and partially endocytosed by the infected cell. Viral assembly is then targeted to this contact zone, and new virions 'surf' along the cytoneme to the target cell body, where they enter. A similar structure, termed a nanotube, has been observed between T cells in culture and can facilitate the transfer of cellular components. Although nanotubes are not necessarily virally induced per se, HIV-1 particles have been visualized trafficking along their length. CA, capsid; NC, nucleocapsid; $PtdIns(4,5)P_2$, phosphatidylinosotol-4,5-bisphosphate.

Total internal reflection fluorescence microscopy
A microscopy technique that is based on the selective illumination of the molecules in a thin section near the coverslip. This technique is particularly suitable to the study of events that occur near or at the plasma membrane.

Fluorescence resonance energy transfer
A phenomenon of quantum mechanics that occurs when a pair of donor and acceptor fluorophores are less than 10 nm from each other, allowing excitation of the acceptor by emission from the donor.

analysis of HIV-1 assembly in infected macrophages suggests that viral particles form at both the 'internal compartment' and at the surface[32]. However they arise, the accumulation of virions in these structures may have profound pathogenic consequences. Macrophages are long-lived cells and can maintain HIV-1 production with little cytotoxicity, suggesting that infectious, cell-associated virions can be 'archived' for long periods of time[33]. Furthermore, the plasma membrane-derived internal compartments have similarities to those to which HIV-1 virions are targeted in dendritic cells after interactions with surface C-type lectins[34]. In both cases, these internal compartments can be sent to the surface upon interaction with target $CD4^+$ T cells, and virions can be transferred across an infectious synapse structure[32,35,36] (see below).

Polarized assembly and cell-to-cell transfer

Many retroviruses, including HIV-1 and human T-lymphotropic virus 1 (HTLV-1), spread more efficiently in culture through cell-to-cell contact, probably representing a major route of retroviral transfer *in vivo*[37,38] (FIG. 1b). In both HTLV-1- and HIV-1-infected T cells, viral assembly is directed towards polarized conjugations between infected and uninfected cells, called virological synapses[39,40]. For HIV-1, the virological synapse is induced by Env interactions with CD4 on the target cell, with much of the membrane-bound Gag assembly directed to the contact zone[40]. Virological synapses are reminiscent of the immunological synapses that form between T cells and antigen-presenting cells[41]. Both types of synapse require polarization of the actin cytoskeleton, use membrane microdomains and are further regulated by a range of adhesion molecules and tetraspanins[37,38]. Infected cells can form synapses with more than one uninfected cell (known as polysynapses)[42]. Glycan-rich extracellular matrix components have been shown to accumulate into layers that superficially resemble bacterial biofilms, and this accumulation has been implicated in concentrating HTLV-1 particles at the virological synapse[43], although whether this occurs with HIV-1 is unknown. Recent data suggest that the virological synapse results from HIV-1 assembly being primarily targeted to uropods when T cells adopt a polarized morphology, and these uropods then form adhesion molecule-rich contact zones with uninfected cells[44]. Thus, virological synapses are long-lasting structures that permit multiple rounds of HIV-1 transfer between T cells, and their disruption reduces viral replication in culture[45,46].

The nature of virological synapse-mediated HIV transfer is currently a matter of debate. Cryo-EM tomography of T cell virological synapses suggest that they are partially enclosed spaces or clefts between donor and target cells, are maintained by adhesive contacts and contain multiple mature HIV-1 particles[47]. Thus, these studies suggest that cell-to-cell spread resembles cell-free virus transfer over a very short distance. By contrast, live-cell studies using fluorescently labelled viruses have shown that virological synapse-mediated transmission is accompanied by what seems to be target cell endocytosis of packets of donor cell material that are derived from a Gag-positive disc-like structure termed a synaptic button[48]. These packets contain membranous structures and budding virions that are proposed to fuse in endosomal compartments. Opposing data exist concerning the relative sensitivity of virological synapse transmission to neutralizing antibodies, reflected by these differing cryo-EM tomography and live-cell observations[38,46,47]. It should be borne in mind, however, that these observations might be influenced by the assembly defects of Gag–GFP fusions (BOX 1). Full maturation of the virion is essential for the transfer of infectious HIV-1 across the virological synapse — protease inhibitors potently block viral replication *in vitro* and *in vivo*, and the L-domain is essential for viral replication in T cells[49].

In adherent cells, the study of cell-to-cell spread of murine leukaemia virus (MLV) has led to the identification of an analogous virally induced process for facilitating spreading replication[37] (FIG. 1b). MLV virions move laterally on the plasma membrane along filopodial bridges (termed cytonemes) that are derived from the target cell. These contact and become invaginated by the donor cell. Cytoneme formation requires both Env–receptor interactions and actin-dependent endocytic processes, and results in viral assembly becoming polarized towards the contact site. Similar (but not identical) structures termed nanotubes (FIG. 1b) have been observed to act as long-range conduits between T cells in culture[50]. Although this is not virally induced per se, HIV-1 transfer along nanotubes has been observed, suggesting that these structures are functionally important.

Host factors recruited by L-domains

Pioneering work on HIV-1 established the concept of L-domains as functional determinants that facilitate detachment of virions from the cell surface[51]. L-domain inactivation causes the accumulation of immature viral particles that are attached to the cell via a thin stalk, indicating that L-domains facilitate the membrane fission event that releases the nascent virion into the extracellular milieu. L-domains in divergent retroviruses are functionally interchangeable, short amino acid motifs that work in a position-independent manner[52], features that suggested their role as docking sites for cellular proteins. Evidence that several L-domains promote Gag ubiquitylation and that proteasome inhibitors block L-domain activity suggested that ubiquitin itself and ubiquitin ligases have key roles in retroviral budding[53–55]. Subsequent discoveries established that L-domains function by recruiting the ESCRT proteins[56–59], a highly conserved cellular machinery that mediates membrane scission events resembling retroviral budding (BOX 2).

Modular recruitment of the ESCRT machinery by L-domains. The core ESCRT machinery was shown to be required for retroviral budding. Consistent with the modular nature of this machinery (FIG. 2), the expression of a catalytically inactive vacuolar protein-sorting-associated protein 4 (VPS4), which therefore prevents

Fluorescence recovery after photobleaching
A technique that consists of photobleaching fluorescent molecules in a defined region of the cell, followed by assessing the recovery of fluorescence in this region. The results provide diffusion coefficients of the fluorescent protein as an indication of its mobility.

Virus-like particles
Non-infectious viral particles that lack viral genetic material.

Electron tomography
An experimental strategy that is based on electron microscopy and allows three-dimensional reconstruction of biological samples.

Tetraspanins
A large group of structurally related, small membrane proteins that share the capacity to associate with themselves and with transmembrane receptors. They control viral infections and several cellular processes, including migration, cell-to-cell fusion and signalling.

Uropods
Cytoplasmic protrusions at the rear of a polarized cell. In activated T cells, uropods are enriched for cell adhesion molecules.

Box 2 | **The ESCRT machinery**

The ESCRT machinery is a set of cellular proteins that are involved in sorting ubiquitylated membrane proteins into multivesicular bodies (MVBs) for degradation. The ESCRT machinery is also essential for the resolution of the membranous bridge that connects the daughter cells at the end of cell division, a scission event that is topologically identical to MVB formation and retroviral budding. The ESCRT pathway contains four specialized complexes termed ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III[82,172]. The emerging picture in the field shows a modular nature for the ESCRT machinery, with function-specific entry routes such as ESCRT-0 for endosomal sorting and CEP55 (a centrosomal protein that interacts with TSG101 and ALIX) for cytokinetic abscission, whereas every ESCRT-mediated function requires the membrane deformation and scission activities that are mediated by the core machinery, namely ESCRT-III and vacuolar protein sorting-associated protein 4 (VPS4; of which there are two homologues, VPS4A and VPS4B, in mammals)[106,173]. Another key component is ALIX (also known as PDCD6IP), an adaptor protein that binds ESCRT-I and ESCRT-III.

ESCRT-III is formed of structurally similar subunits, the charged MVB proteins (CHMPs). Humans express 12 known CHMPs (CHMP1A, CHMP1B, CHMP2A, CHMP2B, CHMP3, CHMP4A–C, CHMP5, CHMP6, CHMP7 and IST1). These ESCRT-III subunits have a common architecture, with a positively charged amino-terminal region that is thought to bind membranes and an acidic carboxyl terminus that interacts with basic residues at the N terminus[82,106]. ESCRT-III interacts with the Bro1 domain in ALIX and with the MIT domain in VPS4 and other accessory MIT domain-containing proteins through short amphipathic helices that are present at the very C-terminal residues of the CHMPs[77,174,175].

Deletion of the acidic C-terminal region or binding to downstream effector proteins reveals the ability of the CHMPs to bind to themselves and inhibit HIV-1 budding[176], suggesting that inactive ESCRT-III subunits undergo a conformational change that opens the molecules and activates their function[177]. Subsequent disassembly of the ESCRT complexes for recycling purposes requires the formation of two hexameric rings by VPS4 and its cofactor LIP5 (also known as VTA1)[178]. The requirement for the VPS4 ATPase activity and the essential role of residues at the centre of the rings suggest that the individual CHMPs are 'pumped' through the central pore of the VPS4 double ring into the cytoplasm for recycling.

ESCRT-III recycling, results in potent inhibition of different L-domains[58,60]. Similarly, general L-domain inhibition is observed on disruption of ESCRT-III integrity in cells overexpressing individual ESCRT-III subunits that are fused to a bulky tag[58,61–63]. Conversely, different L-domain classes recruit the ESCRT machinery through direct interactions with multiple adaptor proteins in this pathway, as described below.

P(S/T)AP motifs were initially described in HIV-1 p6, although they are present in other retrovirus and filovirus proteins[18,19]. These motifs bind ESCRT-I through a direct interaction with the ubiquitin E2 variant (UEV) domain in TSG101 (REF. 64), thereby mimicking the PTAP motifs that are found in cellular proteins such as HRS (also known as HGS)[65], a component of ESCRT-0. P(S/T)AP-dependent budding requires an intact ESCRT-I, including the subunits VPS28, one of the four VPS37 variants and one of the two multivesicular body sorting factor 12 (MVB12) variants[60,66–68]. Several hypotheses have been put forward to explain the recruitment of ESCRT-III by ESCRT-I in viral budding, including the interaction of TSG101 with EAP30 (also known as SNF8) and EAP45 (also known as VPS36)[61], two components of ESCRT-II that could potentially bridge ESCRT-I and ESCRT-III through charged multivesicular body protein 6 (CHMP6), an ESCRT-III subunit that also binds VPS28 (REF. 69). Alternatively, the interaction of the adaptor protein ALIX (also known as PDCD6IP) with TSG101 and the three CHMP4 proteins from ESCRT-III could be relevant for P(S/T)AP-dependent budding[61,63]. However, functional studies have shown that ESCRT-II, CHMP6 and ALIX are not required for P(S/T)AP-dependent budding[63,70]. Consequently, ESCRT-III recruitment by the P(S/T)AP–TSG101 axis is a major question that needs to be answered to fully understand HIV-1 egress.

(L)YPX$_n$L motifs are described mainly in lentiviruses, and the best characterized L-domains in this category are found in the equine infectious anaemia virus (EIAV) protein p9 and the HIV-1 protein p6 (both of which are part of the Gag polyproteins of these viruses)[18,20], although variations that match a looser consensus ($\Phi YX_{0/2}\Phi X_{1/3}L$; in which Φ is any non-polar amino acid and the fractions denote the range of numbers of X residues) have been identified in simian immunodeficiency virus *Macaca mulatta* isolate 239 (SIV_{mac239}) and SIV *Chlorocebus tantalus* isolate 1 ($SIV_{agm.tan1}$)[71]. These motifs bind a conserved hydrophobic groove on arm 2 of the V-domain of ALIX dimers[72–75], perhaps mimicking similar interactions with cellular proteins, although the only known physiological example is the interaction between the *Aspergillus* spp. ALIX homologue (PalA) and its binding partner (PacC)[76]. The recruitment of ESCRT-III by the (L)YPX$_n$L motif occurs through the interaction of the ALIX Bro1 domain with amphipathic C-terminal helices in the three human paralogues of CHMP4 (REF. 77). Crucially, the ESCRT-III-binding interface in ALIX is essential for HIV-1 budding[73,78].

PPXY motifs are present in a wide range of enveloped viruses, and some of the best characterized L-domains of this class are found in MLV, HTLV-1 and Ebola viruses[19,20]. PPXY motifs promote viral budding by binding to the WW domains of a subset of NEDD4-like HECT ubiquitin ligases[79], primarily WWP1, WWP2 and ITCH. PPXY motifs are found in cellular substrates of the NEDD4 family and, notably, these peptides exhibit L-domain activity[53]. The mechanism of ESCRT-III recruitment during PPXY-dependent budding remains elusive. However one important clue is the absolute requirement for the ubiquitin ligase activity of the HECT proteins[80], particularly their ability to synthesize K63-linked ubiquitin chains[81]. Nonetheless, the identity of the relevant ubiquitin acceptor has not been unequivocally established. One possibility is that ubiquitin molecules attached to Gag could serve as a platform for the recruitment of ubiquitin-binding components in the ESCRT machinery, such as ALIX, TSG101 or EAP45 (REFS 53,82). This notion is supported by the fact that an L-domain-defective Gag can be rescued by direct fusion of ubiquitin, and this rescue is ESCRT dependent[83,84]. However, viral budding can occur in the complete absence of Gag ubiquitylation[85], so Gag cannot be the only relevant substrate for PPXY-dependent ubiquitylation, although self-ubiquitylation of HECTs provides a hypothetical platform for ESCRT recruitment in this context. Alternatively, HECTs could promote viral budding through ubiquitylating adaptor proteins[80], a hypothesis that is supported by the existence

NEDD4-like HECT ubiquitin ligases
A family of E3 ubiquitin ligases that contain WW domains which bind to PPXY-dependent late-budding domains (L-domains) and are required for recruitment of the ESCRT machinery.

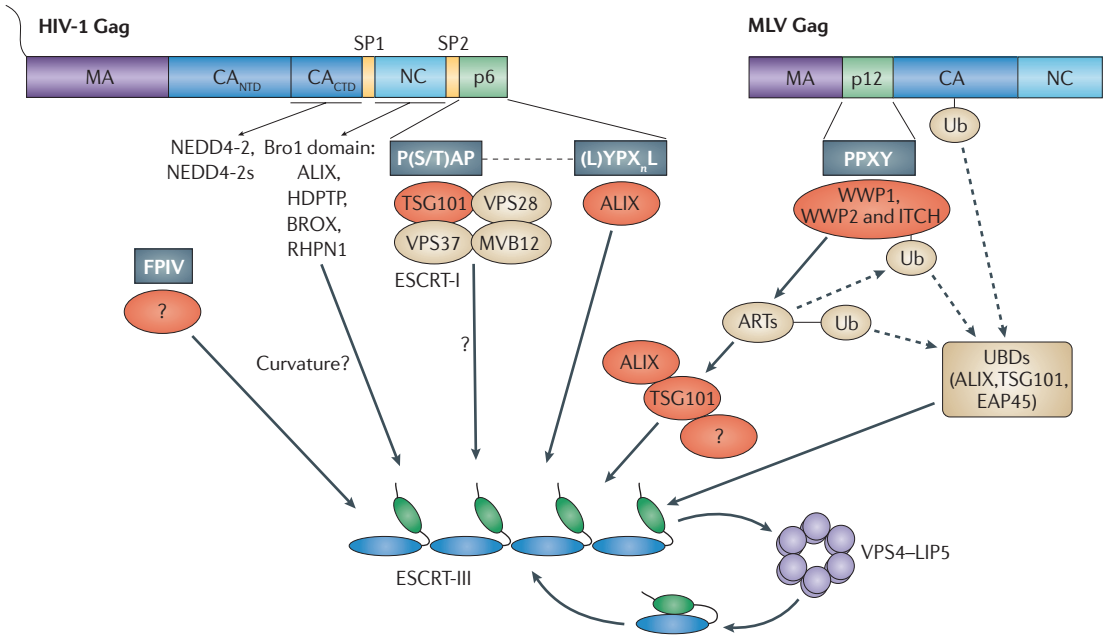

Figure 2 | **Recruitment of host factors by L-domains.** Late-budding domains (L-domains) encoded by enveloped viruses are short amino acid motifs (grey boxes in the figure) that recruit the ESCRT machinery through direct interactions with adaptor proteins in this pathway (red). Every known L-domain requires components of the core ESCRT machinery, such as ESCRT-III and vacuolar protein sorting-associated protein 4 (VPS4), to facilitate the final separation of virions from the infected cell. Auxiliary interactions that have been described for HIV-1 Gag with the NEDD4-2 and NEDD4-2s isoforms and with Bro1 domain-containing proteins are shown in the figure. In the case of PPXY motifs, ubiquitin has a key role in ESCRT recruitment, perhaps through its interaction with the ubiquitin-binding domains (UBDs) that are encoded by several ESCRT subunits. Arrestin-related trafficking (ART) proteins are also thought to have a role in PPXY-dependent budding. CA, capsid; CTD, carboxy-terminal domain; MA, matrix; MLV, murine leukaemia virus; MVB12, multivesicular body sorting factor 12; NC, nucleocapsid; NTD, amino-terminal domain; RHPN1, rhophilin 1; Ub, ubiquitin.

of factors such as the arrestin-related trafficking (ART) proteins that have the potential to provide a physical link between HECTs and components of the core ESCRT machinery[86]. Interestingly, ARTs are substrates for HECT-mediated ubiquitylation, and they may recruit the ubiquitin-binding proteins in the ESCRT pathway as described above[86,87]. Accordingly, ARTs can be recruited to the site of viral budding, and their overexpression specifically inhibits PPXY-dependent budding[86]. Thus, the emerging view is that multiple, possibly redundant, mechanisms have the potential to link HECT ubiquitin ligases with the core ESCRT machinery (FIG. 2).

FPIV motifs have been described in the M protein of paramyxovirus simian virus 5 (REF. 88), an enveloped virus that requires the core ESCRT machinery for budding. Peptides containing the FPIV motif work as heterologous L-domains in the HIV-1 context, and mutagenesis analysis indicates a consensus sequence (ØPXV; in which Ø is any aromatic amino acid)[88] that could be present in other enveloped viruses. However, there are no additional examples of FPIV motifs to date, and the cellular protein that is recruited by these motifs remains unknown.

Auxiliary interactions in viral budding. In addition to those L-domains that have the main role in viral budding, a more complex picture of multiple auxiliary L-domains is gradually emerging. This concept is highlighted by evidence that the P(S/T)AP motif in HIV-1 acts synergistically with sequences at the C terminus of p6 to facilitate egress[89]. Subsequent work has established the presence of a (L)YPX$_n$L motif at the p6 C terminus that binds ALIX with low affinity and has an auxiliary role in HIV-1 replication[61,73]. Importantly, the ALIX-binding site in p6 can substitute the P(S/T)AP motif in cells overexpressing ALIX[73,78], suggesting that auxiliary L-domains allow viral replication in different cellular environments. This suggestion is supported by the cell type-specific importance of the ALIX-binding site in p6. Replication of HIV-1 bearing inactivating mutations in this site is markedly defective in T cells as compared with more modest phenotypes in macrophages[49]. A similar scenario has been described for Rous sarcoma virus, which relies on a PPXY motif and encodes an auxiliary LYPSL motif[90]. Likewise, adjacent PPXY and P(S/T)AP motifs promote the release of Ebola viruses and HTLV-1 (REF. 18).

The rescue of a p6-deleted HIV-1 by overexpression of the NEDD4-2s isoform (which has a truncated C2 domain) has uncovered other auxiliary interactions in retroviral budding[91,92]. This is a surprising result, because HIV-1 lacks PPXY motifs and, in fact, this rescue seems to be dependent on the CA C-terminal domain–SP1 region in Gag and the residual C2 domain

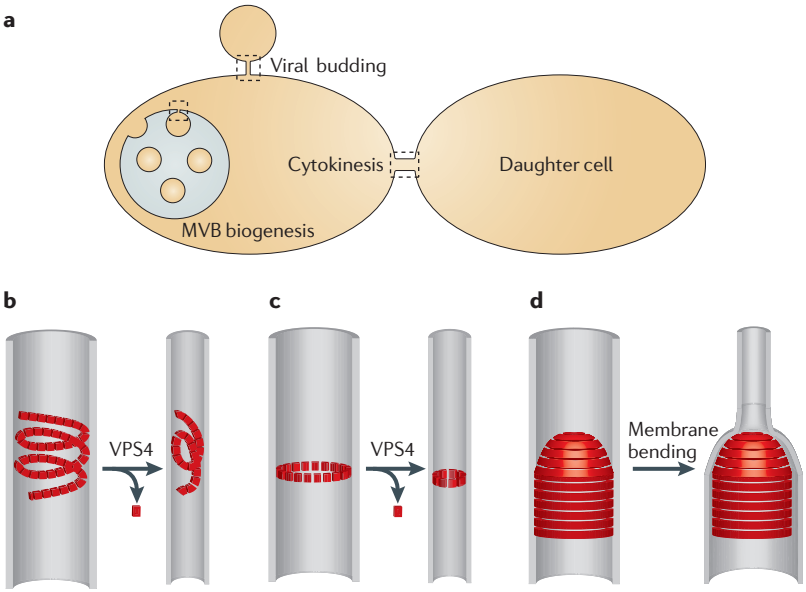

Figure 3 | **Membrane scission by the ESCRT machinery. a** | The topology of the membrane scission events that are catalysed by the ESCRT machinery are shown: multivesicular body (MVB) formation, resolution of the midbody during cytokinesis, and budding of enveloped viruses. **b** | One of the proposed models for membrane scission facilitated by ESCRT-III filaments (red block ribbons) is that the filaments form a spiral that could enclose the endosomal cargo and simultaneously constrict the membrane until scission occurs. **c** | The formation of tubular structures by charged MVB protein 2A (CHMP2A; also known as VPS2-1) and CHMP3 suggests an alternative model whereby the outside of these tubes exposes the membrane-binding region of the CHMPs, while the region that binds vacuolar protein sorting-associated protein 4 (VPS4) faces towards the inside of the tube. Thus, it is possible that active removal of the ESCRT-III subunits by VPS4 could reduce the tube diameter up to a point at which membrane scission is energetically favourable. **d** | The presence of lipids results in shorter ESCRT-III tubes that are often cone-shaped, suggesting another model for ESCRT-mediated scission, whereby the membrane would follow the dome shape and, as a consequence of this interaction, the neck diameter could be thinned to ~3 nm for spontaneous membrane fission. One prediction of this model is the lack of requirement for VPS4 in ESCRT-mediated fission, as opposed to the VPS4-dependent constriction of tubular structures in the spiral and tube models. In accordance with the dome model, *in vitro* reconstitution models for MVB formation suggest that VPS4 is dispensable for scission, although more work is needed to establish this point unequivocally.

in NEDD4-2s[81]. Similarly, overexpression of ITCH promotes MLV budding in a PPXY-independent manner, and the C2 domain of ITCH is required for this activity[93]. These findings suggest that certain C2 domains contain targeting modules for retroviral Gag proteins, although this concept needs to be characterized further.

Recent work has elucidated the interactions of the Bro1 domain in ALIX with the zinc-fingers in the NC protein of HIV-1 (REFS 94,95). The importance of this interaction is highlighted by the fact that viral budding is inhibited by mutations in NC that impair the interaction with ALIX, and by overexpression of the N-terminal region of the ALIX Bro1 domain[94–96]. One caveat is that depletion of ALIX by siRNA (short interfering RNA) has a minor effect on HIV-1 release, although this paradoxical result could be explained by a potential compensation through additional interactions of NC with other Bro1 domain-containing proteins (BROX, HDPTP (also known as PTPN23) and rhophilin 1)[97]. Interestingly, overexpression of isolated Bro1 domains from these proteins promotes budding of a minimal Gag construct, although this activity does not require binding to ESCRT-III[97]. Therefore, Bro1 domains could assist viral budding through their interaction with additional cofactors or, alternatively, could generate negative curvature through their intrinsic boomerang shape. Intriguingly, the dependence of HIV-1 on ESCRT function is conferred, at least in part, by the NC–p1 region of Gag[98], thus raising the possibility that Bro1 domains also overcome an inhibitory effect that is imposed by NC on viral release.

siRNA
(Small interfering RNA). A double-stranded, short RNA molecule that induces RNA interference and silencing of a target mRNA.

Mechanisms of membrane scission

The study of the cellular mechanisms that are subverted by L-domains has immensely benefited from the study of basic cellular processes. Conversely, parallels with retroviral budding have been essential in uncovering the unexpected role of the ESCRT machinery in the last step of cell division[99]. This fascinating intersection between cell biology, retrovirology and structural biology has illuminated the mechanisms that are employed by the ESCRT machinery in membrane deformation and scission.

A direct involvement of ESCRT-III in membrane deformation was first indicated by the assembly of CHMP4 filaments in circular arrays that could bend the plasma membrane away from the cytoplasm[100] and, at least in this system, catalytically inactive VPS4 binds to these filaments and promotes membrane evagination. These observations are consistent with the tendency of ESCRT-III to spontaneously form 15 nm filaments or helical polymeric structures[101,102]. More sophisticated *in vitro* systems have revealed an obligatory sequence of events during ESCRT-III assembly and membrane scission: CHMP6 interaction with membranes nucleates the polymerization of CHMP4 filaments that are 'capped' by the recruitment of the CHMP2A–CHMP2B–CHMP3) subcomplex, which subsequently binds VPS4 (REFS 103,104). The formation of the ESCRT-III filaments has suggested several scission models that are extensively reviewed elsewhere[105,106] and summarized in FIG. 3. Intriguingly, ring-like striations consistent with these models have been observed in the membrane stalks of arrested HIV-1 virions[61,107].

A systematic functional analysis has shown that HIV-1 requires a surprisingly small subset of ESCRT-III subunits, namely CHMP4 and CHMP2 (REF. 107). Interestingly, the essential interaction between these two proteins might explain the dispensable role of CHMP3, which bridges the yeast homologues of CHMP4 and CHMP2. The lack of requirement for CHMP6 is particularly intriguing given its role in nucleating the above-mentioned ESCRT-III filaments. It is interesting to note that ALIX can bind CHMP4 filaments *in vitro*[75], suggesting that, in the absence of CHMP6, ALIX could provide an alternative mechanism for positioning these filaments during viral budding.

Two recent imaging studies have started to elucidate the kinetics of ESCRT recruitment during HIV-1 budding[108,109]. Remarkably, ESCRT-III and VPS4

associate with Gag transiently at the end of assembly, while early-acting factors such as ALIX are associated with Gag throughout assembly. These results suggest that assembly of the ESCRT-III filaments might require a threshold level of adaptor proteins such as ALIX, although it is also possible that this process is triggered by an unknown signalling event. Interestingly, ESCRT-III still localizes to the sites of viral assembly in cells expressing a dominant-negative VPS4, suggesting that VPS4 is not only involved in ESCRT-III recycling but also directly involved in membrane scission *in vivo*.

Last, influenza virus budding is one fascinating exception to the widespread dependence of enveloped viruses on the ESCRT machinery[110]. The absence of L-domain equivalents in influenza viruses has been a mystery for a long time, but recent work has shown that these viruses rely on an amphipathic helix in the M2 protein to facilitate egress[110]. More interestingly, M2 has the intrinsic property of inducing membrane curvature and scission, raising the possibility that viral proteins such as M2 promote viral budding by mimicking the filaments or cone-shaped tubes described above for ESCRT-III.

Inhibition of retroviral release

The past decade has seen great interest in the innate immune mechanisms that mammalian cells use to resist viral replication. For retroviruses, this has led to the identification of cellular proteins (often inducible by type I interferons) that directly inhibit stages of the viral life cycle[111]. Of these, tetherin, a type II membrane glycoprotein, is the most prominent antiviral factor that inhibits the release of retroviral particles (FIG. 4a). However, there are hints that the assembly process itself could also be an antiviral target. Tetherin was originally identified as the factor responsible for the restriction of virion release from certain cell types for HIV-1 mutants lacking the accessory gene *vpu*[112,113]. It is now well established that the mode of action of tetherin allows it to potentially inhibit the release of many diverse enveloped viruses by targeting the one basic molecular feature that is common to all of them: the host cell-derived lipid bilayer. The importance of host restriction at the level of this immutable target is underlined by the acquisition of viral proteins that inactivate tetherin[114].

Tetherin structure and mechanism. In cells in which efficient viral release requires Vpu expression, mature Vpu-defective virions accumulate on cell surfaces and in endosomal compartments[115]. Unlike L-domain mutants, these virions can be liberated by protease treatment, demonstrating the existence of a protein-based rather than lipid-based 'tether'. Moreover, this phenotype can be induced by type I interferon[116]. It was these attributes that allowed for the identification of tetherin[112,113].

Tetherin is expressed by mature B cells in humans and by plasmacytoid dendritic cells in mice, and can be upregulated in many cell types by type I interferon and other pro-inflammatory stimuli[114]. Tetherin localizes to the plasma membrane, recycling compartments and the *trans*-Golgi network (TGN)[114]. The unusual topology of tetherin is indicative of its antiviral mechanism[117] (FIG. 4a). The extracellular domain forms an extended parallel coiled coil that is anchored at both ends to the plasma membrane[118–120] — at the N terminus by a peptide transmembrane domain, and at the C terminus by a glycosyl phosphatidylinositol (GPI) linkage that allows the protein to partition into cholesterol-rich microdomains[114]. Three extracellular cysteine residues mediate disulphide linkages, allowing tetherin to dimerize[114]. The short N-terminal cytoplasmic tail promotes endocytic recycling of tetherin through its interaction with clathrin adaptors AP1 and AP2 (REF. 121), and in polarized epithelial cells this tail links tetherin to subapical cortical actin filaments through an interaction with the BAR–RAC GTPase-activating protein family member RICH2 (also known as ARHGAP44)[122]. Tetherin becomes incorporated into the nascent budding virion[117,123–125]. Biochemical and structural evidence strongly favours a model whereby parallel tetherin dimers crosslink the virion with either the cellular membrane or membranes of other virions[117–120], leading to the characteristic retention of mature virions in protease-sensitive 'layers' on the plasma membrane and the subsequent endocytic uptake of these virions[112] (FIG. 4b). Tetherin dimerization, the GPI anchor and the coiled coil are essential for inhibiting the release of retroviral particles[112,117,126]. Tetherin can be mimicked by an artificial chimeric protein comprising equivalent domains from unrelated proteins in a similar topological arrangement, suggesting that tetherin may not require cellular cofactors for its activity[117]. Recent crystal structures have suggested a degree of flexibility around an N-terminal hinge in the extracellular domain[118–120]. In the reduced state, the tetherin extracellular domain forms a rod-like structure of head-to-head dimers, which are unlikely to be functionally relevant. In its native state, the N terminus and two of the disulphide bonds cannot be resolved, leading to questions of whether conformational changes in this region are required for efficient virion restriction. Finally, it is unlikely that the sole function of tetherin is the physical retention of viral particles — it has putative signalling activities, and it may regulate innate immune responses in plasmacytoid dendritic cells[127].

Tetherin can be visualized in virion membranes by immunoelectron microscopy, which shows it to be often associated with electron-dense 'tethers' between viral particles and cells[123,124]. Enrichment in particles seems to be mainly driven by the transmembrane domain, and protease-mediated release of tethered virions leaves vestigial dimeric N-terminal 'stumps' of tetherin in purified viral particles[117]. However, images of the 'tethers' between virions and cells show some to be considerably longer than the estimated 17 nm length of a tetherin molecule[116,123,124]. The nature of these structures is unclear, although tetherin can be visualized along their length, suggesting them to be strips of plasma membrane. How these structures affect the direct tethering model that is favoured by biochemical and structural studies will require further in-depth electron tomography of accumulated virions.

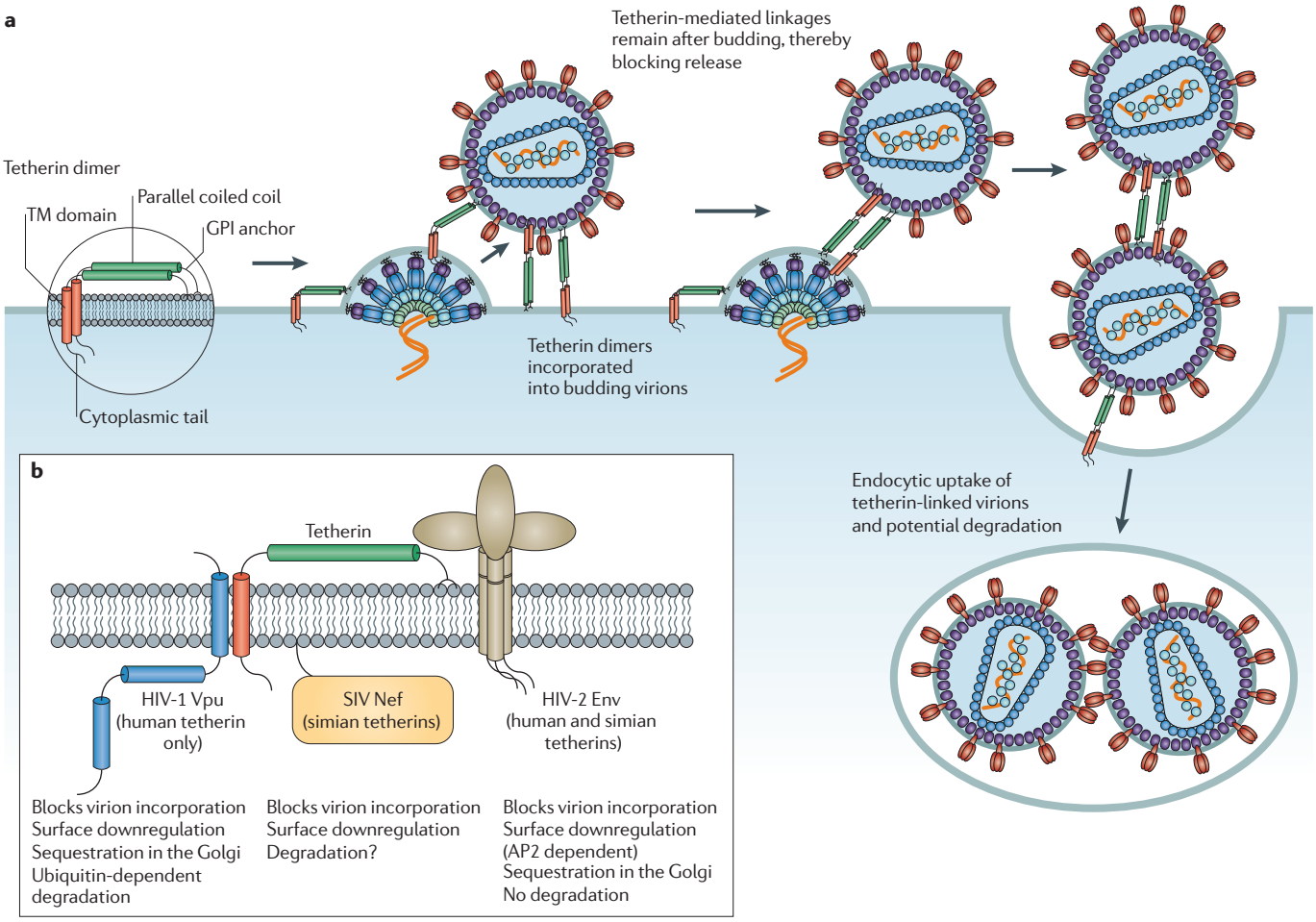

Figure 4 | **Restriction of retroviral release by tetherin, and lentivirus-encoded countermeasures. a** | In macrophages and in interferon-stimulated $CD4^+$ T cells, the antiviral membrane protein tetherin (also known as BST2 or CD317) becomes incorporated into the nascent virion. Although the parallel tetherin dimers do not inhibit viral assembly and membrane scission, they are thought to form physical crosslinks between the cell and the virion by virtue of their dual-membrane anchors. This leads to virion accumulation on the cell surface and subsequent internalization to late endosomes. **b** | Primate immunodeficiency viruses encode countermeasures that interact with tetherin (Vpu for HIV-1, Nef for most simian immunodeficiency viruses (SIVs) and envelope glycoprotein (Env) in the case of HIV-2). These interactions can be species specific and result in inhibition of the antiviral activity of tetherin, often accompanied by cell surface removal of the protein, its intracellular sequestration and, ultimately, its degradation. GPI, glycosyl phosphatidylinositol; TM, transmembrane.

In adherent cell types that express tetherin, Vpu-defective HIV-1 particles accumulate in late endosomes[112]. This is the result of RAB5A-dependent endocytosis of surface-tethered particles and suggests that these virions can be targeted for endosomal degradation. A recent study has implicated Rabring7 (also known as BCA2 or RNF115) binding to tetherin in the uptake and degradation of restricted HIV-1 particles[128]. Whether such targeted virion degradation leads to enhanced viral-antigen presentation in primary cells such as macrophages is an interesting question.

Retroviral antagonism of tetherin. Tetherin is perfectly placed to be a generalized inhibitor of enveloped-virus release, because it directly interacts not with a virally encoded structure, but with one of cellular origin: the membrane. Tetherin can restrict the release of all retroviral particles tested to date, including those such as foamy viruses and betaretroviruses, which pre-assemble their capsids in the cytosol and bud through internal membranes[129]. In addition, mammalian tetherins can restrict the release of various RNA viruses (filoviruses, arenaviruses and rhabdoviruses), as well as at least one herpesvirus (human herpesvirus 8)[114] and, potentially, influenza viruses[130]. In many cases, these viruses have themselves evolved countermeasures that inhibit tetherin function[131] (FIG. 4c).

The prototypical viral countermeasure to tetherin, the HIV-1 protein Vpu, is a small integral membrane phosphoprotein that may multimerize to form cation-permeable channels[132]. Vpu interacts with human tetherin through species-specific determinants in their respective transmembrane domains[133–138], resulting in downregulation of tetherin from the plasma membrane and its subsequent lysosomal degradation[139,140]. This degradation is ubiquitin dependent, requiring the

recruitment of a SKP1–cullin 1–βTRCP2 E3 ubiquitin ligase complex that binds to the phosphorylated Vpu cytoplasmic tail[136,139–142]. Furthermore, a recent study has implicated ESCRT-mediated degradation of tetherin through the recruitment of HRS to Vpu–tetherin complexes[143]. However, tetherin degradation seems to be dispensable for the ability of Vpu to counteract restriction. Vpu localizes predominantly to the TGN, where it colocalizes with tetherin[135], and Vpu truncation mutants with defects in localization to the TGN are impaired in antagonism, indicating that differential trafficking of tetherin is key to the activity of Vpu[144]. The rate of tetherin endocytosis is unaffected by Vpu expression, suggesting that Vpu instead affects tetherin trafficking to the plasma membrane[135,140]. Again, this could be ubiquitin dependent; in response to Vpu, the tetherin cytoplasmic tail can be ubiquitylated on lysine and non-lysine residues[145,146]. Although lysine-linked ubiquitylation of tetherin is required for degradation, these lysine residues are completely dispensable for Vpu-mediated antagonism[145,147]. Recent data have implicated serine- and threonine-linked ubiquitylation in tetherin inactivation and surface downregulation by Vpu[146]. The underlying cell biology of these effects on tetherin localization remain uncharacterized, and observations that Vpu can antagonize tetherin function in the absence of substantial surface downregulation of the protein[148] and that Vpu downregulates tetherin specifically from outside budding virions[124] demonstrate that many outstanding questions remain unanswered.

Vpu is present in HIV-1 and its direct evolutionary precursors but is absent in most other primate immunodeficiency viruses, including HIV-2. However, tetherin counteraction is highly conserved in primate lentiviruses, indicating that the modulation of tetherin function may be essential *in vivo*[149]. In most of the SIVs that do not encode Vpu, the accessory protein Nef can antagonize tetherin-mediated restriction[150,151]. Nef, a membrane-associated adaptor protein, induces the surface downregulation of primate tetherin molecules that contain a (G/D)DIWK motif in their cytoplasmic tails, although the mechanistic details of this process remain to be determined. The absence of this sequence in human tetherin renders it completely resistant to SIV Nef proteins[150,151]. The importance of tetherin activity *in vivo* is further highlighted by the adaption of the Env proteins of HIV-2 and SIV to target human or macaque tetherin when the Nef countermeasure is compromised (because of either the lack of target sequence in human tetherin for HIV-2 Nef, or experimental infection of macaques with Nef-defective SIV_{mac} that reverts to pathogenicity)[152–154]. Recent evolutionary studies suggest that the efficient adaption of HIV-1 Vpu to human tetherin may be one of the virological factors associated with the pandemic spread of HIV-1 group M[149]. Tetherin itself has been subjected to positive selection during mammalian evolution[133,134,155,156]. Finally, several tetherin countermeasures have been identified in other mammalian viruses, providing further evidence that tetherin serves an important antiviral function[131].

HIV-1 group M
The major phylogenetic group of HIV-1 that resulted in the HIV/AIDS pandemic. This group was originally derived from a zoonotic transfer of simian immunodeficiency virus from chimpanzees (SIV_{cpz}) to a human. HIV-1 groups N, O and P represent separate zoonotic events, and the spread of these viruses is geographically limited.

Tetherin and cell-to-cell transmission. Tetherin-mediated accumulation of virions on infected cell surfaces raises important questions about the role of this protein in cell-to-cell transmission of viruses across the virological synapse. In some T cell culture systems, Vpu-defective viruses spread faster than their wild-type counterparts, despite having a reduced cell-free virion output[132]. Indeed, culture selection of HIV-1 for enhanced cell-to-cell transfer in T cell lines results in inactivating mutations in Vpu, suggesting that tetherin-induced accumulation of virions promotes rather than restricts viral transfer[157]. Recent studies directly addressing this issue have arrived at opposing conclusions[158–160]. In two of the studies, tetherin was recruited to the virological synapse[158,159]. Indeed, tetherin has also been observed in the virological synapse induced by HTLV-1, a virus that spreads almost exclusively through cell-to-cell contact[43]. Casartelli *et al.* showed that Vpu-defective HIV-1 transfer from adherent cells to T cells was inhibited by tetherin in part because large accumulations of virions were transferred as a 'package' that was poorly fusogenic[158]. By contrast, Jolly *et al.* showed that in $CD4^+$ T cells infected with Vpu-defective HIV-1, there were more virological synapse contacts and there was an enhanced transfer of virus to new target cells owing to tetherin-induced viral accumulation on the cell surface. This investigation also hinted that synapse formation itself requires tetherin[159]. Why there is a discrepancy between these studies is unclear at present, but it may be related to tetherin expression level. There is currently no evidence of tetherin antagonism in gammaretroviruses or HTLV-1, suggesting that the role of tetherin in cell-free versus cell-to-cell spread may be a double-edged sword for host and virus alike.

Other inhibitory mechanisms of retroviral assembly. Our increasing understanding of the interferon-induced effector mechanisms that inhibit viral replication has suggested that other stages of the retroviral assembly pathway could be targets for antiviral inhibition. As described above, late stages of retroviral assembly are dependent on ubiquitylation–deubiquitylation events. When overexpressed, the interferon-induced ubiquitin-like molecule ISG15 can block the assembly of HIV-1 and avian leukosis virus particles. Although ISG-ylation of HIV-1 Gag blocks TSG101 binding under conditions in which E2 and E3 conjugating enzymes are overexpressed (in addition to overexpression of ISG15)[161], a more recent study suggests that ISG15 disrupts the recruitment of VPS4 by ESCRT-III, inhibiting budding mediated by both P(S/T)AP- and PPXY-dependent L-domains[162]. It is yet to be demonstrated that ESCRT function is inhibited by an interferon-mediated physiological induction of ISG15 expression. However, as ESCRT-III and VPS4 are essential for all ESCRT-dependent budding events irrespective of the nature of the viral L-domain, this may be another generalized antiviral strategy used by the innate immune system to target assembly of enveloped viruses.

Concluding remarks

Recent progress in the study of retroviral assembly has led the way in illuminating how mammalian enveloped viruses exploit cellular proteins to facilitate

their release and transfer to new target cells. Because of the incredible tractability of retroviral experimental systems, this progress has in turn stimulated huge advances in our understanding of the roles of many of these factors in fundamental cellular processes such as endosomal sorting and cell division. Furthermore, through the study of HIV-1, we have become aware of the ability of the host antiviral response to impede the assembly and release of enveloped viruses, with potentially important implications for the pathogenesis and spread of HIV-1. There is much still to learn. A full mechanistic understanding of how the ESCRT pathway regulates the processes of retroviral assembly, endosomal sorting and cell division may allow for the development of pharmalogical inhibitors that specifically block budding not just for HIV-1, but for enveloped viruses in general, and therefore multiple classes of medically important viruses. Furthermore, the ability of the innate immune response to target the budding of enveloped viruses through factors such as tetherin, and the concomitant evolution of specific virally encoded countermeasures, suggests that this aspect of enveloped-virus replication is a battleground in host–pathogen interactions. Innate responses such as tetherin-mediated inhibition of viral release clearly have important roles in host-to-host transmission, in the generation of antiviral immune responses and in the spread of viruses such as HIV-1 from the portal of entry to establish a systemic infection. A better understanding of these roles will enhance both our knowledge of viral pathogenesis and the potential for novel broadly acting therapeutics.

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Competing interests statement

The authors declare no competing financial interests.

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