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Minireview

Mechanisms for enveloped virus budding: Can some viruses do without an ESCRT?

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Abstract

Many enveloped viruses complete their replication cycle by forming vesicles that bud from the plasma membrane. Some viruses encode "late" (L) domain motifs that are able to hijack host proteins involved in the vacuolar protein sorting (VPS) pathway, a cellular budding process that gives rise to multivesicular bodies and that is topologically equivalent to virus budding. Although many enveloped viruses share this mechanism, examples of viruses that require additional viral factors and viruses that appear to be independent of the VPS pathway have been identified. Alternative mechanisms for virus budding could involve other topologically similar process such as cell abscission, which occurs following cytokinesis, or virus budding could proceed spontaneously as a result of lipid microdomain accumulation of viral proteins. Further examination of novel virus–host protein interactions and characterization of other enveloped viruses for which budding requirements are currently unknown will lead to a better understanding of the cellular processes involved in virus assembly and budding.

Keywords: Virus budding; Enveloped virus assembly; Virus exit; Late domain; ESCRT pathway

Introduction

The mechanism by which enveloped viruses complete their replication cycle by budding through a cellular membrane presents a biophysical problem. After a complex, multistep process involving the proper transport and organization of viral proteins on the membrane, followed by incorporation of the genetic material, enveloped viruses must then form viral particles by deforming the membrane and, finally, pinching off from the membrane in a membrane fission step. Much as enveloped viruses entering a cell have to do so by mediating fusion of their membrane with a cellular membrane, viruses exiting a cell encounter the problem of membrane fission to release the nascent particles from the host membrane. To facilitate fusion, many enveloped viruses have encoded fusion machines in the form of fusion proteins present on the viral surface. These fusion proteins undergo dramatic conformational changes, converting the free energy released by refolding of the fusion protein into energy that is used to merge together viral and cellular membranes. However, for budding, no such virus-encoded protein is known to exist to overcome the energy barrier required for membrane fission.

As budding is an essential step in the life cycle of enveloped viruses, the mechanisms employed by viruses to accomplish this task have been an area of intense recent study. Whereas these mechanisms have previously been categorized under the rubric of "push vs. pull" processes (Garoff et al., 1998; Welsch et al., 2007), it is likely that a continuum or combination of forces is employed by viruses to induce membrane vesicularization and fission.

Host-assisted budding

The concept that viruses might utilize a cellular process to assist in virus budding arose initially from observations of retroviruses that failed to be released from cells during the late stages of virus budding. For human immunodeficiency virus

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Table 1
Enveloped viruses that possess L domains that interact with cellular proteins in the VPS pathway

Virus	Viral protein: L domain [a]	Direct interactions [b]	References [c]
Retroviruses			
Human immunodeficiency virus-1	Gag (p6):		Göttlinger et al. (1991)
	PTAP	Tsg101	Huang et al. (1995)
	YPDL	AIP1/Alix	Garrus et al. (2001)
		(ESCRT I, III)	Martin-Serrano et al. (2001)
		(Vps4A/B)	Martin-Serrano et al. (2003b)
			Strack et al. (2003)
			Martin-Serrano et al. (2003a)
			von Schwedler et al. (2003)
			Stuchell et al. (2004)
Rous sarcoma virus	Gag (p2b):		Wills et al. (1994)
	PPPPYV	Nedd4	Xiang et al. (1996)
		(Vps4A)	Kikonyogo et al. (2001)
			Vana et al. (2004)
			Medina et al. (2005)
Equine infectious anemia virus	Gag (p9):		Puffer et al. (1997)
	YPDL	AIP1/Alix	Puffer et al. (1998)
		AP-2	Li et al. (2002)
			Strack et al. (2003)
			Martin-Serrano et al. (2003a)
			Shehu-Xhilaga et al. (2004)
			Chen et al. (2005b)
Mason-Pfizer monkey virus	Gag (p24):		Yasuda and Hunter (1998)
	PSAP	Tsg101	Gottwein et al. (2003)
	PPPY	Nedd4	
		(Vps4A)	
Moloney murine leukemia virus	Gag (MA, p12):		Yuan et al. (1999)
	PSAP	Tsg101	Segura-Morales et al. (2005)
	PPPY	AIP1/Alix	
		(Nedd4)	
Human T cell leukemia virus-1	Gag (MA):		Le Blanc et al. (2002)
	PPPY	Nedd4.1	Bouamr et al. (2003)
		WWP1	Wang et al. (2004)
	(PTAP)	Tsg101	Blot et al. (2004)
		(Vps4A/B)	Heidecker et al. (2004)
		(AIP1/Alix)	Dorweiler et al. (2006)
			Urata et al. (2007b)
			Heidecker et al. (2007)
Prototypic foamy virus	Gag:		Stange et al. (2005)
	PSAP	Tsg101	Patton et al. (2005)
	(PPPI)		
	(YEIL)		
		(Vps4A/4B)	
		(CHMP3)	
Rhabdoviruses			
Vesicular stomatitis virus	M:		Harty et al. (1999)
	PPPY	Nedd4	Craven et al. (1999)
	(PSAP)		Jayakar et al. (2000)
			Irie et al. (2004b)
			Irie et al. (2004a)
			Irie and Harty (2005)
Rabies virus	M: PPEY	Nedd4	Harty et al. (1999)
Filoviruses			
Ebola virus	VP40:		Harty et al. (2000)
	PTAPPEY	Tsg101	Martin-Serrano et al. (2001)
		Nedd4	Licata et al. (2003)
			Timmins et al. (2003)
			Yasuda et al. (2003)
			Irie et al. (2005)

Table 1 (*continued*)

Virus	Viral protein: L domain[a]	Direct interactions[b]	References[c]
Marburg virus	VP40:		Kolesnikova et al. (2004)
	PPEY	Tsg101	Urata et al. (2007a)
Arenaviruses			
Lymphocytic choriomeningitis virus	Z:		Perez et al. (2003)
	PTAP	Tsg101	
	PPPY		
Lassa virus	Z:		Perez et al. (2003)
	PTAP	Tsg101	Urata et al. (2006)
	PPPY		
		(Vps4A)	
Paramyxoviruses			
Parainfluenza virus-5	M:		Schmitt et al. (2005)
	FPIV	n.d.	
		(Vps4A)	
Nipah virus	M:		Ciancanelli and Basler (2006)
	YMYL	n.d.	
Sendai virus	C: ?	AIP1/Alix[d]	Sakaguchi et al. (2005)
	M: YLDL	AIP1/Alix[d]	Irie et al. (2007)
		(Vps4A)[d]	Gosselin-Grenet et al. (2007)
Other RNA viruses			
Japanese encephalitis virus	NS3	Tsg101	Chiou et al. (2003)
Bluetongue virus	NS3/3A:		Wirblich et al. (2006)
	PSAP	Tsg101	
	(PPRY)	Nedd4	
DNA viruses			
Hepatitis B virus		(Vps4A/4B)	Kian Chua et al. (2006)
		(ESCRT-III)	Watanabe et al. (2007)
		(AIP1/Alix)	
		(Nedd4)	Lambert et al. (2007)
Vaccinia virus	F13L: YPPL	n.d.	Honeychurch et al. (2007)
Herpes simplex virus-1		(Vps4)	Crump et al. (2007)
			Calistri et al. (2007)
Epstein–Barr virus	Rta	Tsg101[e]	Chua et al. (2007)

n.d. — Not determined.

[a] L domains in parentheses represent secondary domains that contribute subtly to budding.

[b] Direct interactions as determined by yeast two-hybrid, coimmunoprecipitation, or colocalization studies. Proteins in parentheses indicate functional importance in budding as determined by dominant-negative or cellular depletion studies.

[c] References are listed in chronological order.

[d] Conflicting reports.

[e] EBV requires Tsg101 for late gene transcription only.

type-1 (HIV-1), disruption of the p6 region of Gag resulted in defective virus budding, characterized by vesicles that appeared to be fully formed but remained tethered to the plasma membrane (Göttlinger et al., 1991). Analysis of the p6 region revealed a PTAP amino acid motif, mutation of which was responsible for this defect in HIV-1 budding (Göttlinger et al., 1991; Huang et al., 1995). A similar analysis of the p2b region of Rous sarcoma virus Gag identified a PPPY motif (Wills et al., 1994). For the filovirus Ebola virus, overlapping motifs (PTAPPPEY) in the VP40 structural protein were each found to contribute to efficient budding of subviral particles (Harty et al., 2000; Licata et al., 2003; Martin-Serrano et al., 2001). These motifs along with other motifs characterized subsequently, including YPxL and FPIV, have been identified in a variety of viruses (Table 1) and termed late (L) domains. Disruption of these domains typically results in defects during the late stages of budding, particularly at the final step of vesicle fission (reviewed in Bieniasz, 2006; Morita and Sundquist, 2004; Schmitt and Lamb, 2004).

The feature that makes the existence of L domains in the proteins of a variety of enveloped viruses so compelling is that these motifs have now been demonstrated, by biochemical and structural studies, to interact with cellular proteins. Under normal circumstances, these cellular proteins are involved in pathways that promote the formation of vesicles that bud away from the cytoplasm, a process analogous to virus budding and distinct from inward budding processes such as endocytosis (Fig. 1). The most studied of these pathways is the vacuolar protein sorting (VPS) pathway, a branch of which gives rise to multivesicular bodies (MVB) (Fig. 1A). Another topologically equivalent cellular process includes abscission during cytokinesis, which utilizes an overlapping subset of cellular proteins (discussed below, Fig. 1C).

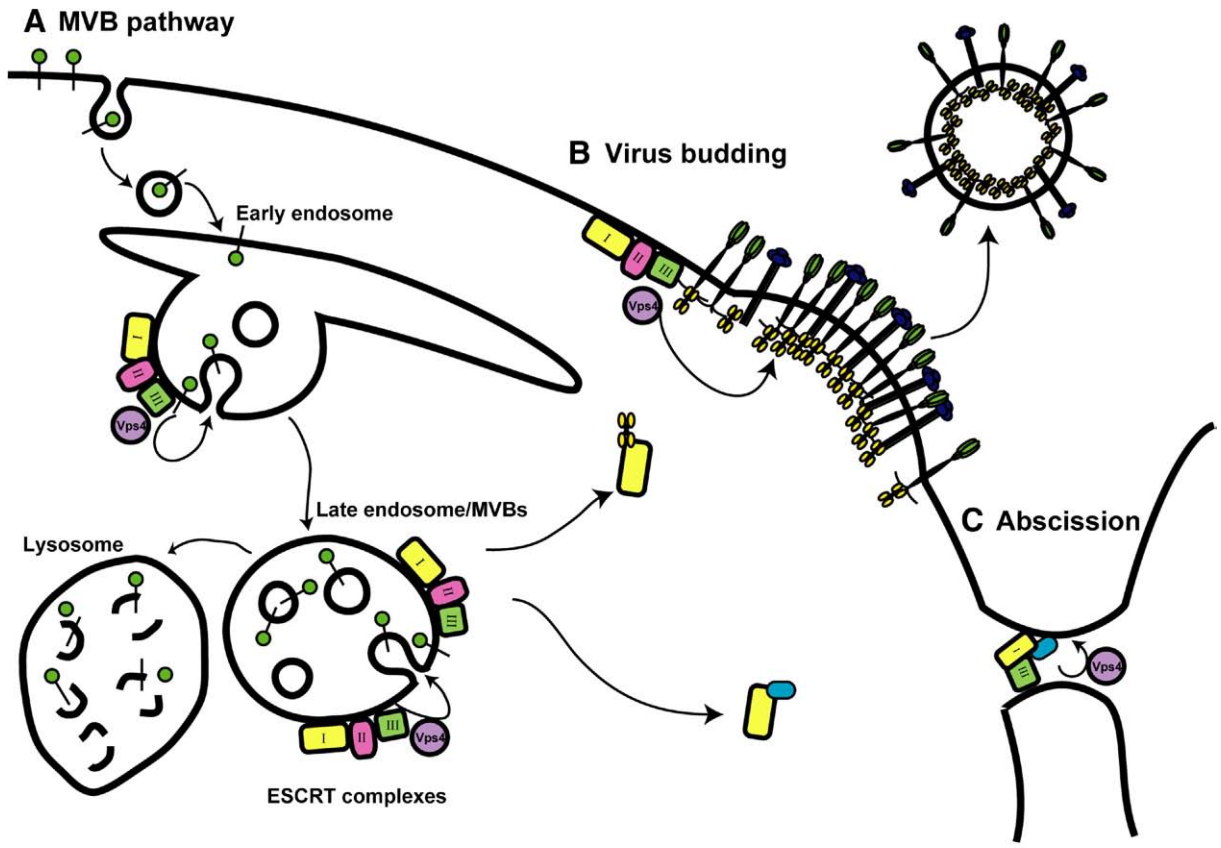

Fig. 1. Vesicularization pathways. (A) Cellular VPS pathway and MVB biogenesis. Receptors on the plasma membrane destined for degradation are internalized into vesicles by endocytosis which fuse with other vesicles to form endosomes en route to the lysosome. Ubiquitination of cargo proteins recruits the ESCRT complexes. To form MVBs, Vps4 AAA-ATPase activity is required to remove ESCRT-III proteins from the membrane and induce vesicularization. (B) Enveloped virus budding from the plasma membrane. For some enveloped viruses, viral proteins recruit components of the ESCRT complex through L domains, redirecting the MVB formation machinery from endosomes to sites of virus budding. (C) Abscission. To complete cytokinesis, abscission requires the recruitment of ESCRT proteins Tsg101 and AIP1/Alix by Cep55 to the midbody, and the activity of Vps4. The membrane topology of abscission, when the daughter cell pinches off, is equivalent to both MVB formation and virus budding.

MVB vesicles are formed on late endosomes and contain cargo destined for lysosomal degradation. MVB formation requires the activity of a network of cytoplasmic protein complexes, known as ESCRT (endosomal sorting complex required for transport) complexes I, II, and III, that are sequentially, or perhaps concentrically, recruited to the endosomal membrane to sequester cargo proteins and drive vesicularization into the endosome (reviewed in Hurley and Emr, 2006; Nickerson et al., 2007) (Fig. 2). Proteins comprising the ESCRT I, II, and III intracellular trafficking machinery are evolutionarily conserved from yeast to humans. The atomic structure of two key components of this pathway – Tsg101, a component of ESCRT-I, and AIP1/Alix, a protein that bridges the interaction between ESCRT-I and ESCRT-III – revealed the binding pockets for the L domain motifs P(T/S)AP and YPxL, respectively (Fisher et al., 2007; Lee et al., 2007; Pornillos et al., 2002). These interactions between viral proteins and ESCRT proteins are thought to redirect the ESCRT machinery to the site of virus budding where viral particle vesicularization is required, and they are thought to constitute a "pushing" force for virus budding (Figs. 1B and 2). A third L domain motif, PPxY, is able to bind the protein Nedd4 and similar HECT domain-containing E3 ubiquitin ligases, proteins which presumably feed into the VPS pathway upstream of the ESCRT complexes (Martin-Serrano et al., 2005) (Fig 2).

The final step of budding when vesicles pinch off from the membrane is perhaps the least well understood. A key protein, however, appears to be the AAA-ATPase Vps4, which is present in humans as two isoforms, Vps4A and Vps4B (Babst et al., 1998; Scheuring et al., 2001). Based on structural and functional studies, Vps4 is thought to remove catalytically the ESCRT-III complex from the endosomal membrane resulting in the contraction of the membrane surrounding the nascent vesicle, leading to membrane fission (Scott et al., 2005a,b). This activity is required for MVB formation because dominant-negative forms of Vps4, in which the ATPase catalytic site is ablated by mutation, induce a phenotype in which incomplete and aberrantly formed vesicles accumulate and fail to separate from the membrane (Fujita et al., 2003; Scheuring et al., 2001).

The list of viruses that have been shown to utilize the VPS pathway and ESCRT proteins during budding is now quite extensive (Table 1). Our understanding of the proteins that make up the complexes involved in MVB biogenesis is also expanding as new components are identified (reviewed in Hurley and Emr, 2006; Williams and Urbé, 2007). As different viruses are shown to require

components of this vesicularization pathway, the importance of this mechanism to virus budding becomes satisfyingly reinforced.

However, this mechanism does not appear to apply universally to, nor is it sufficient for, budding of all enveloped viruses. Notable exceptions have gradually accumulated in the literature that suggest that requirements for enveloped virus budding can be more complex than simply redirecting the ESCRT components. In some cases, recruitment of ESCRT factors may not be sufficient for budding. In other cases, budding appears to be entirely independent of known cellular pathways.

The VPS pathway is required but not sufficient for some viruses

Virus-like particle (VLP) systems and reverse genetics have become important tools with which to dissect the budding requirements for a number of enveloped viruses. Internal structural proteins of enveloped viruses are attractive candidates for organizing virus budding as they are often thought to form bridging interactions between the viral envelope proteins and genome-containing core. Whereas many L domain-containing structural proteins are able to drive budding and thus are believed to contain sufficient information to direct budding independently of other viral proteins, there are several enveloped viruses that, although they appear to encode L domains and require VPS components, they also require additional viral proteins for budding.

Among the retroviruses, foamy virus budding is different in that Env is essential for particle formation in addition to Gag (Fischer et al., 1998; Shaw et al., 2003). Whereas the Gag proteins of all other retroviruses tested thus far are able to form VLPs when expressed alone, prototype foamy virus (PFV) Gag is unable to form VLPs. Despite this observation, analysis of the PFV Gag protein revealed a dominant PSAP L domain similar to that of other retrovirus Gag proteins. Furthermore, budding of viral particles was sensitive to dominant-negative mutants of Tsg101, CHMP-3, and Vps4 (Patton et al., 2005; Stange et al., 2005).

Similar to PFV, the matrix (M) protein of the paramyxovirus parainfluenza virus 5 (PIV5) also contains an L domain (Schmitt et al., 2005), yet expression of the M protein alone in cells does not result in vesicle formation (Schmitt et al., 2002). In VLP studies, the M protein is only released efficiently into VLPs upon

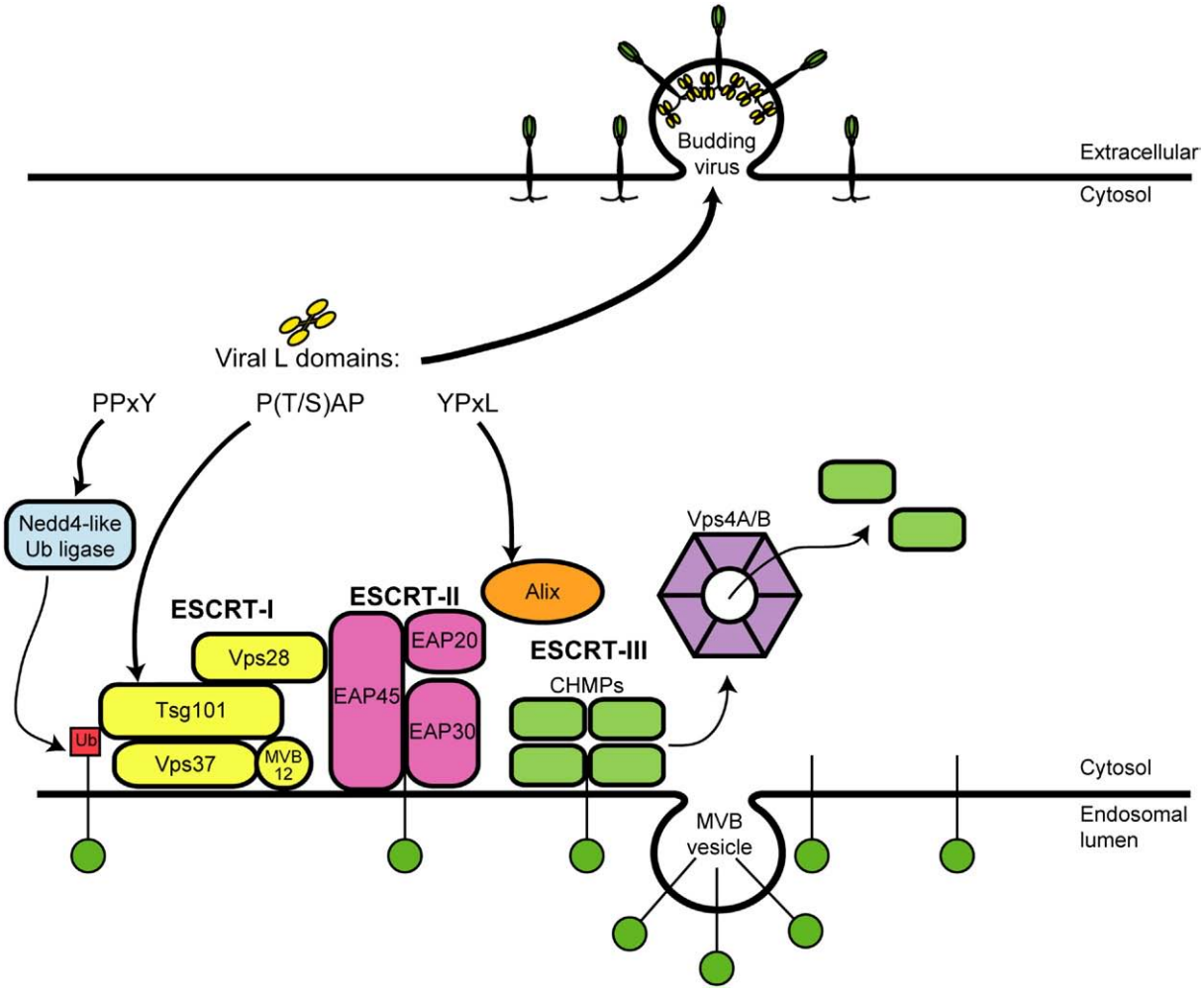

Fig. 2. ESCRT complexes and vesicle formation. ESCRT complexes I, II, and III are recruited to the endosomal membrane by ubiquitinated cargo proteins and inter-complex interactions. The AAA-ATPase Vps4A/B facilitates MVB vesicle formation by removing ESCRT-III subunits from the endosomal surface. Viral L domains found in viral proteins interact with components of the VPS pathway, redirecting the complexes to the site of virus budding on the plasma membrane.

coexpression of the nucleocapsid protein (NP) and the spike glycoproteins (F or HN). Furthermore, the cytoplasmic tails of F or HN were shown to be required for VLP budding as well as virus replication, suggesting a role for these proteins in directing proper assembly of the virion prior to budding (Schmitt et al., 2002). Indeed, truncation of the cytoplasmic tails of F or HN resulted in a diffuse redistribution of M within cells infected with recombinant mutant viruses (Schmitt et al., 1999; Waning et al., 2002).

In the case of both PFV and PIV5, there appears to be a requirement for proper assembly of viral components prior to budding. This function appears to be supplied by the integral membrane proteins of the viral envelope. For PFV, it has been shown that targeting of Gag, which lacks an N-terminal myristylation signal usually found on retroviral Gag proteins, to the membrane requires Env, and that this targeting is specific as budding is only observed in the presence of homologous Env proteins (Eastman and Linial, 2001; Pietschmann et al., 1999). For PIV5, the recruitment of M by F and HN may be crucial for proper assembly prior to budding.

Compared to the budding of other enveloped viruses, the failure of PFV and PIV5 structural proteins to bud independently may represent differences in assembly efficiency. Clearly, it is advantageous to bud only after proper assembly has occurred. Thus, a limiting step to virus budding may be the recruitment of internal viral proteins by the surface membrane proteins to sites of virus budding. Targeting of the internal components to the surface may then redirect the budding machinery bound to L domains, leading to particle formation.

At least two virus families have envelope proteins, rather than internal proteins, that encode L-domain-like motifs in their cytoplasmic domains. Budding of the orbivirus bluetongue virus requires the transmembrane proteins NS3 and NS3A for VLP budding (Hyatt et al., 1993). NS3/NS3A contains a dominant PSAP L domain motif in its N-terminal cytoplasmic domain that can complement budding-deficient Gag constructs, and budding of VLPs and virus was reduced when Tsg101 was depleted by using RNAi techniques (Wirblich et al., 2006). Within the vaccinia virus F13L envelope protein cytoplasmic tail, a YPPL L domain motif, conserved in many poxviruses, has been described (Honeychurch et al., 2007). A modest decrease in virus replication was observed when this motif was mutated and when AIP1/Alix was depleted from cells, suggesting the use of VPS proteins in the envelopment of this large DNA virus.

VPS pathway-independent virus budding

For some enveloped viruses, there is evidence that budding can occur independent of an active VPS pathway. This category of enveloped viruses is characterized by the ability to bud and replicate in the presence of dominant-negative forms of Vps4. As Vps4 is believed to function at the final steps of MVB biogenesis and is required for the final pinching off of MVBs, the finding that several viruses do not require its function suggest either the presence of alternative vesicularization pathways or complete independence of virus budding from cellular assistance.

The budding requirements of vesicular stomatitis virus (VSV) have been examined extensively and VSV was one of the first non-retrovirus examples of a virus containing a putative L domain motif (Craven et al., 1999; Harty et al., 1999). Although the M protein of VSV contains both PPPY and PSAP motifs, it has been shown that only the PPPY motif is required for efficient virus replication (Irie et al., 2004a; Jayakar et al., 2000). Thus, it was not entirely surprising that VSV VLP budding was not inhibited by Tsg101 depletion; however, the ability of VSV VLPs and infectious virions to bud in the presence of dominant-negative Vps4A was unexpected (Irie et al., 2004b). The VSV M protein has been shown to interact with the ubiquitin ligase Nedd4 in a PPPY-dependent manner suggesting an alternative pathway linking cellular machinery to VSV budding, possibly through ubiquitination (Harty et al., 2001, 1999). Other viruses with PPPY motifs that interact with Nedd4-like proteins, however, do require Vps4A activity (Table 1) highlighting the unusual nature of VSV budding.

Studies examining the requirements for Sendai virus budding have yielded conflicting data. Initially, it was reported that coexpression of both M and F proteins was required for Sendai VLP budding suggesting that both proteins were required to drive budding (Takimoto et al., 2001). However, additional studies found that the nonstructural C protein enhanced VLP release (Sugahara et al., 2004). One explanation for the enhanced budding contributed by the C protein is that the C protein can bind AIP1/Alix, despite lacking a YPxL motif (Sakaguchi et al., 2005). This finding was somewhat surprising in that it was the first example of a nonstructural protein potentially linking virus budding to the ESCRT pathway. Later studies, however, identified a YLDL motif in the Sendai virus M protein and it was shown that this motif could also interact with AIP1/Alix (Irie et al., 2007). The YLDL motif was shown to be essential for VLP budding independent of the C protein, consistent with the mechanism used by other enveloped viruses that utilize the ESCRT pathway. However, a recent study suggests that Sendai virus budding in the context of a biological infection, rather than a VLP context, is not sensitive to AIP1/Alix depletion or dominant-negative Vps4 (Gosselin-Grenet et al., 2007). Currently, it is difficult to reconcile the discrepancies between the conflicting studies, although it is apparent that there may be differences in ESCRT utilization between VLP systems and biological infections, perhaps due to overexpression from transfected plasmids. For Ebola virus, a mutant virus was recovered containing an altered VP40 L domain and it was found that only a small decrease in replication occurred (Neumann et al., 2005). This raises the question of whether cellular assistance simply enhances budding efficiency. Why the requirement for the VPS pathway differs among viruses during an actual virus infection as compared to the effects observed with VLP systems is a topic worthy of additional investigation.

For influenza virus, there have also been discrepancies among different studies of the requirements for budding. Similar to findings with other enveloped viruses, early influenza VLP studies suggested that the matrix protein (M1) served as the major driving force for virus budding (Gómez-Puertas et al., 2000). This conclusion was consistent with the notion that internal viral structural proteins are sufficient to drive virus

budding due to the recruitment of cellular factors, similar to the example of the retroviruses and filoviruses.

In contrast to previous reports, it was found recently that if a noncytotoxic VLP system that reflects a biological infection is used, M1 expressed on its own does not produce VLPs and is not required for VLP formation (Chen et al., 2007). The major requirement for VLP budding was found to be the hemagglutinin (HA) protein, in particular the cytoplasmic tail of HA. Numerous studies have examined the contribution of the HA and neuraminidase (NA) cytoplasmic domains to influenza virus assembly and budding (Jin et al., 1997; Zhang et al., 2000a,b). These studies suggest the that HA and NA tails are functionally redundant and that the secondary structure or orientation of the cytoplasmic domains could be important for proper assembly of the virus, as removal of an essential proline residue in the NA tail (Bilsel et al., 1993) or the palmitoylation sites in the HA tail (Chen et al., 2005a) affect the viability of recombinant viruses and affect M1 incorporation into the virion. Taken together, these studies suggest that the envelope glycoproteins HA and NA serve to direct assembly and are required for virus budding. Indeed, VLPs failed to bud in the absence of HA, but VLPs without M1 resembled authentic influenza virions (Chen et al., 2007) (Fig. 3). Furthermore, budding of influenza VLPs was not sensitive to dominant-negative Vps4 in contrast to budding of HIV-1 and PIV5, but similar to the findings with VSV budding (Chen et al., 2007) (Fig. 4).

Placed in the context of other enveloped viruses that have been examined to date, it appears that influenza virus budding is different in that it does not require the AAA-ATPase activity of Vps4 and it does not contain a known L domain motif. The importance of the cytoplasmic tails of the viral membrane proteins suggests a functional role for these proteins in the assembly of the virus, similar to findings with PIV5 and PFV assembly and budding. The organizing role played by the influenza virus membrane proteins is also reminiscent of alphavirus assembly where the interaction between the spike glycoprotein E2 and nucleocapsid is required for organization of an icosahedral capsid (Forsell et al., 2000; Lopez et al., 1994; Suomalainen et al., 1992).

Thus, if budding of some viruses does not require the AAA-ATPase activity of Vps4, are there alternative cellular pathways that lead to vesicle formation that are Vps4-independent? Do these pathways also use ESCRT complexes, or are there completely novel vesicularization pathways in the cell? For both VSV and Sendai virus, budding appears to be independent of the VPS pathway despite containing L-domain-like motifs. This raises the possibility that there are multiple entry and exit points within the VPS network and possibly cross talk with other cellular networks where Vps4 activity is not a required component.

Certainly, a trend has emerged where enveloped virus budding in the presence of dominant-negative Vps4 is treated as a gold standard test applied to numerous enveloped viruses to determine whether budding occurs through the VPS pathway. Budding of a number of enveloped viruses has now been shown

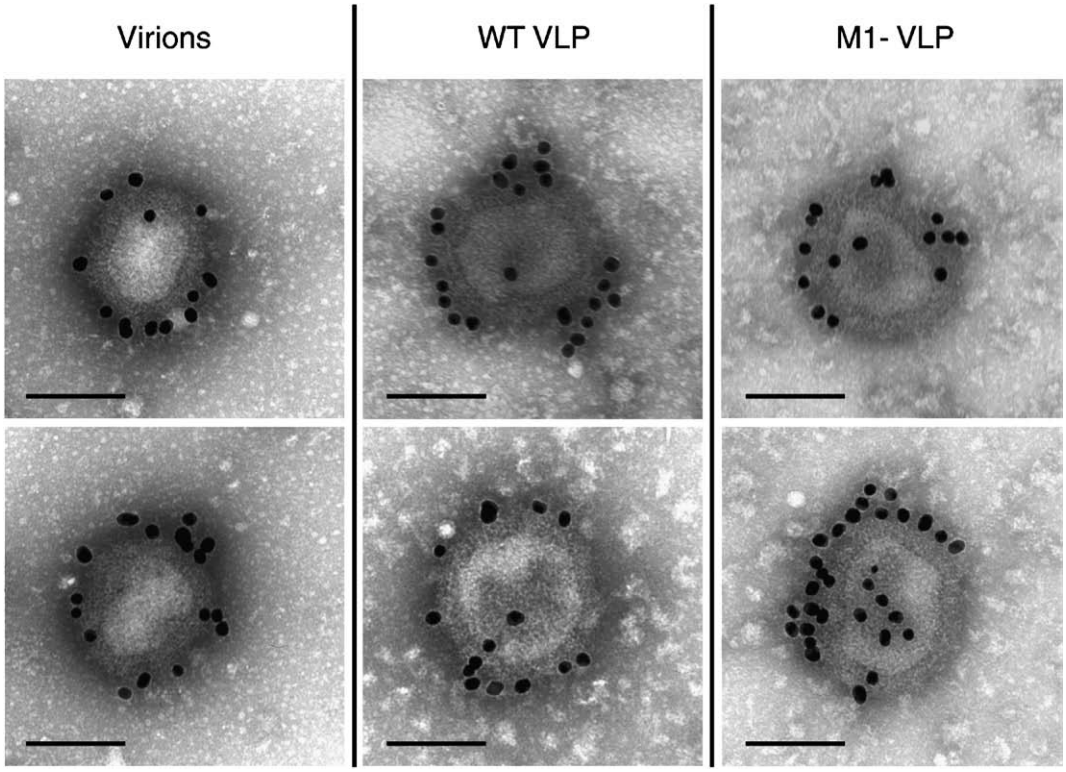

Fig. 3. Influenza VLP morphology as shown by EM. Influenza virions, VLPs containing all VLP proteins (WT VLP), and VLPs lacking M1 protein (M1- VLP) were prepared by infecting or transfecting 293T cells. Purified virions and VLPs were immunostained with a monoclonal anti-HA antibody followed by IgG conjugated to 15-nm gold and then negative stained. Scale bar, 100 nm. From Chen et al. (2007) with permission from the American Society for Microbiology.

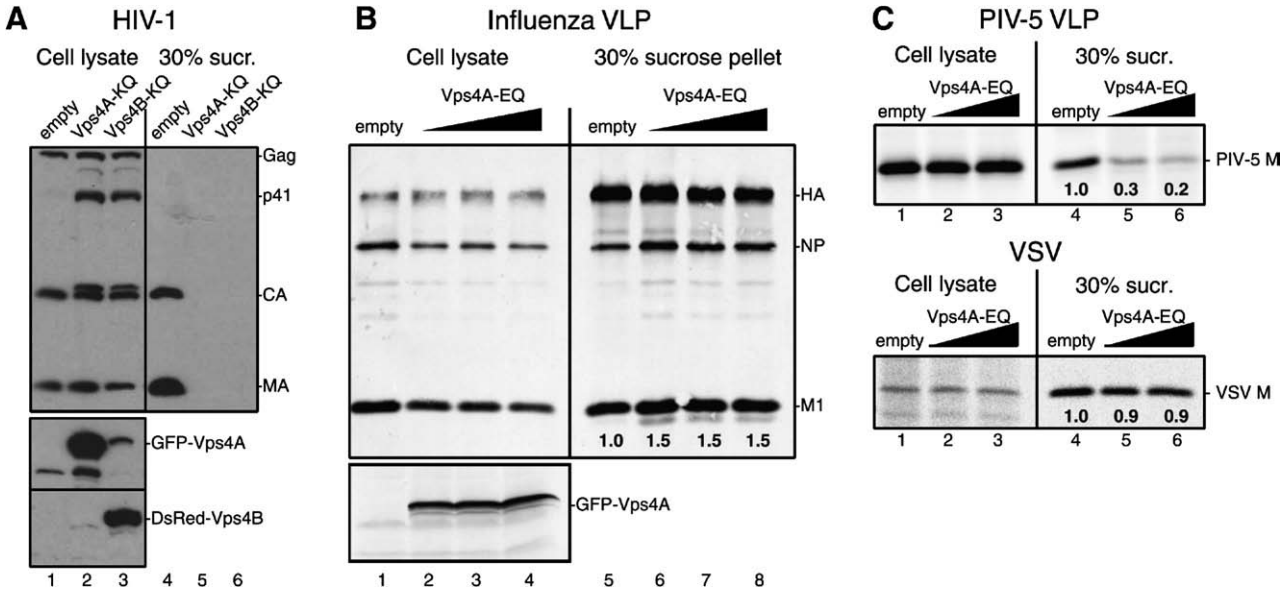

Fig. 4. Budding of HIV-1, influenza VLPs, PIV5 VLPs, and VSV in the presence of dominant-negative Vps4. Budding of HIV-1 in the presence of dominant-negative Vps4A-KQ or Vps4B-KQ (A), or budding of influenza VLPs (B), PIV5 VLPs (C), or VSV (D) in the presence of increasing amounts of dominant-negative Vps4A-EQ was tested. HIV-1 and PIV5 VLP budding was dramatically reduced whereas VSV and influenza VLP budding was not inhibited. Adapted from Chen et al. (2007) with permission from the American Society for Microbiology.

to be sensitive to dominant-negative Vps4, including viruses such as hepatitis B virus (Kian Chua et al., 2006; Lambert et al., 2007; Watanabe et al., 2007) and herpes simplex virus-1 (Calistri et al., 2007; Crump et al., 2007) (Table 1). As more viruses are categorized in this manner, it will be interesting to determine whether these viruses contain conventional or novel L domains and whether they interact with known ESCRT components. Greater advances will be made, however, by identifying viruses that are insensitive to dominant-negative Vps4, and by uncovering pathways that diverge from the currently understood cellular pathways by identifying novel viral–cellular protein interactions.

Besides the well-studied MVB pathway, one mechanism related topologically to MVB formation and virus budding is the final step of cytokinesis, known as abscission, during which two daughter cells separate (Fig. 1C). A recent study found that both Tsg101 and AIP1/Alix are recruited to sites of abscission by Cep55, a centrosome protein required for abscission (Carlton and Martin-Serrano, 2007). Depletion of either Tsg101 or AIP1/Alix increased the number of cells arrested during cell division, suggesting the ESCRT complexes are involved in this related membrane fission event (Carlton and Martin-Serrano, 2007). Cytokinesis was also sensitive to dominant-negative Vps4 and other components of the VPS pathway. Although the processes of MVB formation and abscission are related through common cellular machinery, there are also unique elements. For example syntaxin-2 and vamp-8 were both required for cell division but dominant-negative forms of each did not inhibit HIV-1 budding (Carlton and Martin-Serrano, 2007). Also, although Cep55 overexpression inhibited both cytokinesis and HIV-1 budding, Cep55 itself was not required for virus budding as a Tsg101 mutant that could not bind to Cep55 was still able to rescue HIV-1 budding (Carlton and Martin-Serrano, 2007). This raises the intriguing possibility that the cell is able to relocate the ESCRT machinery through the use of adapter proteins to various locations within the cell to participate in different budding events. It will be interesting to investigate whether viruses are able to recruit similar adapter proteins as a means of commandeering the VPS pathway for their own use.

Intrinsic budding potential

Although hijacking the cellular machinery used in MVB biogenesis or cytokinesis for virus budding is an attractive and satisfying mechanism for virus budding, there is evidence that suggests that virus budding can also proceed spontaneously without the help of host factors.

Budding of viruses such as alphaviruses, PIV5, and influenza virus appears to be driven by integral membrane proteins as discussed above. Although host proteins may still be involved with budding of these viruses, the intrinsic association of viral proteins into microdomains on the plasma membrane may be sufficient to drive virus budding by inducing membrane curvature and supplying a “pulling” force for virus formation. The lipid raft association of many enveloped viruses, such as HIV-1 (Lindwasser and Resh, 2001; Ono and Freed, 2001), Ebola virus (Bavari et al., 2002), VSV (Brown and Lyles, 2003), and influenza virus (Scheiffele et al., 1999), has suggested that these microdomains serve as virus “budozones” where viral proteins are concentrated to facilitate efficient virus budding (reviewed in Schmitt and Lamb, 2004, 2005). For influenza virus, lipid raft association is an intrinsic property of HA (Leser and Lamb, 2005), and HA raft association is required for efficient virus replication (Takeda et al., 2003). Combined with evidence that the HA and NA cytoplasmic tails (Ali et al., 2000; Enami and Enami, 1996; Zhang et al., 2000a,b), along

with the M2 cytoplasmic tail (McCown and Pekosz, 2005), recruit M1 and contribute to packaging of the viral RNA-containing ribonucleoprotein complex, lipid microdomain association may play an additional role in budding by promoting the clustering of a critical mass of viral proteins to nucleate membrane vesicularization independent of host budding machinery.

Recent computer simulations have shown quite dramatically that spontaneous membrane vesicularization can occur in environments analogous to that of viral budozones (Reynwar et al., 2007). In these simulations, when small proteins were placed in a simulated lipid environment, similar in scale to lipid microdomains (160×160 nm), vesicular structures formed spontaneously despite a lack of inter-protein interactions. Rather, the vesicularization was induced by local hydrophilic attraction between the proteins and their immediate lipid environment. These local attractions led to aggregation and local membrane curvature which summed over the membrane to induce vesicle formation. Additionally, when virus capsid-sized particles were placed in the lipid environment, these also induced spontaneous vesicle formation (Reynwar et al., 2007) (Fig. 5).

Thus, in the case of influenza virus, it is possible that the accumulation of viral proteins into lipid raft microdomains is sufficient to induce vesicle formation due to attractive forces between the viral proteins and the plasma membrane. This mechanism may serve as a default process for other lipid microdomain-associated viruses as well. In vitro analysis of VSV M protein has demonstrated that the M protein alone, reconstituted in artificial liposomes, can induce membrane curvature reminiscent of virus budding (Solon et al., 2005). However, complete, budded vesicles were not observed in this system (Solon et al., 2005) nor in computer simulations (Reynwar et al., 2007), suggesting that host factors may still be required for the final pinching off step.

Perspectives

The biophysical problem posed by virus budding may therefore not be as difficult as it seems. What is intriguing is how different viruses accomplish this task in seemingly disparate ways. How is assembly that is organized by the integral membrane proteins on the cell surface translated into efficient virus budding? Is there a balance between microdomain association (pulling forces) and host protein recruitment (pushing forces)? Do differing requirements for budding reflect fundamentally different mechanisms for virus budding; or, do they illustrate spatial and temporal restrictions or targeting strategies unique to different viruses that ultimately feed into a similar vesicularization pathway? Does the structure of a given virus increase or decrease the amount of assistance required to form a viral particle? Dissecting the relative contributions of lateral membrane protein interactions, interactions between internal and membrane proteins, and "pushing" forces from recruited internal proteins to virus budding will increase our understanding of how different viruses escape from the cell. Furthermore, determining the consequences of interactions between viral proteins and cellular budding machinery on physiologic processes may reveal an additional mechanism by which viruses disable the host cell.

As the budding requirements of more enveloped viruses are cataloged, the network of cellular proteins involved in this process will likely expand and perhaps lead to the identification of novel cellular pathways. The membrane fission step remains

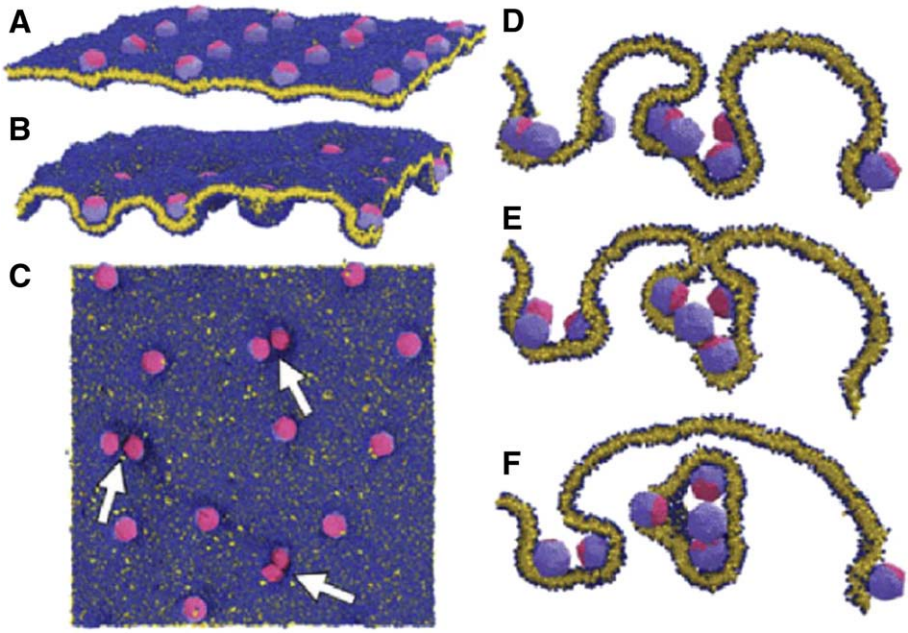

Fig. 5. Computer simulation of spontaneous membrane vesicularization. In coarse-grained computer simulations modeling membrane vesicularization, capsids placed in the simulated lipid environment drove vesicle budding through attractive and cooperative forces. Panels A–F show a series of simulation snapshots over a time course of approximately 0.3 ms. Adapted from Reynwar et al. (2007) with permission from the Nature Publishing Group.

the least well understood, both for virus budding and cellular budding. The identity, recruitment, and mechanism of action of host factors involved in fission should be a priority, much as the in-depth examination of viral fusion proteins and cellular SNARE proteins helped define membrane fusion. Technological advancements in mass spectrometry and large-scale proteome mapping will be crucial to finding new and pertinent interactions between viral and cellular proteins. Visualization of virus budding by using real time high-resolution single-particle light microscopy and by using cryoelectron tomography and correlative electron microscopy techniques may shed light on this process as well.

Gaining a better understanding of virus budding and appreciating the diversity of strategies employed by different viruses will not only lead to a better understanding of fundamental cellular processes but also has the potential to lead to alternative therapeutic approaches to treating enveloped virus infections.

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