44 Brown, S.A. *et al.* (1998) Holoprosencephaly due to mutations in ZIC2, a homologue of *Drosophila* odd-paired. *Nat. Genet.* 20, 180–183
45 Aruga, J. *et al.* (2002) Zic2 controls cerebellar development in cooperation with Zic1. *J. Neurosci.* 22, 218–225
46 Brewster, R. *et al.* (1998) Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. *Nature* 393, 579–583
47 Koyabu, Y. *et al.* (2001) Physical and functional interactions between Zic and Gli proteins. *J. Biol. Chem.* 276, 6889–6892
48 Kim, P.C. *et al.* (2001) Murine models of VACTERL syndrome: Role of sonic hedgehog signaling pathway. *J. Pediatr. Surg.* 36, 381–384
49 Koike, C. *et al.* (2002) Introduction of wild-type patched gene suppresses the oncogenic potential of human squamous cell carcinoma cell lines including A431. *Oncogene* 21, 2670–2678
50 Sato, N. *et al.* (1999) Induction of the hair growth phase in postnatal mice by localized transient expression of Sonic hedgehog. *J. Clin. Invest.* 104, 855–864
51 Sato, N. *et al.* (2001) Effect of adenovirus-mediated expression of Sonic hedgehog gene on hair regrowth in mice with chemotherapy-induced alopecia. *J. Natl. Cancer Inst.* 93, 1858–1864
52 Tsuboi, K. and Shults, C.W. (2002) Intrastriatal injection of sonic hedgehog reduces behavioral impairment in a rat model of Parkinson's disease. *Exp. Neurol.* 173, 95–104
53 Pepinsky, R.B. *et al.* (2002) Long-acting forms of Sonic hedgehog with improved pharmacokinetic and pharmacodynamic properties are efficacious in a nerve injury model. *J. Pharm. Sci.* 91, 371–387
54 Ahlgren, S.C. *et al.* (2002) Sonic hedgehog rescues cranial neural crest from cell death induced by ethanol exposure. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10476–10481
55 Taipale, J. *et al.* (2000) Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. *Nature* 406, 1005–1009

Mechanisms of enveloped RNA virus budding

Owen Pornillos, Jennifer E. Garrus and Wesley I. Sundquist

To spread infection, enveloped viruses must bud from infected host cells. Recent research indicates that HIV and other enveloped RNA viruses bud by appropriating the cellular machinery that is normally used to create vesicles that bud into late endosomal compartments called multivesicular bodies. This new model of virus budding has many potential implications for cell biology and viral pathogenesis.

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Enveloped RNA viruses include pathogens with exceptional histories of morbidity, such as HIV (60 million people infected, and 20 million people killed since 1981 [1]) and influenza (about 500 million infections each year, and 40 million people killed in the 1918 pandemic alone [2]), as well as important emerging pathogens such as the Ebola virus. Their medical importance and fascinating biology has made them (and indeed all viruses) the subject of intense study, which has revealed recurring themes that underlie many viral replication strategies. First, the limited coding capacity of RNA viruses forces them to use host cell factors to extend their capabilities. Second, viral proteins often achieve this by mimicking the structures and functions of cellular proteins. Third, different viruses, as well as cells themselves, frequently use similar mechanisms to accomplish difficult molecular transformations. Finally, whenever possible, viruses modify their cellular environment to maximize replication and to minimize cellular responses (whereas cells do the converse).

These principles are nicely illustrated by the process of enveloped RNA virus entry, which is initiated when viral envelope proteins bind to cell-surface receptors. These interactions frequently mimic ligand–receptor interactions and can thereby initiate signaling cascades that trigger endocytosis and/or increase the permissivity of the host cell environment. Analyses of the molecular basis of virus entry have identified the unexpected conservation of a three-stranded, coiled-coil architecture in viral envelope proteins that mediates membrane fusion [3] and have also revealed surprising similarities between this machinery and the SNARE (soluble NSF attachment protein receptor) complexes that mediate cellular vesicle fusion [4,5].

Here we review our emerging understanding of the reciprocal process: that is, how HIV and other enveloped RNA viruses exit cells. We also speculate on how these processes, although not yet fully elucidated, can be understood ultimately in terms of the principles of viral replication outlined above.

HIV assembly

Late in the infectious cycle of HIV [6], the viral Gag polyprotein captures the RNA genome, binds to the plasma membrane and assembles into spherical, enveloped particles that bud from the cell [7]. Gag is organized into four distinct regions (see Fig. 1), which carry out different primary functions in the coordinated processes of viral assembly and egress: the *N*-myristoylated MA domain targets Gag to the plasma membrane, CA makes important protein–protein interactions that are required for particle assembly, NC captures the viral RNA genome and couples RNA binding to particle assembly, and p6 recruits cellular proteins that function in the final stages of virus release.

Although Gag is processed by the viral protease to produce infectious virions, extracellular particles are still produced in the absence of the viral protease,

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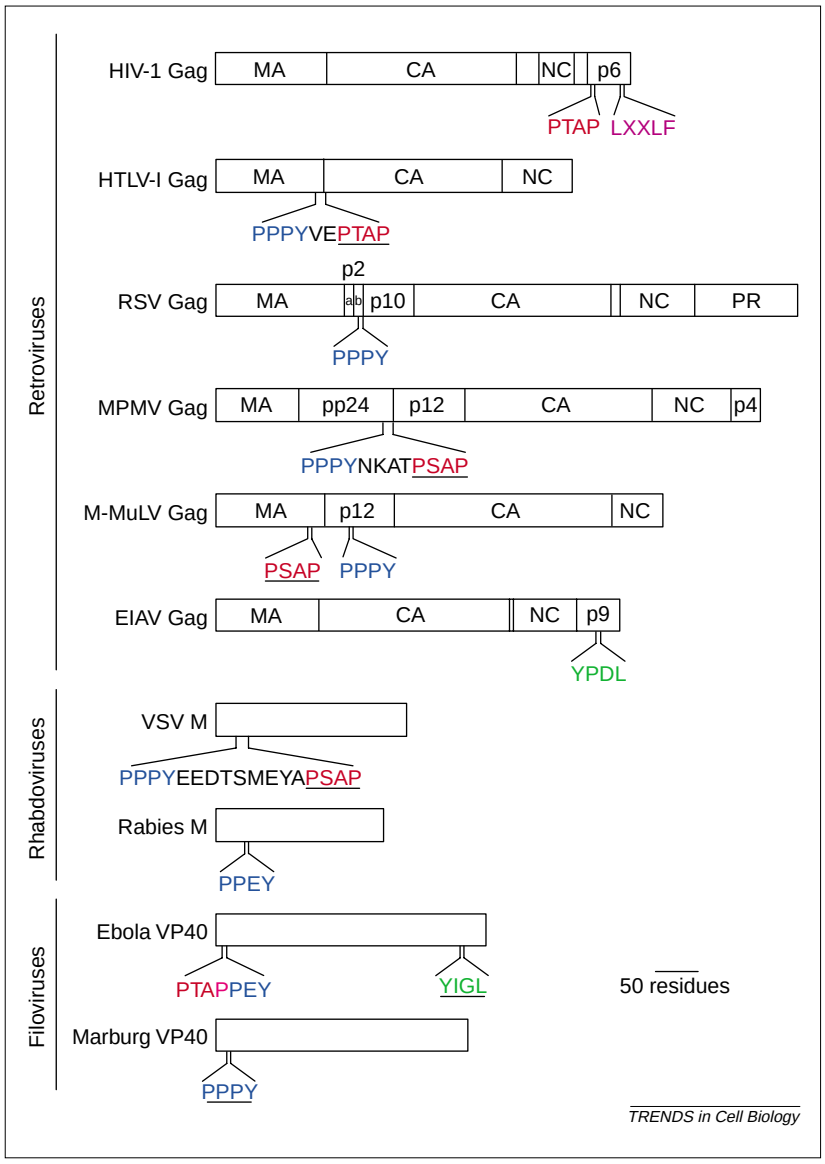

Fig. 1. Domain organization and locations of PTAP, PPXY, YXXL and LXXLF late domain motifs found in the membrane-associated structural proteins of enveloped RNA viruses [47]. Domains are colored as follows: PTAP, red; PPXY, blue; YXXL, green; and LXXLF, purple. Apart from those that are underlined, late domain activities have been demonstrated experimentally. Abbreviations: EIAV, equine infectious anemia virus; HIV, human immunodeficiency virus; HTLV, human T cell leukemia virus; M-MuLV, Moloney murine leukemia virus; MPMV, Mason–Pfizer monkey virus; RSV, Rous sarcoma virus; VSV, vesicular stomatitis virus.

and the intact Gag polyprotein is therefore considered to be the 'machine' that drives viral assembly and release.

HIV budding

Enveloped virus budding is a fission event in which the continuous cell membrane that connects the assembling virion to the cell is severed to create discreet viral and cellular membranes. Many enveloped viruses exit the cell by budding directly from the plasma membrane, although budding into intracellular compartments can also lead to productive release, as discussed below. Because HIV Gag can assemble and bud in the absence of other viral proteins, any additional machinery necessary for viral budding and membrane fission must be supplied by the cell (and recruited by Gag).

Such cellular factors are, in fact, recruited by an invariant tetrapeptide motif, Pro-(Thr/Ser)-Ala-Pro (PTAP), which is located within the p6 domain of HIV Gag (Fig. 1). Point mutations in this motif block the release of virus at a late stage [8,9], and it is therefore termed a 'late domain' [10]. Recent studies have shown that the cellular protein Tsg101 (tumor susceptibility gene 101) binds to the PTAP late domain of HIV Gag and facilitates the final stages of virus release [11–14] (Fig. 2a). Tsg101 also seems to be involved in the release of other pathogenic human viruses because structural proteins in both Ebola virus (VP40) and human T cell leukemia virus type I (Gag) contain PTAP motifs, and the Ebola VP40 protein has been shown to recruit Tsg101 to assembly sites on the plasma membrane [13].

Tsg101 and its yeast ortholog, Vps23p, normally function in the cellular vacuolar protein sorting pathway, where they play central roles in selecting cargo for incorporation into vesicles that bud into the maturing endosome to create multivesicular bodies (MVBs) (Figs 3,4a) [15]. This is an unusual intracellular vesiculation event because MVB vesicles bud away from (rather than into) the cytoplasm (Fig. 3). Hence, vesicle budding into the MVB and viral budding at the plasma membrane are topologically equivalent [16], and the same machinery could, in principle, catalyze both processes. We have argued previously that HIV budding and MVB vesicle formation are analogous processes [12], and we return to this idea below after reviewing the process of MVB biogenesis.

Vacuolar protein sorting and MVB biogenesis

Genetic screens in yeast have identified a series of proteins that are essential for membrane protein trafficking from the Golgi and plasma membrane through the endosomal system to the lysosome (vacuole in yeast) (reviewed in [17]). Although the mammalian vacuolar protein sorting (Vps) pathway is more complex, the yeast and human pathways are similar in outline. Our discussion is therefore based mainly on studies of yeast proteins, and the relevant human orthologs and references are provided in Table 1.

The essential sorting step in vacuolar protein sorting occurs during MVB formation, when ubiquitinated proteins and lipids present on the limiting endosomal membrane are recognized and sorted into endosomal membrane microdomains, which ultimately invaginate and form vesicles that bud into the lumen to create the MVB (Figs 3 and 4a). The MVB can then go on to fuse with a lysosome, thereby exposing the internal vesicles to the degrading lipases and proteases in this organelle (whereas proteins that remain on the limiting membrane escape degradation).

MVB biogenesis and protein sorting are coordinated mainly by the class E Vps proteins (Table 1 and Fig. 4a). In class E *vps* mutants, lysosomal protein targeting fails because early endosomes do not mature into

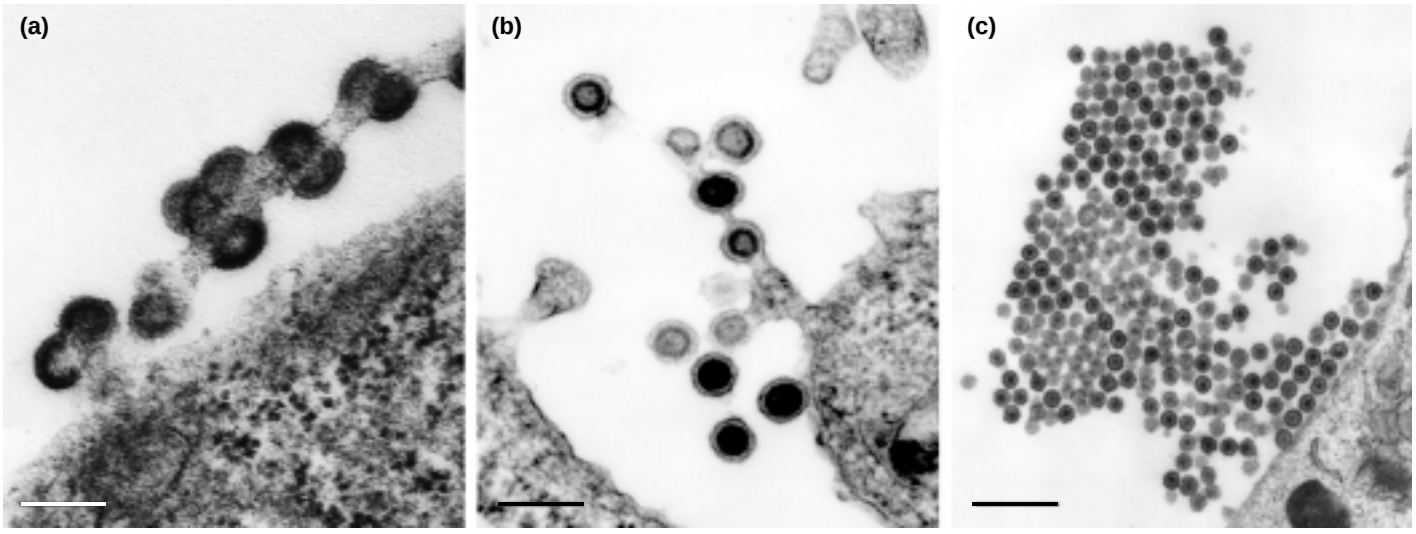

Fig. 2. Viruses arrested at late stages of budding. (a) Arrested HIV-1 budding from 293T cells depleted of Tsg101 protein [12]. Note that this phenotype, in which viruses remain attached to the plasma membrane and to other particles via membrane stalks, is very similar to that seen in viruses with mutations in the PTAP late domain [8]. In some cases 'clumps' of connected viruses can escape from the cell surface [57]. (b) Arrested budding of Moloney murine leukemia virus from 293T cells expressing dominant-negative, ATPase-defective Vps4A protein [12]. (c) Arrested budding of Rous sarcoma virus from QT6 cells depleted of ubiquitin using proteasome inhibitors [16]. To help orient the reader, scale bars have been added to (a), (b) and (c), which correspond to approximately 150, 200 and 500 nm, respectively.

MVBs and instead enlarge into aberrant organelles called 'class E' compartments [18–20]. In wild-type cells, the initial recognition of cargo proteins seems to involve an oligomeric complex that includes the class E proteins Hrs (yeast Vps27p), as well as Stam, Eps15 (Ede1p), the endosomal lipid phosphatidylinositol-3-phosphate [PtdIns(3)*P*] and clathrin [20–25]. Elegant studies from the Emr laboratory have shown that a complex comprising three yeast class E proteins (Vps23p, Vps28p and Vps37p) termed ESCRT-I (endosome-associated complexes required for transport I) is then recruited from the cytoplasm to the surface of maturing endosomes [15].

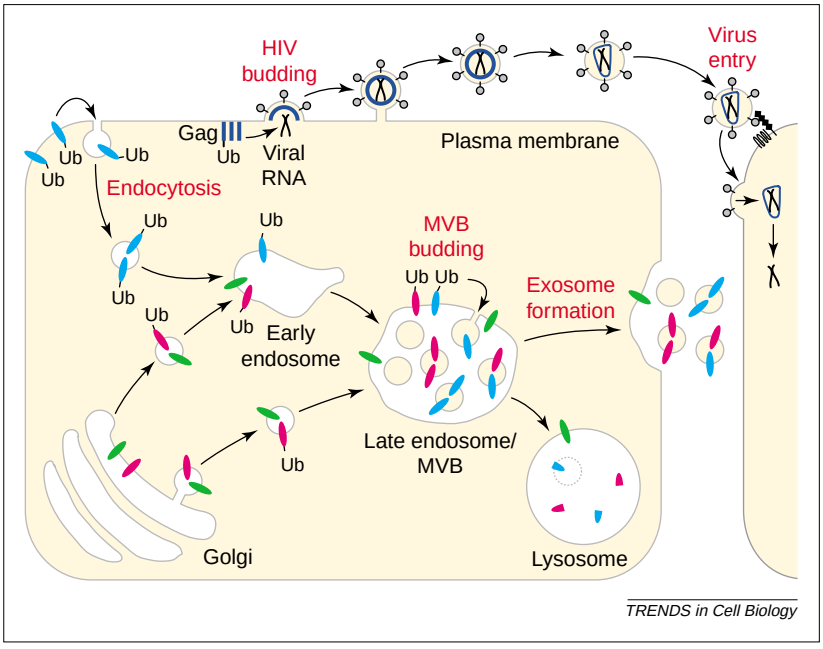

Fig. 3. HIV assembly and budding, vacuolar protein sorting and exosome formation. HIV Gag proteins are shown in dark blue, and protein cargos that are sorted through the Vps pathway are shown in magenta, green and light blue. For clarity, not all of the possible protein trafficking pathways are shown; for example, some proteins seem to be sorted to the vacuolar lumen in the absence of ubiquitination [63].

ESCRT-I recognizes the ubiquitinated protein cargos (see below) and recruits two more class E protein complexes (ESCRT-II and ESCRT-III) that participate in protein sorting and vesicle formation [26,27] (Fig. 4a). ESCRT-III components also recruit yet another class E protein, Vps4p, which is an AAA-type ATPase [28,29]. Genetic analyses indicate that Vps4 is involved in catalyzing the disassembly and recycling of the membrane-bound ESCRT complexes, because Vps4p mutations that block ATP binding or hydrolysis trap assembled ESCRT complexes on endosomal membranes [28,30], although Vps4p could also have a more active role in protein sorting.

Lipids are also important in MVB biogenesis, where they seem to function in protein recruitment and in vesicle formation. MVB vesicles themselves are enriched in both PtdIns(3)*P*, which is sorted and degraded through the pathway [31], and the cone-shaped lipid, lysobisphosphatidic acid, which may help to generate vesicle curvature [32,33]. PtdIns(3,5)P_2 also accumulates at the site of vesicle formation through the action of Fab1p PtdIns(3)*P* 5-kinase [20], and this lipid probably participates in protein recruitment.

Involvement of class E Vps proteins in HIV budding

A plausible cascade of the cellular events involved in HIV budding is shown in Fig. 4b. In this scheme, HIV budding from a human cell is modeled in analogy to vesicle formation in the yeast MVB. Consistent with this model, emerging studies indicate that HIV budding also requires several class E proteins. For example, HIV budding also seems to require an interaction between Tsg101 and Vps28, suggesting that the whole ESCRT-I complex probably participates in the budding process (J. Garrus, unpublished, and P. Bieniasz, pers. commun.).

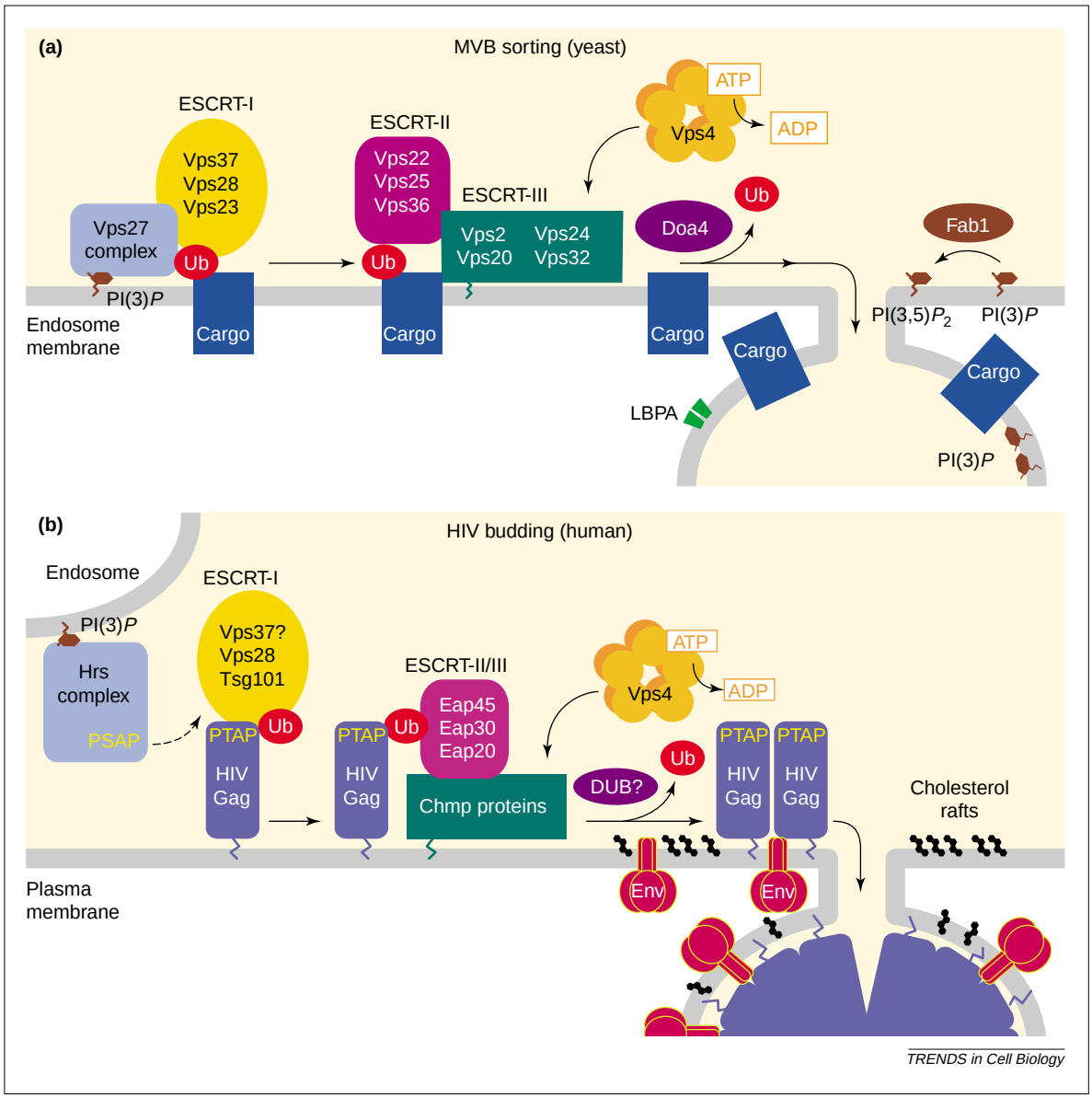

Fig. 4. Potential similarities between HIV budding and MVB sorting. (a) Yeast Class E Vps protein complexes and auxiliary factors that function in protein sorting and vesicle formation during MVB biogenesis. (b) Model of the involvement of human Class E Vps protein complexes in HIV budding at the plasma membrane. This speculative model emphasizes potential similarities between MVB vesicle formation and HIV budding. Abbreviations: DUB, deubiquitinating enzyme; Ub, ubiquitin. See Table 1 for a list of the proteins involved.

HIV budding is also blocked efficiently by dominant-negative mutations that disrupt the ATPase activity of Vps4 [12]. This observation supports the idea that viral budding depends on the machinery that acts late in MVB vesicle formation because Vps4 functions after assembly of the ESCRT complexes. These issues clearly need to be addressed much more directly, however – for example, by testing whether other ESCRT components also function at the sites of virus budding.

Mechanisms of vesicle formation

There is still much to be learned about the biochemical mechanisms of vesicle formation during both MVB biogenesis and virus budding. Unresolved issues include: first, the chemical and conformational changes that allow sequential recruitment of ESCRT complexes and give directionality to the pathway; second, the mechanisms by which proteins are sorted into plasma and endosomal membrane microdomains; third, how membrane curvature is generated; and last, what actually catalyzes the membrane fission step that allows vesicle release.

This last issue is particularly intriguing, because the mechanism of membrane fission during enveloped virus budding and MVB vesicle formation must be distinct from the well-studied membrane fission events that occur in the conversion of clathrin-coated pits to endocytic vesicles (where dynamin 'pinchases' form external collars that surround the necks of budding vesicles) [34]. Instead, the unique topology of virus and MVB budding requires a very different mechanism that must involve catalysis of membrane fission by molecules located inside (or adjacent to) the neck of the budding vesicle. Given these unusual topological requirements, it is perhaps not surprising that HIV and other viruses have evolved to appropriate the cellular machinery that can accomplish such vesiculation events.

Table 1. Proteins involved in MVB biogenesis

Yeast	Human[a]	Function	Refs[g]
Vps27p	Hrs/Hgs	Class E Receptor recycling, ESCRT recruitment (?)	[1–4]
ESCRT-I:		Class E	[5–7]
Vps23p/Stp22p	Tsg101	Recognition of	
Vps28p	hVps28	monoubiquitinated	
Vps37p	?	cargo proteins	
ESCRT-II:		Class E	[8,9]
Vps22p/Snf8p	Eap30	ESCRT assembly	
Vps25p	Eap20		
Vps36p	Eap45		
ESCRT-III[b]:		Class E	[10–12]
Vps2p/Did4p	Chmp2, Chmp2.5	Core sorting complex	
Vps20p	Chmp6		
Vps24p	Chmp3		
Vps32p/Snf7p	Chmp4, CAC14088, XP_044996		
Vps60p/Mos10p	Chmp5		
Did2p/Chm1p	Chmp1, Chmp1.5		
Fab1p[c]	PIKfyve	PI(3)P 5-kinase	[13–16]
Vps4p	Vps4A, Vps4B/Skd1	Class E ESCRT complex disassembly	[6,17–19]
Doa4p[d]		Ubiquitin recycling	[10,20]
Bro1 domain proteins:[e]	Hp95, KIAA1471,	Class E (Vps31p)	[21–23]
Vps31p/Npi3p/Bro1p	KIAA1929	Receptor	
Rim20p		downregulation	
Vps44p/Nhx1p[f]	SLC9A6	Na^+/H^+ exchanger	[24]

[a]Some human proteins were identified solely by sequence similarity and have not yet been characterized functionally.
[b]The membrane-associated ESCRT-III sorting complex is an oligomeric array of related proteins, all of which have basic N-termini, acidic C-termini, putative central coiled-coil domains and, in one case (Vps20p), an N-myristoyl modification. In yeast, four of these proteins make up the essential core complex (Vps2p, Vps20p, Vps24p and Vps32p), while two play non-essential roles (Vps60p and Did2p). Functions of the 10 apparent human orthologs of the yeast ESCRT-III proteins have not been characterized extensively, but Chmp1 has been shown to be involved in MVB protein sorting and to bind to Vps4B.
[c]Yeast Fab1p is not formally a Class E gene, but the PI(3)P kinase activity of the protein is required for protein sorting into the MVB.
[d]The yeast ubiquitin hydrolase, Doa4p, removes ubiquitin from protein cargos immediately prior to incorporation into MVB vesicles. This activity is not absolutely required for protein sorting into MVB vesicles, however.
[e]Yeast Vps31p is a Class E gene, whose precise function is not yet well characterized, but is implicated in ubiquitin-dependent downregulation of the yeast permease Gap1p. The protein binds to Vps4p and Vps32p and has an identifiable domain (the 'Bro1 domain') found in other yeast and human proteins listed here.
[f]Yeast Vps44p is also a Class E gene, which functions as a sodium/proton exchanger and may help acidify the endosomal compartment.
[g]References: 1 Shih, S.C. *et al.* (2002) *Nat. Cell Biol.* 4, 389–393; 2 Raiborg, C. *et al.* (2002) *Nat. Cell Biol.* 4, 394–398; 3 Bishop, N. *et al.* (2002). *J. Cell Biol.* 157, 91–101; 4 Lloyd, T.E. *et al.* (2002) *Cell* 108, 261–269; 5 Katzmann, D.J. *et al.* (2001) *Cell* 106, 145–155; 6 Bishop, N. and Woodman, P. (2001) *J. Biol. Chem.* 276, 11735–11742; 7 Babst, M. *et al.* (2000) *Traffic* 1, 248–258; 8 Babst, M. *et al.* (2002) *Dev. Cell* 3, 283; 9 Kamura, T. *et al.* (2001) *J. Biol. Chem.* 276, 16528–16533; 10 Babst, M. *et al.* (2002) *Dev. Cell* 3, 271; 11 Howard, T.L. *et al.* (2001) *J. Cell Sci.* 114, 2395–2404; 12 Kranz, A. *et al.* (2001) *Mol. Biol. Cell* 12, 711–723; 13 Odorizzi, G. *et al.* (1998) *Cell* 95, 847–858; 14 Gary, J.D. *et al.* (1998) *J. Cell Biol.* 143, 65–79; 15 Ikonomov, O.C. *et al.* (2002) *J. Biol. Chem.* 277, 9206–9211; 16 Sbrissa, D. *et al.* (2002) *J. Biol. Chem.* 277, 6073–6079; 17 Babst, M. *et al.* (1998) *EMBO J.* 17, 2982–2993; 18 Scheuring, S. *et al.* (2001) *J. Mol. Biol.* 312, 469–480; 19 Yoshimori, T. *et al.* (2000) *Mol. Biol. Cell* 11, 747–763; 20 Amerik, A.Y. *et al.* (2000) *Mol. Biol. Cell* 11, 3365–3380; 21 Nickas, M.E. and Yaffe, M.P. (1996) *Mol. Cell. Biol.* 16, 2585–2593; 22 Wu, Y. *et al.* (2001) *Differentiation* 67, 139–153; 23 Springael, J.Y. *et al.* (2002) *FEBS Lett.* 517, 103–109; 24 Bowers, K. *et al.* (2000) *Mol. Biol. Cell* 11, 4277–4294

Other viral late domains

Several enveloped RNA viruses lack PTAP motifs and apparently cannot bind Tsg101, yet are still able to bud efficiently from cells. In addition to PTAP, two other short sequence motifs have been shown to function as viral late domains, and it is likely that even more late domains remain to be identified. The Pro-Pro-Xaa-Tyr (PPXY) motif (where Xaa is typically proline) carries out late domain functions in many oncoviruses, filoviruses and rhabdoviruses [35–44], and a Tyr-Xaa-Xaa-Leu (YXXL) motif functions in the release of the retrovirus equine infectious anemia virus (EIAV) [45] (Fig. 1). Although these three different late domains mediate binding to different cellular receptors, they can often function interchangeably [36,42,46–49], which indicates that they mediate similar processes.

Considerable progress has been made in identifying the cellular receptors for the PPXY and YXXL late domains. PPXY late domains can bind Nedd4-like proteins, which are E3 ubiquitin ligases that interact with the PPXY motifs through their WW domains [39,47,50–53]. The prototypic family member, Nedd4 (yeast Rsp5p), can ubiquitinate

and thus promote the endocytosis and lysosomal degradation of surface receptors such as the epidermal growth factor receptor [54]. Receptor recognition is mediated by PPXY motifs, acidic sequences and/or phosphorylation-based signals. Nedd4 can also ubiquitinate the VP40 proteins of vesicular stomatitis virus and Ebola virus *in vitro* [39,52]. It is not yet clear which of the numerous Nedd4-like proteins of higher eukaryotes can function as PPXY late domain receptors, but at least one, BUL1 (budding-associated ubiquitin ligase 1), binds the PPXY late domain of the Mason–Pfizer monkey virus Gag protein in cells and enhances viral budding when overexpressed [53]. Other family members that might function as late domain receptors include Nedd4 itself [47] and late domain-interactor 1 (LDI-1) [51].

Clues to the identity of the receptor for the YXXL late domain have come from the realization that this late domain matches the consensus binding sequence of the μ2 subunit of AP-2, a complex involved in selecting cargo for clathrin vesicles [55]. Indeed, AP-2 can bind the YXXL sequence of EIAV Gag *in vitro* and colocalizes with EIAV Gag in cells [56], which is consistent with the idea that AP-2 is the receptor for the EIAV late domain.

It is clear that some viruses carry more that one late domain (see Fig. 1), which might carry out synergistic or redundant functions and extend viral tropism. Ultimately, the use of several late domains could prove to be common, as illustrated by recent studies of HIV Gag. As discussed above, the universally conserved PTAP sequence of the p6 domain of Gag has been considered to be the primary late domain of HIV, because mutations in this motif alter virus release in almost all cell lines that have been tested, and because the PTAP motif can rescue virus budding and infectivity in viruses that lack their normal PPXY motifs [46,48]. However, HIV strains lacking PTAP sequences can replicate, albeit with delayed kinetics, in some T cell lines (but not in many others) [8,57], and recent evidence shows that a conserved Leu-Xaa-Xaa-Leu-Phe (LXXLF) motif located downstream in p6 can also function as a late domain (H. Göttlinger, pers. commun.). In general, the search for new viral late domains represents an important area of study that should increase our understanding of the biology of specific viruses and help to define the range of cellular partners that facilitate virus budding.

Do other viral late domains also usurp the MVB machinery?

The functional redundancy of different viral late domains suggests that these domains might provide different routes of entry into the same MVB pathway. Consistent with this model, the release of both HIV (PTAP late domain) and Moloney murine leukemia virus (PPPY late domain) is blocked efficiently at a late stage by dominant-negative mutants of Vps4 [12] (Fig. 2b). However, Moloney murine leukemia virus is not strongly inhibited by Tsg101 depletion or by overexpression of dominant-negative Tsg101 constructs [12,14], which implies that the PPPY late domain is independent of Tsg101 function and probably acts downstream of (or redundantly with) Tsg101.

Although it is not yet clear how the Nedd4-like proteins link the PPXY late domain to MVB formation, mutations in Rsp5p (the Nedd4 ortholog in yeast) give rise to MVB defects, suggesting that there is a link [58,59]. It is less clear how the μ2 subunit of AP-2 links the YXXL late domain to the MVB vesicle machinery, although AP-2 does function in both endocytosis [60] and lysosomal trafficking [61]. It is therefore important to establish that AP-2 is truly the functional receptor for the YXXL late domain and, if so, to understand how it functions.

Ubiquitin in MVB protein sorting and viral budding

Both retroviral budding [16,47,52,62] and MVB protein sorting [15,63–65] require ubiquitin, providing two more examples of biological pathways in which ubiquitin functions other than to target proteins for proteasomal degradation. Ubiquitination targets proteins for incorporation into MVB vesicles [15,63,65] and could well have additional roles in vacuolar protein sorting, given that several components of the endosomal sorting apparatus are themselves also ubiquitinated [66]. Hrs (yeast Vps27p), Eps15 (Ede1p) and Tsg101 (Vps23p) all have ubiquitin-binding activities that are required for cargo sorting, which indicates that ubiquitin is recognized at several stages along the pathway [15,23,66,67]. As cargo proteins enter vesicles, ubiquitin is removed and recycled by the deubiquitinating enzyme, yeast Doa4p, which is recruited by the Vps24p protein of the ESCRT-III complex [27,68].

Ubiquitin transfer is also required for the efficient release of viruses that use PTAP and PPXY late domains (although not for EIAV [69,70], which, in addition to its YXXL motif also has a sequence in the p9 domain of Gag that may mimic ubiquitin [70]). It is therefore tempting to speculate that ubiquitination causes Gag proteins to be treated as 'cargos' that are 'sorted' into viral vesicles as they bud from the plasma membrane. In support of this model, viral late domains recruit ubiquitin E3 ligase activities that ubiquitinate Gag proteins at low levels [69,71,72], and Gag ubiquitination is correlated with successful virus release [47]. However, a direct role for ubiquitination of HIV Gag in virus release has not been demonstrated as yet [72], and it remains possible that cellular proteins are the functional targets of ubiquitination. Identification of the E2 and E3 ligases that ubiquitinate viral structural proteins will presumably help us to understand the role of ubiquitin in virus budding.

Late domain recognition

Learning how HIV and other enveloped RNA viruses redirect cellular machinery to the sites of

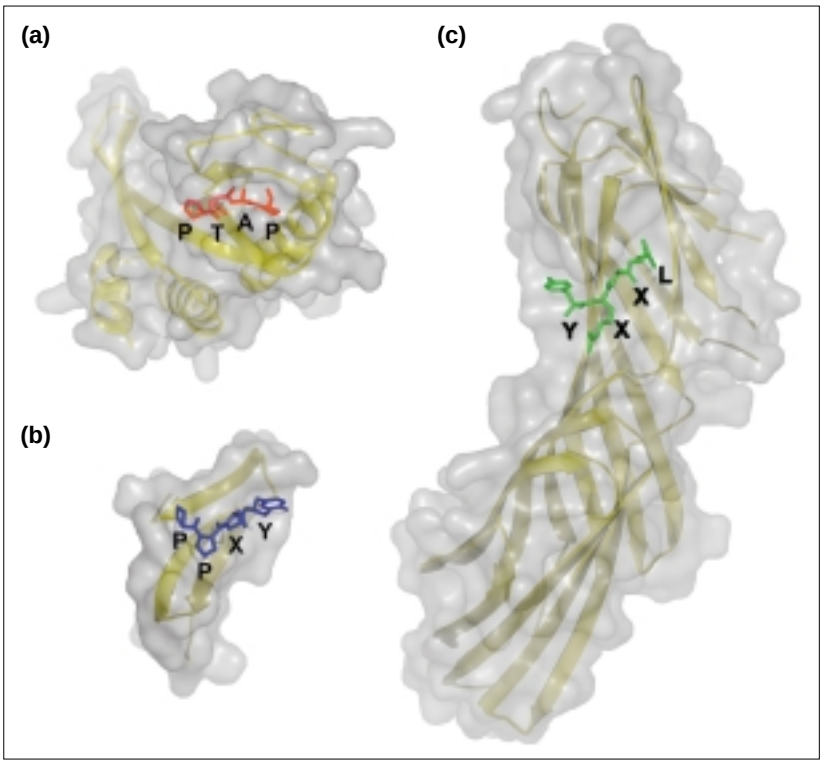

Fig. 5. Cellular protein recognition of late domains. (a) Structure of the Tsg101 UEV domain in complex with the HIV-1 PTAP late domain (HN-PEPTAPPEE-CO_2) [76]. (b) Structure of the WW domain of dystrophin in complex with a PPXY peptide from β-dystroglycan (H_2N-KNMTPYRSPPPYVPP-CO_2) [112]. (c) Structure of the μ2 subunit of AP-2 in complex with a YXXL peptide (H_2N-FYRALM-CO_2) from the epidermal growth factor receptor [78].

budding is of fundamental interest and could also contribute to the development of viral inhibitors. Tsg101 is recruited to HIV budding sites by a direct interaction between its N-terminal UEV (ubiquitin E2 variant) domain and the PTAP late domain. The Tsg101 UEV domain can also bind ubiquitin and this activity is essential for MVB sorting, although its functional relevance for virus budding remains to be established [12,15,67]. UEV domains show significant sequence similarity to E2 ubiquitin conjugating enzymes, but are unable to catalyze ubiquitin transfer because they lack the active-site cysteine that forms a transient thioester bond with the C-terminus of ubiquitin [73,74].

Recent structures of the Tsg101 UEV domain, both free and in complex with the PTAP motif on the p6 domain of HIV Gag, have shown that the protein contains the α-helix/β-strand/loop/α-helix fold that is found in all E2 enzymes, but it lacks the two C-terminal E2 helices [75,76]. The PTAP peptide binds in a groove that is exposed by the absence of the C-terminal E2 helix, and all four PTAP residues make important contacts (Fig. 5). Notably, the binding site for the central Ala-Pro dipeptide is similar to the Xaa-Pro binding pockets of the SH3 (Src-homology domain 3) and WW domains, revealing convergence in the mechanisms of proline recognition in these different protein classes [77].

Structures of complexes between WW domains and PPXY peptides, and between the μ2 domain of AP-2 and YXXL peptides, have also been determined [77,78]. Although these studies used peptides from cellular proteins rather than authentic viral late domains, the structures provide very useful models for understanding PPXY and YXXL recognition.

Molecular mimicry

Recent evidence suggests that viral late domains might recruit the ESCRT-I complex by mimicking the activities of the cellular Hrs protein (Vps27p), which is required for the Vps-mediated sorting of ubiquitinated cargos [23,25,66,67] (Fig. 4b). Like HIV Gag, Hrs oligomerizes on membrane surfaces, is monoubiquitinated [66] and contains a PSAP motif. Hrs also contains a PPXY motif and can be monoubiquitinated by Nedd4, suggesting that PPXY motifs might also mimic Hrs activity(ies).

Recently, our group has shown that the C-terminal two-thirds of Hrs can rescue viral budding when fused to HIV Gag proteins, which formally demonstrates that the protein-recruitment functions of Hrs are sufficient for viral late domain activity (D. Higginson *et al.*, unpublished). There is a fundamental difference between Hrs and HIV Gag, however, in that the two proteins contain different membrane-targeting signals. Specifically, Hrs is directed to early endosomal membranes through an interaction between its FYVE domain and PtdIns(3)*P* lipids [24,79], whereas HIV Gag is directed to sites of budding on the plasma membrane by signals in its *N*-myristoylated MA domain [7,10].

Processes unique to virus budding

Although the analogy to cellular MVB biogenesis is increasingly compelling, viruses must also carry out some unique functions to escape cells. In particular, viruses must target their structural proteins to appropriate sites of assembly (e.g. the plasma membrane), couple membrane binding to particle assembly, penetrate the cortical actin cytoskeleton shell that lies beneath the plasma membrane, and recruit appropriate viral and cellular factors at the correct times.

Recent progress in these areas includes the identification of an HIV assembly chaperone [80], the demonstration that phosphatidylinositol phosphates promote assembly of more authentic Gag particles *in vitro* [81], the realization that HIV [82–84], Ebola [85] and influenza [86] bud from specialized, cholesterol-rich microdomains on the plasma membrane (lipid 'rafts'), and the identification of p6 as the principal phosphoprotein of HIV [87]. Although it is not yet possible to put all of these observations into a coherent biochemical pathway, these and other processes presumably work together to achieve the successful spatial and temporal coordination of viral particle release. For example, Harty and coworkers now have evidence that the Ebola virus VP40/TPsg101 recruits VP40 into lipid rafts (R. Harty, pers. commun.)

Roles for other viral proteins in virus budding

Although HIV Gag drives particle assembly and budding, other virion-associated viral proteins can also interact with cellular trafficking molecules and might thereby influence particle assembly and release. A highly conserved YXXΦ (where Φ is a large, hydrophobic residue) motif in the intracellular domain of the viral Env protein binds AP-2 with high affinity and recycles unpackaged Env glycoproteins through a clathrin-dependent endocytic pathway [88]. This motif functions to restrict the surface expression of envelope glycoproteins and can also mediate directional virus budding in polarized cells.

The viral Nef protein contains a dileucine motif that interacts with AP-1 and downregulates CD4 from the cell surface, thereby enabling viral assembly and budding to occur without the potentially deleterious effects of CD4 interactions [89]. Finally, deletion of the transmembrane viral accessory protein Vpu causes HIV to arrest with a late-domain-like phenotype in some cell lines [90], suggesting that Vpu might also contribute 'late domain' activity in some contexts. It will be interesting to dissect how these (and other) viral protein trafficking signals help to regulate the timing, efficiency and sites of virion assembly and release.

Alternative sites of viral budding

Remarkably it seems that, in addition to budding directly from the plasma membrane, HIV can also use the MVB compartment to escape from cells ([91]; and M. Marsh, M. Thali and W. Mothes, pers. commun.). Extracellular virus release from MVBs is possible because these compartments can, in some contexts, act as stable storage compartments (e.g. in immature dendritic cells) and can even fuse with the plasma membrane to release their internal vesicles (e.g. in mature dendritic cells), which are then termed 'exosomes' [92] (Fig. 3).

In some cell types, particularly macrophages, HIV particles are known to accumulate primarily in intracellular compartments [93–95]. Recent work from Raposo and coworkers [91] shows that these compartments correspond to MVBs and can fuse with the plasma membrane to release mature HIV particles. Intriguingly, the Marburg VP40 protein also localizes primarily to MVBs in some cell types [96], suggesting that the budding of enveloped RNA viruses into the MVB compartment might be a general phenomenon.

Potential advantages of exit through the exosome pathway include the acquisition of surface molecules that might increase infectivity (e.g. MHC class II molecules in HIV [97]), the suppression of immune responses that normally occur by this pathway (discussed below), long-term storage in an infectious state, and directional release. In addition, the fact that HIV can escape from cells through the exosome pathway raises the intriguing possibility that viral particles can also be passed on to T cells through this same pathway after they enter dendritic cells via endocytosis mediated by DC-SIGN [98–100].

Given these recent discoveries, it is now essential to understand what dictates the differences in viral protein trafficking in different cell lines [93], to determine the relative contributions to infectivity of HIV particles that escape through exosomes versus the plasma membrane, and to identify the signals that dictate whether MVB compartments migrate to the plasma membrane and release viral particles as exosomes or fuse with lysosomes and degrade viral particles. It is indeed exciting to think that viruses and cells might be battling for control of both the machinery and the fate of the multivesicular body.

Consequences of viral usurpation of the MVB pathway

Endosomal trafficking and the MVB pathway help to regulate several important cellular processes, including the downregulation of growth factor receptors [101,102] and the trafficking of MHC class I and II molecules [92,103,104]. It is possible that viruses could gain replicative advantages by maintaining cellular proliferation signals or by altering antigen presentation, and it will be therefore interesting to determine whether the amounts of Gag produced during an HIV infection can titrate sufficient quantities of class E Vps proteins to alter the flux of cellular molecules through the MVB pathway. In addition, there are also reports that Tsg101 helps to regulate the p53 [105], p21Cip1/WAF1 [106] and cdk2 [107] pathways, and thus these processes might also be perturbed upon infection.

Concluding remarks

The cellular cofactors and viral budding mechanisms described here can provide both useful models for understanding viral egress in other systems, as well as potential targets for therapeutic intervention. Although budding is still understood poorly in several important families of enveloped RNA viruses (e.g. the paramyxo- and orthomyxoviruses), it will be surprising if at least a subset do not use the MVB pathway. Indeed, Schmitt and Lamb have recently obtained evidence indicating a requirement for both ubiquitin and Vps4A in the budding of paramyxovirus SV5 (A. Schmitt and R. Lamb, pers. commun.).

In addition, tantalizing clues suggest that the MVB machinery might also participate in the release of even more distantly related viruses. An example is the non-enveloped bluetongue virus (an RNA orbivirus), which temporarily acquires a membrane coat as it buds through the plasma membrane. Virus release depends on the viral NS3 protein [108], which contains adjacent PPAY and PSAP motifs that could, in principle, act as late domains during the budding step [47].

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Another example is the enveloped human cytomegalovirus (a DNA herpesvirus): Marsh and colleagues [109] have recently obtained electron microscopy images of human cytomegalovirus particles that seem to be budding into multivesicular bodies [109]. These observations raise the exciting possibility that many different classes of virus might exit the cell through the MVB pathway.

In principle, virus budding also represents an attractive target for therapeutic intervention because the pathway seems both essential and general, and because cellular targets might have better drug resistance profiles than those of conventional viral targets. Although viruses can spread through cell-to-cell contact in some contexts, the absolute conservation of the PTAP motif in HIV Gag, together with the severe reductions in viral infectivity observed upon mutation of the late domain or blocking the MVB pathway, indicates that inhibitors of virus budding should also block replication. The number of apparent targets in the MVB pathway and the emergence of biochemical and structural data bode well for the discovery and development of inhibitors.

The possibility of cellular toxicity is a legitimate cause for concern, however, because the MVB pathway carries out important cellular functions, and both Tsg101 and Hrs are essential for murine development [107,110,111]. But the class E Vps genes are not essential in yeast, and short-term toxicity is not observed in cultured cells depleted of Tsg101 [12]. Thus, it will be important to identify inhibitors of budding and to test whether they have therapeutic windows that make them attractive candidates as broad-spectrum antiviral agents.

References

1 Marais, H. and Wilson, A. (2002) The report on the global HIV/AIDS epidemic. The Barcelona report. *XIV International Conference on AIDS*

2 Ghendon, Y. (1992) Influenza-its impact and control. *World Health Stat. Q.* 45, 306–311

3 Eckert, D.M. and Kim, P.S. (2001) Mechanisms of viral membrane fusion and its inhibition. *Annu. Rev. Biochem.* 70, 777–810

4 Skehel, J.J. and Wiley, D.C. (2000) Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu. Rev. Biochem.* 69, 531–569

5 Brunger, A.T. (2001) Structural insights into the molecular mechanism of calcium-dependent vesicle-membrane fusion. *Curr. Opin. Struct. Biol.* 11, 163–173

6 Greene, W.C. and Peterlin, B.M. (2002) Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. *Nat. Med.* 8, 673–680

7 Gottlinger, H.G. (2001) The HIV-1 assembly machine. *AIDS* 15 (Suppl. 5), S13–S20

8 Gottlinger, H.G. *et al.* (1991) Effect of mutations affecting the p6 gag protein on human immunodeficiency virus particle release. *Proc. Natl. Acad. Sci. U. S. A.* 88, 3195–3199

9 Huang, M. *et al.* (1995) p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. *J. Virol.* 69, 6810–6818

10 Wills, J.W. and Craven, R.C. (1991) Form, function, and use of retroviral gag proteins. *AIDS* 5, 639–654

11 VerPlank, L. *et al.* (2001) Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55Gag. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7724–7729

12 Garrus, J.E. *et al.* (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107, 55–65

13 Martin-Serrano, J. *et al.* (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

14 Demirov, D.G. *et al.* (2002) Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. *Proc. Natl. Acad. Sci. U. S. A.* 99, 955–960

15 Katzmann, D.J. *et al.* (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 106, 145–155

16 Patnaik, A. *et al.* (2000) Ubiquitin is part of the retrovirus budding machinery. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13069–13074

17 Piper, R.C. and Luzio, J.P. (2001) Late endosomes: sorting and partitioning in multivesicular bodies. *Traffic* 2, 612–621

18 Raymond, C.K. *et al.* (1992) Morphological classification of the yeast vacuolar protein sorting mutants: evidence for a prevacuolar compartment in class E vps mutants. *Mol. Biol. Cell* 3, 1389–1402

19 Rieder, S.E. *et al.* (1996) Multilamellar endosome-like compartment accumulates in the yeast vps28 vacuolar protein sorting mutant. *Mol. Biol. Cell* 7, 985–999

20 Odorizzi, G. *et al.* (1998) Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body. *Cell* 95, 847–858

21 Asao, H. *et al.* (1997) Hrs is associated with STAM, a signal-transducing adaptor molecule. Its suppressive effect on cytokine-induced cell growth. *J. Biol. Chem.* 272, 32785–32791

22 Bean, A.J. *et al.* (2000) Hrs-2 regulates receptor-mediated endocytosis via interactions with Eps15. *J. Biol. Chem.* 275, 15271–15278

23 Shih, S.C. *et al.* (2002) Epsins and Vps27p/Hrs contain ubiquitin-binding domains that function in receptor endocytosis. *Nat. Cell Biol.* 4, 389–393

24 Raiborg, C. *et al.* (2001) FYVE and coiled-coil domains determine the specific localisation of Hrs to early endosomes. *J. Cell Sci.* 114, 2255–2263

25 Raiborg, C. *et al.* (2002) Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat. Cell Biol.* 4, 394–398

26 Babst, M. *et al.* (2002) Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev. Cell* 3, 283

27 Babst, M. *et al.* (2002) Escrt-III. An endosome-associated heterooligomeric protein complex required for mvb sorting. *Dev. Cell* 3, 271

28 Babst, M. *et al.* (1998) The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. *EMBO J.* 17, 2982–2993

29 Howard, T.L. *et al.* (2001) CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins. *J. Cell Sci.* 114, 2395–2404

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

31 Wurmser, A.E. and Emr, S.D. (1998) Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires lumenal vacuolar hydrolase activities. *EMBO J.* 17, 4930–4942

32 Gillooly, D.J. *et al.* (2000) Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J.* 19, 4577–4588

33 Kobayashi, T. *et al.* (2001) Localization of lysobisphosphatidic acid-rich membrane domains in late endosomes. *Biol. Chem.* 382, 483–485

34 Schmid, S.L. *et al.* (1998) Dynamin and its partners: a progress report. *Curr. Opin. Cell Biol.* 10, 504–512

35 Wills, J.W. *et al.* (1994) An assembly domain of the Rous sarcoma virus Gag protein required late in budding. *J. Virol.* 68, 6605–6618

36 Xiang, Y. *et al.* (1996) Fine mapping and characterization of the Rous sarcoma virus Pr76gag late assembly domain. *J. Virol.* 70, 5695–5700

37 Yuan, B. *et al.* (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

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

39 Harty, R.N. *et al.* (2000) A PPxY motif within the VP40 protein of ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13871–13876

40 Craven, R.C. *et al.* (1999) Late domain function identified in the vesicular stomatitis virus M protein by use of rhabdovirus-retrovirus chimeras. *J. Virol.* 73, 3359–3365

41 Jayakar, H.R. *et al.* (2000) Mutations in the PPPY motif of vesicular stomatitis virus matrix protein reduce virus budding by inhibiting a late step in virion release. *J. Virol.* 74, 9818–9827

42 Strack, B. *et al.* (2002) Late assembly domain function can exhibit context dependence and involves ubiquitin residues implicated in endocytosis. *J. Virol.* 76, 5472–5479

43 Wang, H. *et al.* (2002) Analysis of bovine leukemia virus gag membrane targeting and late domain function. *J. Virol.* 76, 8485–8493
44 Le Blanc, I. *et al.* (2002) The PPPY motif of human T-cell leukemia virus type 1 Gag protein is required early in the budding process. *J. Virol.* 76, 10024–10029
45 Puffer, B.A. *et al.* (1997) Equine infectious anemia virus utilizes a YXXL motif within the late assembly domain of the Gag p9 protein. *J. Virol.* 71, 6541–6546
46 Parent, L.J. *et al.* (1995) Positionally independent and exchangeable late budding functions of the Rous sarcoma virus and human immunodeficiency virus Gag proteins. *J. Virol.* 69, 5455–5460
47 Strack, B. *et al.* (2000) A role for ubiquitin ligase recruitment in retrovirus release. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13063–13068
48 Yuan, B. *et al.* (2000) Infectivity of moloney murine leukemia virus defective in late assembly events is restored by late assembly domains of other retroviruses. *J. Virol.* 74, 7250–7260
49 Li, F. *et al.* (2002) Functional replacement and positional dependence of homologous and heterologous L domains in equine infectious anemia virus replication. *J. Virol.* 76, 1569–1577
50 Harty, R.N. *et al.* (1999) A proline-rich motif within the matrix protein of vesicular stomatitis virus and rabies virus interacts with WW domains of cellular proteins: implications for viral budding. *J. Virol.* 73, 2921–2929
51 Kikonyogo, A. *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. U. S. A.* 98, 11199–11204
52 Harty, R.N. *et al.* (2001) Rhabdoviruses and the cellular ubiquitin-proteasome system: a budding interaction. *J. Virol.* 75, 10623–10629
53 Yasuda, J. *et al.* (2002) Functional involvement of a novel Nedd4-like ubiquitin ligase on retrovirus budding. *EMBO Rep.* 3, 636–640
54 Rotin, D. *et al.* (2000) Ubiquitination and endocytosis of plasma membrane proteins: role of Nedd4/Rsp5p family of ubiquitin-protein ligases. *J. Membr. Biol.* 176, 1–17
55 Collins, B.M. *et al.* (2002) Molecular architecture and functional model of the endocytic AP2 complex. *Cell* 109, 523–535
56 Puffer, B.A. *et al.* (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
57 Demirov, D.G. *et al.* (2002) The late domain of human immunodeficiency virus type 1 p6 promotes virus release in a cell type-dependent manner. *J. Virol.* 76, 105–117
58 Dunn, R. and Hicke, L. (2001) Domains of the rsp5 ubiquitin-protein ligase required for receptor- mediated and fluid-phase endocytosis. *Mol. Biol. Cell* 12, 421–435
59 Wang, G. *et al.* (2001) Localization of the Rsp5p ubiquitin-protein ligase at multiple sites within the endocytic pathway. *Mol. Cell. Biol.* 21, 3564–3575
60 Hirst, J. and Robinson, M.S. (1998) Clathrin and adaptors. *Biochim. Biophys. Acta* 1404, 173–193
61 Traub, L.M. *et al.* (1996) AP-2-containing clathrin coats assemble on mature lysosomes. *J. Cell Biol.* 135, 1801–1814
62 Schubert, U. *et al.* (2000) Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13057–13062
63 Reggiori, F. and Pelham, H.R. (2001) Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J.* 20, 5176–5186
64 Longva, K.E. *et al.* (2002) Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies. *J. Cell Biol.* 156, 843–854
65 Urbanowski, J.L. and Piper, R.C. (2001) Ubiquitin sorts proteins into the intralumenal degradative compartment of the late-endosome/vacuole. *Traffic* 2, 622–630
66 Polo, S. *et al.* (2002) A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature* 416, 451–455
67 Bishop, N. *et al.* (2002) Mammalian class E vps proteins recognize ubiquitin and act in the removal of endosomal protein-ubiquitin conjugates. *J. Cell Biol.* 157, 91–101
68 Amerik, A.Y. *et al.* (2000) The Doa4 deubiquitinating enzyme is functionally linked to the vacuolar protein-sorting and endocytic pathways. *Mol. Biol. Cell* 11, 3365–3380
69 Ott, D.E. *et al.* (2002) Equine infectious anemia virus and the ubiquitin-proteasome system. *J. Virol.* 76, 3038–3044
70 Patnaik, A. *et al.* (2002) Budding of equine infectious anemia virus is insensitive to proteasome inhibitors. *J. Virol.* 76, 2641–2647
71 Ott, D.E. *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
72 Ott, D.E. *et al.* (2000) Ubiquitination of HIV-1 and MuLV Gag. *Virology* 278, 111–121
73 Koonin, E.V. and Abagyan, R.A. (1997) TSG101 may be the prototype of a class of dominant negative ubiquitin regulators. *Nat. Genet.* 16, 330–331
74 Ponting, C.P. *et al.* (1997) The breast cancer gene product TSG101: a regulator of ubiquitination? *J. Mol. Med.* 75, 467–469
75 Pornillos, O. *et al.* (2002) Structure and functional interactions of the Tsg101 UEV domain. *EMBO J.* 21, 2397–2406
76 Pornillos, O.P. *et al.* Structure of the Tsg101 UEV domain in complex with a HIV-1 PTAP 'late domain' peptide. *Nat. Struct. Biol.* (in press)
77 Zarrinpar, A. and Lim, W.A. (2000) Converging on proline: the mechanism of WW domain peptide recognition. *Nat. Struct. Biol.* 7, 611–613
78 Owen, D.J. and Evans, P.R. (1998) A structural explanation for the recognition of tyrosine-based endocytotic signals. *Science* 282, 1327–1332
79 Komada, M. *et al.* (1997) Hrs, a tyrosine kinase substrate with a conserved double zinc finger domain, is localized to the cytoplasmic surface of early endosomes. *J. Biol. Chem.* 272, 20538–20544
80 Zimmerman, C. *et al.* (2002) Identification of a host protein essential for assembly of immature HIV-1 capsids. *Nature* 415, 88–92
81 Campbell, S. *et al.* (2001) Modulation of HIV-like particle assembly *in vitro* by inositol phosphates. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10875–10879
82 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
83 Lindwasser, O.W. and Resh, M.D. (2001) Multimerization of human immunodeficiency virus type 1 Gag promotes its localization to barges, raft-like membrane microdomains. *J. Virol.* 75, 7913–7924
84 Ono, A. and Freed, E.O. (2001) Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13925–13930
85 Bavari, S. *et al.* (2002) Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. *J. Exp. Med.* 195, 593–602
86 Scheiffele, P. *et al.* (1999) Influenza viruses select ordered lipid domains during budding from the plasma membrane. *J. Biol. Chem.* 274, 2038–2044
87 Muller, B. *et al.* (2002) The late-domain-containing protein p6 is the predominant phosphoprotein of human immunodeficiency virus type 1 particles. *J. Virol.* 76, 1015–1024
88 Marsh, M. and Pelchen-Matthews, A. (2000) Endocytosis in viral replication. *Traffic* 1, 525–532
89 Geyer, M. *et al.* (2001) Structure–function relationships in HIV-1 Nef. *EMBO Rep.* 2, 580–585
90 Bour, S. and Strebel, K. (2000) HIV accessory proteins: multifunctional components of a complex system. *Adv. Pharmacol.* 48, 75–120
91 Raposo, G. *et al.* (2002) Human macrophages accumulate HIV-1 particles in MHC II compartments. *Traffic* 3, 718–729
92 Denzer, K. *et al.* (2000) Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J. Cell Sci.* 113, 3365–3374
93 Blom, J. *et al.* (1993) An ultrastructural study of HIV-infected human dendritic cells and monocytes/macrophages. *APMIS* 101, 672–680
94 Pautrat, G. *et al.* (1990) Human immunodeficiency virus type 1 infection of U937 cells promotes cell differentiation and a new pathway of viral assembly. *Virology* 179, 749–758
95 Meltzer, M.S. *et al.* (1990) Macrophages and the human immunodeficiency virus. *Immunol. Today* 11, 217–223
96 Kolesnikova, L. *et al.* (2002) VP40, the matrix protein of Marburg virus, is associated with membranes of the late endosomal compartment. *J. Virol.* 76, 1825–1838
97 Rossio, J.L. *et al.* (1995) HLA class II on HIV particles is functional in superantigen presentation to human T cells: implications for HIV pathogenesis. *AIDS Res. Hum. Retroviruses* 11, 1433–1439
98 Geijtenbeek, T.B. *et al.* (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 100, 587–597
99 Kwon, D.S. *et al.* (2002) DC-SIGN-mediated internalization of HIV is required for trans- enhancement of T cell infection. *Immunity* 16, 135–144
100 Engering, A. *et al.* (2002) The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J. Immunol.* 168, 2118–2126
101 Hicke, L. (1999) Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. *Trends Cell Biol.* 9, 107–112
102 Kramer, H. (2002) Sorting out signals in fly endosomes. *Traffic* 3, 87–91
103 Turley, S.J. *et al.* (2000) Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 288, 522–527
104 Kleijmeer, M.J. *et al.* (2001) Antigen loading of MHC class I molecules in the endocytic tract. *Traffic* 2, 124–137

105 Li, L. *et al.* (2001) A TSG101/MDM2 regulatory loop modulates MDM2 degradation and MDM2/p53 feedback control. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1619–1624
106 Oh, H. *et al.* (2002) Negative regulation of cell growth and differentiation by TSG101 through association with p21Cip1/WAF1. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5430–5435
107 Krempler, A. *et al.* (2002) Targeted deletion of the Tsg101 gene results in cell cycle arrest at G1/S and p53 independent cell death. *J. Biol. Chem.* 29, 29
108 Hyatt, A.D. *et al.* (1991) Localization of the non-structural protein NS3 in bluetongue virus- infected cells. *J. Gen. Virol.* 72, 2263–2267
109 Fraile-Ramos, A. *et al.* (2002) Localization of HCMV UL33 and US27 in endocytic compartments and viral membranes. *Traffic* 3, 218–232
110 Ruland, J. *et al.* (2001) p53 Accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1859–1864
111 Miura, S. *et al.* (2000) Hgs (Hrs), a FYVE domain protein, is involved in Smad signaling through cooperation with SARA. *Mol. Cell. Biol.* 20, 9346–9355
112 Huang, X. *et al.* (2000) Structure of a WW domain containing fragment of dystrophin in complex with beta-dystroglycan. *Nat. Struct. Biol.* 7, 634–638

PTEN and myotubularin phosphatases: from 3-phosphoinositide dephosphorylation to disease

Matthew J. Wishart and Jack E. Dixon

The phosphatase and tensin homolog deleted on chromosome ten (PTEN) and myotubularin (MTM1) represent subfamilies of protein tyrosine phosphatases whose principal physiological substrates are D3-phosphorylated inositol phospholipids. As lipid phosphatases, PTEN- and MTM1-related (MTMR) proteins dephosphorylate the products of phosphoinositide 3-kinases and antagonize downstream effectors that utilize 3-phosphoinositides as ligands for protein targeting domains or allosteric activation. Here, we describe the cellular mechanisms of PTEN and MTMR function and their role in the etiology of cancer and other human diseases.

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Interconversion of phosphorylated inositol lipids is a fundamental eukaryotic signaling mechanism for eliciting an intracellular response to environmental changes. Phosphatidylinositol (PtdIns) is the most abundant membrane inositol lipid under resting conditions. In addition, it serves as the basic building block for differentially phosphorylated derivatives, which, together with PtdIns, are known as phosphoinositides (PI) [1]. In response to extracellular stimulation, specific PtdIns kinases phosphorylate positions 3-, 4- and 5- of the inositol head group to create seven unique phosphoinositides (Fig. 1). Many of these phosphoinositides function as ligands for protein targeting modules, such as pleckstrin homology (PH), phox homology (PX), epsin N-terminal homology (ENTH), band 4.1/ezrin/radixin/moesin (FERM) and Fab1p/YOTB/Vac1p/EEA1 (FYVE) domains (Fig. 1), where lipid binding leads to the translocation of their associated effector domains to membrane sites of protein complex formation, and, in certain cases, to allosteric regulation of target protein activity [2]. Subsequent dephosphorylation of the inositol head group by PtdIns-specific phosphatases removes these targeting and allosteric regulatory signals. In this way, changes in PtdIns kinase and phosphatase activity in response to extracellular stimuli can dramatically alter the relative levels of specific PtdIns species and thereby orchestrate the spatio-temperal localization of proteins that regulate cellular response to its environment [1]. Conversely, dysregulation of PtdIns kinases and phosphatases results in aberrant phosphoinositide signaling and the disruption of normal control of cellular functions, such as growth, proliferation, differentiation and survival that are the hallmarks of disease. While much is known about the synthesis of PIs, their downstream effectors and associated signaling pathways [1], this review focuses on the regulation of PtdIns signaling by two families of 3-phosphoinositide phosphatases, PTEN and MTM, with particular emphasis on their role in the development of cancers and other human diseases.

Function of 3-phosphoinositides

Synthesis of 3-phosphorylated inositol lipids is a crucial regulatory step in PtdIns metabolism (reviewed in [1]). PtdIns(3)*P* comprises 2–5% of singly phosphorylated cellular PtdIns, whereas very little of the other 3-PIs are present under resting conditions. Upon stimulation, three subclasses of PtdIns 3-kinases synthesize PtdIns(3)*P*, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 by direct phosphorylation at the inositol D3 position of precursor lipids, while PtdIns(3,5)P_2 is produced by PtdIns 5-kinase phosphorylation of PtdIns(3)*P*. Unlike other PIs, 3-phosphoinositides are not broken down to diacylglycerol and soluble inositol second messengers by PtdIns-specific phospholipase C. Instead, these lipids remain partitioned to membranes, where they recruit 3-PtdIns-specific binding domains to discrete subcellular locations.

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