

Minireview

Bending out and breaking away: host-cell accomplices in retroviral escape

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Abstract

Budding through the host-cell membrane is a key step in the life cycle of many viruses. Recent studies of retrovirus replication implicate a large number of cellular proteins in this process.

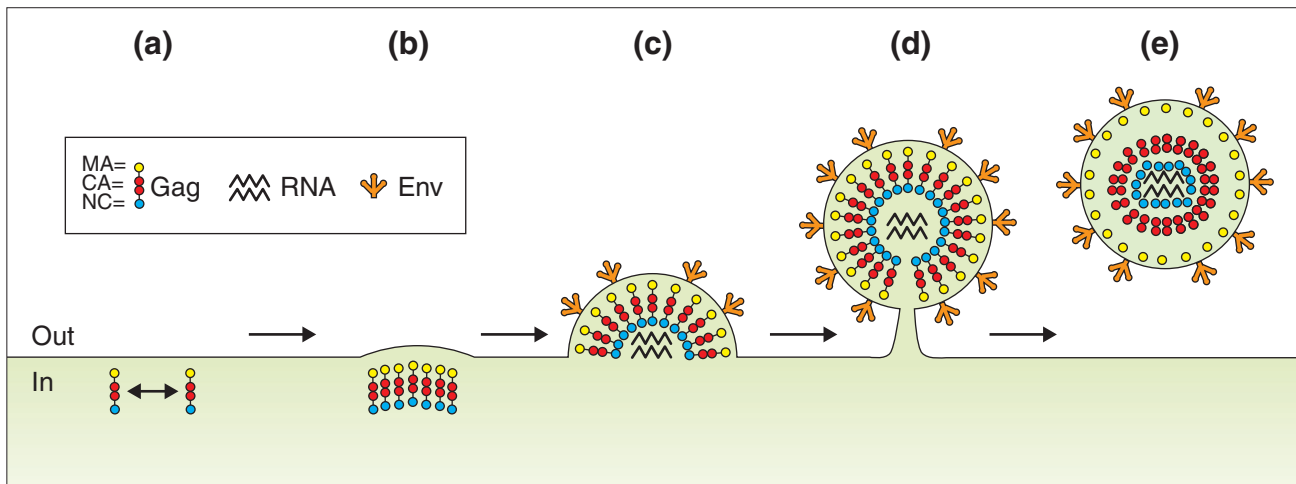
How do enveloped viruses bud from their host cells? To understand how this process is achieved, several fundamental steps must be considered. First, viral structural components must be transported to the appropriate site, typically just under a cell membrane, and there assembled (Figure 1a) [1]. Second, the plasma membrane must be distorted to make a succession of curved budding structures (Figure 1b,c); this requires overcoming the mechanical bending resistance of the plasma membrane [2]. Third, following the formation of the bud, the virus has to pinch off and escape from the cell (Figure 1d,e) [3]. This involves machinery that constricts the neck of the bud, resulting in fusion between the membranes on either side of the neck and the release of the virus from the plasma membrane. Studies with a number of virus types, most prominently retroviruses, have now revealed that cellular proteins that are intimately involved in intracellular membrane trafficking and receptor re-localization play key roles in facilitating these processes.

For a long time, it has been known that the only retroviral component required for assembly and budding is the Gag polyprotein, which ultimately forms the viral core [1]. Gag is cleaved into a variety of smaller components as the virus matures. These include, from amino terminus to carboxyl

terminus, the matrix (MA), capsid (CA) and nucleocapsid (NC). Depending on the virus analyzed, a variety of other protein products are seen after cleavage of Gag. For example, in human immunodeficiency virus-1 (HIV-1) a short peptide called p6 is cleaved from the carboxy-terminal end of NC, whereas in murine leukemia virus (MuLV) a p12 peptide is cleaved from between MA and CA.

Three types of functional domain of Gag can be identified: M, sequences required for transport to and binding of membranes; I, involved in Gag-Gag interactions; and L, late sequences [1,3]. The L domains are short peptide motifs located in different regions of Gag in different viruses; mutation in these sequences results in failure to release budded viruses [4,5]. Many L domains are interchangeable between viruses, suggesting that their role in the late stages of budding is to act as docking sites for cellular proteins [5-7]. A key step in understanding the late budding process came with the demonstration that the L domain of HIV-1 Gag interacted with a component of the cellular machinery responsible for sorting cargo into multivesicular bodies (MVBs) [8-10].

MVBs are formed from early endosomes when their membranes invaginate into the endosomal lumen, resulting in

**Figure 1**

A schematic representation of retrovirus budding. **(a)** Gag proteins move to the plasma membrane and begin to associate with one another. **(b)** Formation of electron-dense aggregates under a deforming plasma membrane follows. **(c)** Bud curvature steadily increases. **(d)** Membrane fusion leads to pinching-off of the virion; **(e)** proteolytic processing of Gag leads to virion maturation and formation of an electron dense core. L-domain mutants of most retroviruses arrest at a stage equivalent to (d) but with an extended stalk [3,4]; in other viruses, such as human T-lymphotropic virus I (HTLV-I), arrest occurs at a stage roughly equivalent to (b) [38]. MA, matrix; CA, capsid; NC, nucleocapsid; Env, envelope proteins.

the release of vesicles into the luminal space [11,12]. Mono-ubiquitination acts as a signal for directing proteins into MVBs, although it might not be the only signal, given that membrane proteins that are not ubiquitinated can also be transported to the MVBs. The formation of MVBs requires three protein complexes, which were first characterized in yeast and are collectively known as the endosomal sorting complexes required for transport (ESCRTs) [13-15]. ESCRTI and ESCRTIII each contain one subunit that binds ubiquitin. ESCRTIII is believed to function downstream of ESCRTI, as overexpression of the former can compensate for the loss of the latter, but the opposite is not the case. ESCRTIII functions to recruit ESCRTIII to the membrane. Recent studies have confirmed the interaction between proteins of ESCRTs I and II and between those of ESCRTs II and III [16,17]. The full ESCRT complex is dissociated by the AAA (ATPase associated with diverse cellular activities) protein, Vps4 [18,19].

HIV-1 interacts with the Tsg101 component of ESCRTI via a late domain within the p6 domain of Gag that contains the sequence P(S/T)AP (in the single-letter amino-acid code). Depletion of Tsg101 results in production of a late-domain phenotype, similar to the stage shown in Figure 1d [8]. Artificially recruiting Tsg101 into another late-domain mutant rescues budding activity [9]. These findings suggest that the ESCRT complexes might facilitate scission of the nascent virion from the cell. Very recent studies have shown that release of HIV-1 can be blocked at a late stage by mutation or deletion of at least eight cellular proteins that are

involved in the biogenesis of MVBs [17]. Other retroviruses containing different L domains, such as MuLV (characterized by a PPXY motif, where X is any amino acid) and equine infectious anemia virus (EIAV, characterized by a YPXL motif), do not interact directly with Tsg101 [3]. Budding of these viruses is arrested by dominant-negative mutants of various components of the MVB pathway, again implicating at least some portions of the endosomal sorting machinery in virus release [20-22]. In addition, some retroviruses appear to contain two L domains that can contribute to virus release [23,24]. L domains are also found in the matrix proteins of rhabdoviruses [25], filoviruses [9] and orthomyxoviruses [26], suggesting that involvement of the MVB pathway may be a common theme in virus budding.

Are these proteins the only cellular factors to play a role in virus budding? The article by Wang and colleagues in this issue of *Journal of Biology* [27] suggests otherwise. It brings several other participants in the field of cell-membrane movement into play, with intriguing possibilities. Wang *et al.* describe the interaction between the Gag protein of the Moloney MuLV and components of the cellular endocytic machinery, the endophilins. The interaction was initially detected in a yeast two-hybrid protein-protein interaction screen using as its 'bait' the Gag protein from the MuLV-related murine acquired immunodeficiency syndrome (MAIDS) virus. Subsequently, endophilin 2 was found to interact with the Gag proteins of MuLV and Rous sarcoma virus (RSV) but not of HIV-1, Mason Pfizer monkey virus

(MPMV) or simian immunodeficiency virus (SIV). MuLV Gag could also interact with rat endophilin 1, another member of the endophilin family [27].

The interaction between endophilin 2 and MuLV Gag was confirmed using a fusion protein made up of glutathione-S-transferase (GST) and endophilin 2, attaching this to beads and using them to pull down Gag from MuLV-infected cells. Significantly, 0.7% of the endophilin 2 present in MuLV-producing cells became incorporated into the virions. Interestingly, α -adaptin and clathrin, two other components of the clathrin-mediated endocytic machinery [28], were also found to be incorporated into MuLV virions. The region required for binding to endophilin 2 was mapped to the MA domain of the Gag protein. An intact endophilin 2 protein was required for Gag interaction, as determined in the yeast two-hybrid system, but various fragments of endophilin 2 could be incorporated into MuLV virions even though they did not interact with Gag in this assay.

Overexpression of full-length endophilin 2 in MuLV-producing cells resulted in a dose-dependent reduction in virion production. Fragments of endophilin 2 were also inhibitory, but to a somewhat lesser degree. In contrast, an excess of endophilin 2 did not affect production of HIV-1 from cells, ruling out the idea that the effects on MuLV are due to direct toxicity. This suggested that the specific binding of endophilin 2 to MuLV Gag plays an important role in MuLV production. It will be of considerable interest to determine where virus production is arrested in overexpressing cells. Inhibiting endophilin 2 levels by 80% using a small interfering (si) RNA, however, did not seem to affect viral production. This was attributed to the potentially low levels of endophilin 2 required for virion production, or the presence of other members of the endophilin family that could make up for the reduction in endophilin 2. Although perfectly plausible, these explanations do not completely dispel the uncertainty introduced by the negative siRNA experiments. Hence, the conclusion that endophilins are absolutely required for MuLV budding remains to be confirmed by further experimentation.

Relatively little is known directly about the function of endophilin 2, but endophilin 1 is a 40 kDa cytoplasmic protein containing an amphipathic domain at the amino terminus as well as a Src homology 3 (SH3) domain near the carboxyl terminus [29]. It is a multifunctional protein that is believed to participate in both early and late stages of endocytosis [28], has lipid transferase activity [30] and is considered capable of affecting membrane curvature [31] as well as binding and deforming liposomes into tubules [32]. It can bind to proline-rich domains in multiple cellular proteins, including dynamin and synaptojanin [33]. The closely

related endophilins 2 and 3, though less well characterized, seem likely to possess similar properties [29].

Given the membrane-bending properties of endophilins, a role for this family of proteins in virus budding seems, at least superficially, an attractive hypothesis. But compared to endocytosis, MVB formation and virus budding are topologically different processes, with endocytosis involving invagination into the cytoplasm whereas MVB formation and virus budding involve evagination, away from the cytoplasm. It seems likely that much of the protein machinery mediating these processes is fundamentally different (for example, involving components of clathrin-coated pits versus the ESCRT complex). It seems quite feasible, however, that some proteins might be involved in both processes, particularly those with the ability to bend and fuse membranes. Certainly there is evidence for some cross-talk, as shown by the interaction between endophilins and ALIX, a key player in formation of ESCRT complexes and virus release [17,34,35].

Although significant steps have been taken towards understanding virus budding during the past couple of years, there are still a number of important issues that remain to be addressed. How is the initial bud formed? It may be that energetic requirements for membrane distortion can be met simply by the I-domain-mediated assembly of Gag molecules, resulting in movement of associated membrane lipid molecules [36]. But what happens in the case of viruses like MPMV that assemble in the cytoplasm? Is there a need for cellular enzymes such as endophilin to introduce negative curvature (bending towards the outside of the cell) by modifying the lipid composition of the membrane? How does membrane pinching-off take place? The ESCRT complex is intimately involved, but is the whole complex required and what is the role of other factors such as the ubiquitin ligase, Nedd4, that are clearly involved in the budding of certain viruses [23,24]? How is the plasma membrane targeted for budding? In macrophages HIV-1 can bud into vacuoles [37], but what targets Gag and associated ESCRT complexes to the cell surface in HIV-infected T cells? Given the pace of progress in this area, driven in part by the urgency of developing novel antiretroviral drugs, we can be optimistic that these and related questions will soon be answered, bringing closer a detailed understanding of the mechanisms of virus budding and membrane remodeling.

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