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How membrane physics rules the HIV envelope

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Abstract

HIV particles incorporate host membrane proteins into their envelope to evade the immune system and infect other cells. A study now shows that Gag assembly on the host cell membrane produces a raft-like nanodomain favourable for protein partitioning due to a transbilayer coupling mechanism assisted by long-saturated chain lipids and cholesterol.

Text

Enveloped viruses such as HIV, Ebola, and Influenza use the plasma membrane of infected cells to wrap virions, forming an envelope through a membrane budding process during viral egress¹. Viruses have been proposed to selectively hijack host proteins, and their envelopes have been reported to be enriched in specific lipids (cholesterol and sphingolipids) found in lipid domains called rafts¹. In the case of HIV-1, adhesion, MHC (Major Histocompatibility Complex) and complement proteins are embedded in the virus envelope and contribute to its transmission and infectivity¹. Several mechanisms have been proposed to explain how the viral envelope composition can differ from the average host membrane composition: either passive, in which the virus assembles and buds at the site of pre-existing lipid rafts, or active, wherein viral components assemble their own raft domain *de novo*. Although such host factors may be potential drug targets against HIV, the partitioning mechanism has not yet been deciphered. In this issue of *Nature Cell Biology*, Sengupta et al. show that clustering of the HIV matrix protein Gag actively reorganizes existing membrane composition into an ordered lipid domain, to which specific proteins are recruited via their affinity for this lipid environment².

Sengupta et al.² investigated HIV envelope assembly using high-resolution TIRF-microscopy and electron microscopy. Using fluorescent lipid analogues, they showed that sphingolipids and cholesterol were enriched *during* the virus wrapping, *not prior* to this step, arguing against the passive hypothesis of budding from a pre-existing lipid raft. These observations are in agreement with previous lipidomic data on viral envelopes³. Sengupta et al. probed membrane proteins that do

not interact with Gag, but have various affinities for liquid ordered (Lo) (raft-like) or liquid disordered (Ld) domains. These affinities were directly measured in giant plasma membrane derived vesicles (GPMVs) extracted from cells, which provided quantitative estimates of raft affinity in intact, isolated plasma membranes. These experiments established a correlation between the preference of a given protein for Lo domains and their recruitment into nascent viral particles in living mammalian cells. Further, the authors evaluated enrichment for marker proteins, showing that proteins with a stronger affinity for a Lo environment were more efficiently enriched in the viral particles, whereas those with affinity for Ld domains were depleted. This result was confirmed by testing a protein called tetherin, which exhibits dual affinity for both types of membrane environments, and was recruited only at the late stage of viral budding, when local membrane curvature was high². Finally, the authors showed that protein redistribution at the virus assembly site was independent of the ESCRT-III machinery required for the final detachment of HIV².

Sengupta et al. thus directly demonstrated that the lipid composition and host protein partitioning into HIV envelope have a physical origin resulting from the following sequence: Gag assembly on the plasma membrane drives a local change in lipid organization, promoting the formation of raft-like domain, which then recruits proteins with affinity for these domains. This partitioning is further amplified by the increasing curvature of the membrane around the assembling virus particle. However, the authors showed that Gag polymerizes on the cytosolic side of the plasma membrane, whereas sphingolipids are mostly present on the membrane other leaflet. How can Gag influence local sphingolipid concentration across the bilayer? Sengupta et al. propose that Gag actively induces ordered domain formation through transbilayer coupling (Fig.1). Gag is a myristoylated protein that binds to negatively charged PIP2 lipids². The myristoyl chain of Gag is exposed upon PIP2 binding, which promotes further Gag anchoring to the plasma membrane⁴. The authors found that assembly of Gag induced clustering of long saturated chains in the cytosolic leaflet (PIP2), which in turn stabilized cholesterol-dependent transbilayer interactions, forming local domains with characteristics of a Lo phase in the external leaflet².

A similar model has been proposed by M. Rao and S. Mayor for the clustering of GPI anchored proteins (which have strong affinity for Lo environment), on the external plasma membrane leaflet⁵. In that model, the clustering of long acyl-chain-containing phosphatidylserine (PS) on the cytosolic membrane leaflet is mediated by dynamic cortical actomyosin asters that actively rearrange their interacting membrane components. The long saturated chains of PS couple across the bilayer in the presence of cholesterol and create a Lo nanodomain in the opposite leaflet, which further recruits proteins with affinity for this environment (i.e. GPI-anchored proteins). Sengupta et al. considered only the role of PIP2 lipids in the transbilayer coupling process in HIV. However, the lipidome of the viral envelope has also revealed a strong enrichment in PS³. Thus, it is also possible that Gag non-specifically recruits PS lipids when bound to the membrane, which in turn reinforces the transbilayer coupling due to PIP2. Other studies have questioned local sphingolipid enrichment during Gag assembly, while confirming the PIP2/cholesterol nanodomain formation⁶. Moreover, the level of sphingolipid enrichment might also depend on cell type^{2, 3, 6}. Protein lipidation such as myristoylation or palmitoylation, along with other protein structural features, have been proposed to be determinants for protein partitioning into Lo domains⁷. Another possibility is that Gag myristoyl chain also contributes to the clustering of lipids with long saturated fatty acids, such as PS or PIP2. Sengupta et al. demonstrated using a palmitoylation-deficient protein that protein lipidation is required for the partitioning of some proteins into the Lo nanodomain of the viral particle².

In addition to GPI-anchored protein nanoclustering via cortical actin and protein partitioning into nanodomains formed by Gag, the transbilayer coupling mechanism could be more generally at work in cells (Fig.2). Such a perspective is particularly attractive given that a number of cellular processes involve precise lipid and protein segregation, such as sorting into transporting vesicles during

membrane traffic, in microvesicles, exosome release, or in endocytic buds^{8, 9}. A similar principle could take place for sphingolipid sorting at the trans-Golgi Network (TGN). Based on physical principles, sphingolipids should be excluded from highly curved membranes, such as transport vesicles, given their highly ordered structure (mainly long, saturated fatty acid chains), due to the increased bending energy of membranes comprised of such stiff lipids compared to soft unsaturated lipids¹⁰. Nevertheless, sphingolipids are transported from the Golgi to the plasma membrane where they are highly enriched¹¹. This observation suggests the potential for cellular machinery that, like Gag, could create a local Lo-like nanodomain across the TGN membrane. Likewise, some endocytic pathways, such as clathrin-independent endocytosis, require clustering of glycosphingolipids and cargo proteins by galectins in the outer leaflet, which induces domain formation, membrane bending and subsequent endocytosis¹². Many signalling events also seem to be dependent on the crosstalk between sphingolipid/cholesterol domains in the outer leaflet and actin assemblies on the cytoplasmic side¹³.

The physical principle proposed by Sengupta et al.² based on the Lo partitioning for protein incorporation into the budding HIV envelope is appealing, but should be moderated. Some proteins known for their affinity for the Lo phase are indeed incorporated into the HIV envelope (GPI-anchored proteins, CD55, CD59, MHC, integrin $\alpha 4\beta 7$), but some are excluded from it (such as CD45 or CD14)¹. Interestingly, CD4 and CD48 are well-established raft resident proteins, present at high levels at the plasma membrane of T-cells and macrophages, but only CD48 is incorporated into the viral envelope whereas CD4 is excluded¹⁴. It is reasonable to hypothesize that HIV possesses additional mechanisms to control the incorporation of certain host proteins in a more refined way. One possibility could be related to membrane curvature-driven protein sorting. Sengupta et al. showed that tetherin associates with the viral particle only at the end of virus assembly when budding is almost complete², and was not enriched in the presence of a Gag mutant that produces flat particles. The authors suggest that the tetherin GPI anchor is insufficient to drive its partition into the forming Lo domain and that budding membrane curvature provides an additional driving force, which can facilitate protein incorporation². Curvature-driven protein sorting depends on membrane protein shape¹⁵, facilitating the inclusion of proteins with a spontaneous curvature similar to that of the viral bud, or conversely preventing the incorporation of proteins with an opposite curvature. Protein sorting can also be driven by steric interaction between disordered regions of the proteins¹⁵. Thus, membrane protein shape and other structural differences could contribute to the fine-tuning of partitioning into viral envelopes.

Further studies will be required to understand where coupling operates in a cell's transbilayer for local reorganization of membrane composition. Rapidly developing lipidomics should help to decipher the key structural determinants for this process. In addition, the role of lipid anchors and the cytoskeleton still needs to be explored. These avenues will enrich physical models and simulations to provide a richer and more complex picture of the structure and function of cell membranes at the nanoscale level.

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Figure Legends

Fig.1. Schematic representation of transbilayer coupling during HIV budding.

Physical origins of host protein partitioning into HIV envelope. A) Gag binds to PIP2 at the inner leaflet of the PM and gets anchored by its myristoyl chain. B) Myristoylated Gag oligomerization at the assembly site triggers PIP2 clustering which is *possibly* followed by the clustering of cholesterol and PS with long saturated fatty acid chains in the inner leaflet of PM. PIP2, PS chains and Gag myristoyl anchor actively promote formation of a raft-like domain in the PM outer leaflet through the transbilayer coupling to SM that also contains long saturated fatty acid chains, and with GPI-anchored proteins. C) Further Gag oligomerization induces membrane bending, which provides additional driving force for certain proteins with affinity for curved compartments (e.g. transmembrane proteins of conical shape).

PIP2 - phosphatidylinositol bisphosphate, PS – phosphatidylserine, SM – sphingomyelin, TM – trans membrane protein

Fig.2. Scheme of cellular processes that might require transbilayer coupling (marked in brown).

Different cellular processes imply precise lipid/protein sorting when a bud is formed, such as during CLIC endocytosis, on early endosomes when forming recycling vesicles or retromer-coated tubules for retrograde transport, or during MVB/exosomes formation. The selectivity for specific lipids and proteins is tightly controlled at the trans-zone of the Golgi apparatus where cargos are precisely sorted to be delivered to different cellular compartments. It is well established that the protein/lipid content of enveloped viruses is enriched in specific molecules but recently it was also shown that the composition of microvesicles is also different from the bulk plasma membrane. Moreover, there are many different domains at the plasma membrane, such as lipid rafts or caveolae, which are composed of very distinct lipid/protein environments, which is further reflected in their functions. Taken together many processes in the cell require precise lipid/protein sorting and communication between both membrane leaflets, which might originate in transbilayer coupling.

MVB – multi vesicular body, GPI – glycosylphosphatidylinositol, CLIC – clathrin-independent carriers



