



Review

Lipids and membrane microdomains in HIV-1 replication

Abdul A. Waheed*, Eric O. Freed**

Virus–Cell Interaction Section, HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702, United States

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ABSTRACT

Several critical steps in the replication cycle of human immunodeficiency virus type 1 (HIV-1) – entry, assembly and budding – are complex processes that take place at the plasma membrane of the host cell. A growing body of data indicates that these early and late steps in HIV-1 replication take place in specialized plasma membrane microdomains, and that many of the viral and cellular components required for entry, assembly, and budding are concentrated in these microdomains. In particular, a number of studies have shown that cholesterol- and sphingolipid-enriched microdomains known as lipid rafts play important roles in multiple steps in the virus replication cycle. In this review, we provide an overview of what is currently known about the involvement of lipids and membrane microdomains in HIV-1 replication.

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1. Introduction

Entry, assembly and release are essential steps in the virus replication cycle. Viruses need to cross the lipid bilayer during both entry into and exit from their host cell. Many families of enveloped viruses enter susceptible target cells upon fusion of the viral membrane with the host plasma membrane; in the case of HIV-1, this process is mediated by the interaction of the viral envelope (Env)

* Corresponding author at: Virus–Cell Interaction Section, HIV Drug Resistance Program, NCI–Frederick, Bldg. 535, Rm. 108, 1050 Boyles St., Frederick, MD 21702-1201, United States. Tel.: +1 301 846 1739; fax: +1 301 846 6777.

** Corresponding author at: Virus–Cell Interaction Section, HIV Drug Resistance Program, NCI–Frederick, Bldg. 535, Rm. 108, 1050 Boyles St., Frederick, MD 21702-1201, United States. Tel.: +1 301 846 6223; fax: +1 301 846 6777.

E-mail addresses: awaheed@ncifcrf.gov (A.A. Waheed), efreed@nih.gov (E.O. Freed).

glycoproteins with cell-surface receptors and coreceptors. Virions that assemble and bud from the infected cell undergo a fission reaction to release progeny particles. Numerous studies during the past decade have indicated that lipids in both viral and cellular membranes play an important role in the HIV-1 replication cycle. Lipids reported to play a critical role in HIV-1 replication include cholesterol, sphingolipids, and phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂]. Manipulating these lipids inhibits virus entry, assembly/release, or both, resulting in impaired viral replication.

Recent advances in the study of membrane biology have superseded the fluid mosaic model of biological membranes (Singer and Nicolson, 1972), which described cellular membranes as uniform and homogeneous, with proteinaceous components floating in a sea of lipid. In contrast, it is now clear that biological membranes contain heterogeneous patches, or microdomains, each with a specific lipid and protein composition. Studies with model membranes, coupled with advanced imaging techniques, identified one such microdomain that is enriched in cholesterol and sphingolipids as “lipid rafts”. The formation of these microdomains occurs as a consequence of the different biophysical properties of lipids found in the plasma membrane, and their differential self-association properties, leading to phase separation.

Since the earliest studies proposing the presence of lipid rafts in mammalian cell plasma membranes, the function and even existence of these microdomains has generated both excitement and controversy. Lipid rafts serve as concentration platforms that promote the interactions of lipids and proteins in the plasma membrane. Many animal viruses associate with lipid rafts during particle entry or egress. In a previous review (Ono and Freed, 2005), we discussed the role of lipid rafts in the replication of a broad range of viruses, both enveloped and nonenveloped. In this review, we focus on the interplay between lipids, membrane microdomains, and HIV-1 replication. Over the past decade, many cell biologists and membrane biochemists have voiced skepticism about the very existence of lipid rafts in living cell membranes (Munro, 2003). However, this has been as much a dispute about semantics and methodologies as a debate about membrane biology. In 2006, as part of the Keystone Symposium on Lipid Rafts and Cell Function (Steamboat Springs, CO, March 23–28, 2006), a definition was developed that appeared to satisfy most participants: “Membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein–protein and protein–lipid interactions” (Pike, 2006).

2. Lipids and membrane microdomains

2.1. Overview

The plasma membrane has a lipid composition that is distinct from that of internal cellular membranes. All mammalian cell membranes consist of three major types of lipids, namely glycerophospholipids, sphingolipids, and sterols (Munro, 2003; Simons and Vaz, 2004). Depending on the head group attached via glycerol to their two acyl chains, glycerophospholipids (GPLs) can be further classified into several groups; namely, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylserine (PS) and phosphatidylinositol (PI). Sphingolipids are derived from ceramide and have either a phosphatidylcholine head group (sphingomyelin, SM) or carbohydrate moiety (glycosphingolipids, GSLs). GSLs are classified into several types based on their carbohydrate modification, the simplest being glucosylceramide (GluCer) and galactosylceramide (GalCer). The acyl chains of sphingolipids are generally saturated, whereas, in the case of

GPLs, one of the acyl chains is usually unsaturated. Cholesterol is the principle sterol found in mammalian cells. Sterols and sphingolipids are synthesized in the endoplasmic reticulum (ER) and Golgi apparatus, respectively. They are present at low levels in most internal membranes but are more abundant on the plasma membrane and in endosomal membranes (Hao et al., 2002; van Meer, 1998).

Although the lipid composition varies depending on cell type, cholesterol, PC, PE, PS, and SM are the primary types of lipids in mammalian cell membranes; PA and PI are also present in significant amounts (Balasubramanian and Schroit, 2003; Feigenson, 2007; van Meer et al., 2008). The distribution of these lipids in the lipid bilayer differs significantly, with most of the sphingolipids and PC localized predominantly in the outer leaflet and PE, PS and PI present primarily in the inner, cytosolic leaflet (Devaux and Morris, 2004). Cholesterol is found in both leaflets, although it may display some asymmetry as well, and is required to establish proper membrane permeability and fluidity. Cholesterol is known to spontaneously flip between the two leaflets and preferentially interacts with sphingolipids rather than phospholipids (Ramstedt and Slotte, 2002). Due to their long and saturated acyl chains, sphingolipids are tightly packed together compared to phospholipids that are rich in kinked, unsaturated acyl chains (Brown, 2006; Brown and London, 2000). This differential lipid packing induces phase separation in membranes, giving rise to a tightly packed liquid-ordered (l_o) phase and a more loosely packed liquid-disordered (l_d) phase (Brown, 2006; Brown and London, 2000). Cholesterol has an important effect on phase behavior; above a threshold concentration of cholesterol the formation of l_o phase is favored (Brown and London, 2000; Simons and Toomre, 2000; Simons and Vaz, 2004). The formation of the l_o phase is also promoted by sphingolipids due to the stronger interaction of long saturated acyl chains with cholesterol, and this effect is enhanced by intermolecular hydrogen bonds that form between sphingolipids (Li et al., 2001; Ramstedt and Slotte, 2002).

Studies with model membranes have clearly established that l_o and l_d phases exist in lipid bilayers. Because sphingolipids are more abundant in the outer leaflet of the plasma membrane, clusters of l_o domains “float” in l_d domains (Brown and London, 1998b; Rietveld and Simons, 1998; Schroeder et al., 1994), hence the term “lipid rafts” for microdomains enriched in cholesterol and sphingolipids (Fig. 1). In addition to these two lipids, certain proteins reside in, or are excluded from, lipid rafts. A major component of the outer leaflet of lipid rafts are proteins that are attached to the bilayer by covalent linkage to glycosylphosphatidylinositol (GPI). The proteins that generally reside in the inner leaflet of lipid rafts are those that are singly or multiply acylated; e.g., G-proteins and nonreceptor tyrosine kinases. Prenylated proteins are generally excluded from rafts. This asymmetric protein distribution allows lipid rafts to function as “signaling platforms” that couple events on the outside of the cell with signaling pathways inside the cell.

2.2. Lipid rafts in living cells

The first method developed to biochemically isolate lipid rafts was based on the resistance of these membrane domains to extraction in nonionic detergents (typically Triton X-100) at low temperature (Brown and Rose, 1992). Because of their high lipid content, these detergent-resistant membrane (DRM) complexes float to a low density in sucrose density gradients (Brown and London, 2000; Simons and Toomre, 2000). A common criticism, or limitation, of isolation methods based on detergent resistance is that DRM fractions are aggregates of raft domains that form after cell lysis and thus do not represent the native state of lipid rafts in living cell membranes (Munro, 2003). The DRM approach was widely used to identify proteins associated with lipid rafts (Brown

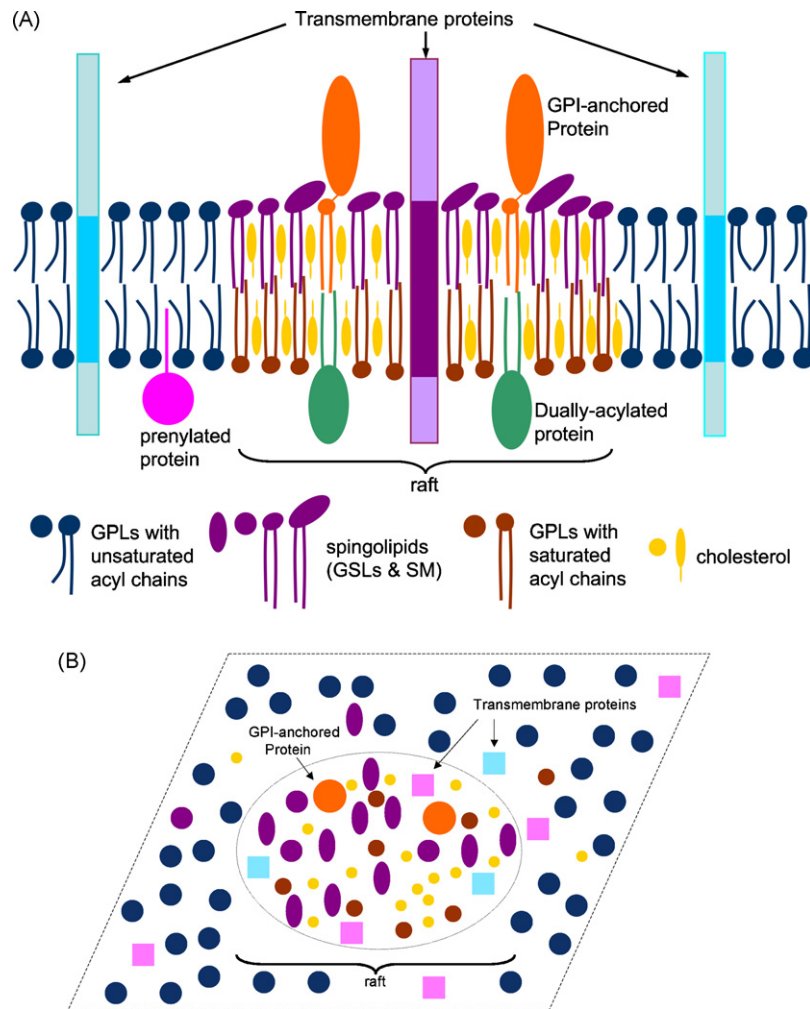


Fig. 1. A simplified model of lipid raft structure in biological membranes. The sphingolipids (violet) are enriched in the outer leaflet of the bilayer, whereas cholesterol (yellow) and phospholipids (brown and blue) are distributed in both leaflets. Note that lipids in the raft domain usually have long and saturated acyl chains (violet and brown), whereas the lipids excluded from these domains have shorter and unsaturated acyl chains (blue). GPI-anchored (orange) and dually acylated proteins (green) are concentrated in raft domains. Transmembrane proteins (blue) and prenylated proteins (pink) generally reside in non-raft regions, whereas some transmembrane proteins (pink) associate with raft domains. Panel A depicts a lipid raft in cross-section; B shows a top view, looking down on the membrane. In panel B, the raft boundary is represented by a dashed oval.

and London, 2000; Simons and Toomre, 2000). These include, as mentioned above, acylated or GPI-anchored proteins. The interactions of these proteins with lipid rafts are mediated by their saturated lipid linkers. Based on their presence or absence in DRMs, some transmembrane proteins (e.g., CD4 and influenza hemagglutinin) were classified as raft-associated whereas other proteins (e.g., transferrin receptor and CD45) appeared to be raft-excluded. These proteins are routinely used as markers to distinguish raft from non-raft membranes. In addition to these proteins, the glycosphingolipid GM1, which is bound by the cholera toxin B subunit, is commonly used as a raft marker.

Although the DRM isolation method is often used to study the composition and properties of lipid rafts, as alluded to above there is a legitimate concern that the complexes detected with this technique could be artifacts of detergent treatment at low temperatures (Lichtenberg et al., 2005; Mayor and Maxfield, 1995; Munro, 2003). For this reason, in combination with detergent treatment analyses, methods that visualize copatching with well-known raft markers (e.g., GM1) by immunofluorescence microscopy provide useful approaches to identify the association of proteins with lipid rafts. However, because of the sub-micron dimensions of lipid rafts, it is difficult to resolve these structures by conventional microscopic techniques. Therefore, more advanced microscopic approaches have been applied to characterize lipid rafts in living

cell membranes. Fluorescence resonance energy transfer (FRET) microscopy, which provides a measure of the distance between fluorescently tagged proteins, is a commonly used technique (Kenworthy and Edidin, 1998; Kenworthy et al., 2000; Sharma et al., 2004; Varma and Mayor, 1998). Single-particle tracking (SPT) of membrane proteins, which is used to study the local diffusion of single raft proteins with high-speed video microscopy, provides information about the dynamic properties of rafts (Kusumi et al., 2004; Kusumi and Suzuki, 2005; Pralle et al., 2000; Ritchie and Kusumi, 2003). Fluorescence recovery after photobleaching (FRAP) is a microscopy-based technique that measures the diffusion rates of fluorescently tagged proteins or lipids (Kenworthy, 2007; Niv et al., 2002; Shvartsman et al., 2003). Two-photon microscopy utilizes a membrane probe that is sensitive to the polarity of the membrane and can visualize microdomains in living cells and tissues (Gaus et al., 2003; Kim et al., 2007, 2008). Immunoelectron microscopy (IEM) of native plasma membrane sheets clearly demonstrates clustering of raft-associated proteins (Prior et al., 2003; Schade and Levine, 2002; Wilson et al., 2001, 2004). These techniques not only allow visualization of lipid rafts in living cells or in native membrane but also provide information about the size of these microdomains *in vivo*. This size varies from ~10 to 200 nm (Dietrich et al., 2002; Pike, 2006; Pralle et al., 2000; Schutz et al., 2000; Varma and Mayor, 1998). Although currently a number of

increasingly sophisticated techniques are used to study lipid rafts, each of these techniques has its advantages and limitations and may in some cases produce conflicting results (Ding et al., 2003; Harder et al., 1998). It is therefore desirable, whenever possible, to use multiple methodologies to identify and characterize the association molecules with membrane microdomains.

2.3. Functions of lipid rafts

Lipid rafts play an important role in many cellular processes, including transmembrane signal transduction at the cell surface, membrane sorting and trafficking, cell polarization, immunological synapse formation, and cell movement. In many cases, lipid rafts serve as concentration platforms for membrane-associated molecules involved in these processes. The abundance of signaling molecules in lipid rafts suggests that these domains are important in signal transduction (Brown and London, 1998a; Fielding and Fielding, 2000; Smart et al., 1999). The signaling molecules that are associated with lipid rafts include GPI-anchored proteins, Src-family tyrosine kinases, and GTP-binding proteins (Brown and London, 1998a, 2000; Campbell et al., 2001; Simons and Ikonen, 1997; Simons and Toomre, 2000). GPI-anchored proteins are known to be involved in signaling across the plasma membrane by binding directly or indirectly to Src-family kinases (reviewed in Brown and London, 1998a). Caveolae, originally observed by electron microscopy (EM) as flask-shaped membrane invaginations found in the plasma membrane of several cell types, are formed from lipid rafts upon multimerization of the cholesterol-binding scaffolding protein caveolin. Caveolae play important roles in signal transduction, non-clathrin-mediated endocytosis, and cholesterol transport (Harris et al., 2002; Kurzchalia and Parton, 1999). Rafts and caveolae function as sorting platforms at various stages of the endocytic and exocytic pathways (Parton and Richards, 2003; Sabharanjak et al., 2002). For example, certain enveloped and nonenveloped viruses are taken into cells by caveolae-mediated endocytosis (as discussed in more detail below). It is widely reported that several groups of pathogens – bacteria, viruses, and parasites – hijack lipid rafts to facilitate their replication and survival (Simons and Ehehalt, 2002; van der Goot and Harder, 2001). It has also been demonstrated that the pathogenesis of a variety of diseases is linked to lipid rafts. For example, the pathogenic roles of the scrapie isoform of the prion protein in prion disease, and β -amyloid in Alzheimer disease, depend on their association with lipid rafts for conversion from non-pathogenic to pathogenic forms (Kaneko et al., 1997; Simons and Ehehalt, 2002; Simons et al., 1998).

Evidence has accumulated over the past decade suggesting that many viruses employ membrane microdomains for their replication (reviewed in Briggs et al., 2003; Campbell et al., 2001; Metzner et al., 2008; Ono and Freed, 2005; Suomalainen, 2002; Suzuki and Suzuki, 2006). Many nonenveloped viruses enter their host cell by internalization after binding to a plasma membrane receptor. This internalization process can take place either by clathrin-mediated or clathrin-independent endocytosis (Marsh and Helenius, 2006). Caveolae are involved in clathrin-independent endocytosis. Nonenveloped viruses that are internalized using caveolae/raft-mediated endocytosis include simian virus 40 (Anderson et al., 1996; Parton and Lindsay, 1999; Pelkmans et al., 2001; Stang et al., 1997), mouse polyomavirus (Richterova et al., 2001), rotavirus (Arias et al., 2002; Cuadras and Greenberg, 2003; Sapin et al., 2002), echovirus type 1 (Marjomaki et al., 2002), rhinovirus (Grassme et al., 2005), coxsackievirus (Stuart et al., 2002; Triantafyllou and Triantafyllou, 2003), human adenovirus (Colin et al., 2005), and enterovirus (Stuart et al., 2002). The role of membrane microdomains in the assembly and release of nonenveloped viruses remains to be elucidated, although rotavirus assembly is reportedly associated with membrane microdomains (Delmas et al., 2004; Sapin et al., 2002).

The role of membrane microdomains in the entry of enveloped viruses is well established. Enveloped virus spike proteins typically attach to receptors on the target cell membrane and, often after undergoing extensive conformational changes, catalyze a membrane fusion reaction either at the plasma membrane or in a low-pH endosomal compartment. This membrane fusion event allows the viral replication complex to access the cytosol. In many cases, viral receptors are raft-associated. Viruses that have been reported to use lipid rafts for entry include herpes simplex virus type 1 (HSV-1) (Bender et al., 2003; Lee et al., 2003), human herpes virus 6 (HHV-6) (Tang et al., 2008) and pseudorabies virus (Desplanques et al., 2008; Lyman et al., 2008). The West Nile virus (a flavivirus) reportedly requires cholesterol-rich membrane microdomains for entry (Medigeschi et al., 2008). Although the spike glycoproteins of the flaviviruses (e.g., West Nile virus) and the alphaviruses (e.g., Semliki Forest virus) are structurally very similar, the Semliki Forest virus glycoprotein binds directly to cholesterol to initiate membrane fusion whereas the West Nile virus glycoprotein does not (Umashankar et al., 2008). Despite the cholesterol requirement for Semliki Forest virus fusion, it may not require lipid rafts for entry (Waarts et al., 2002). These studies highlight the diverse mechanisms by which enveloped viruses use membrane microdomains, and specific lipid components, during virus entry.

Lipid rafts also serve as a platform for the assembly and/or budding of range of enveloped viruses. The filoviruses Marburg and Ebola use caveolae/lipid rafts for assembly (Aman et al., 2003; Bavari et al., 2002; Empig and Goldsmith, 2002; Panchal et al., 2003). The paramyxoviruses measles (Manie et al., 2000; Vincent et al., 2000), respiratory syncytial virus (Brown et al., 2002; Marty et al., 2004; McDonald et al., 2004) and Sendai virus (Ali and Nayak, 2000) assemble within lipid rafts. Influenza virus (an orthomyxovirus) interacts with sphingolipid- and cholesterol-enriched membrane domains during its assembly and release (Barman and Nayak, 2000; Scheiffele et al., 1997). The retroviruses murine leukemia virus (MLV) (Beer and Pedersen, 2006; Beer et al., 2005; Lu and Silver, 2000) and human T cell leukemia virus type 1 (HTLV-1) (Niyogi and Hildreth, 2001; Wielgosz et al., 2005) utilize cholesterol-sphingolipid-rich domains for assembly. In our previous review (Ono and Freed, 2005) we discussed in detail the association of the structural proteins of these enveloped viruses with lipid rafts/caveolae in virus entry, assembly and release. For the remainder of this review, we will focus primarily on the role of lipids and lipid microdomains in HIV-1 replication.

3. Lipid microdomains in HIV-1 replication

3.1. Role of lipids and lipid rafts in HIV-1 entry

HIV-1 enters susceptible target cells primarily by direct fusion of the viral envelope lipid bilayer with the target cell plasma membrane (Berger et al., 1999; Doms, 2000). This fusion reaction is mediated by the viral envelope (Env) glycoproteins (Fig. 2) which are synthesized as a polyprotein precursor, gp160. Cleavage of gp160 to the mature surface glycoprotein gp120 and the transmembrane glycoprotein gp41 is mediated by a cellular protease during

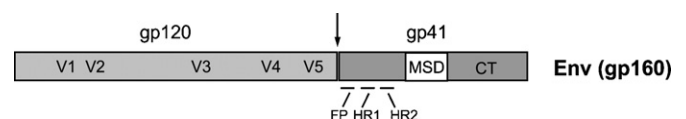


Fig. 2. Schematic representation of HIV-1 Env proteins. The HIV-1 Env glycoprotein precursor (gp160) is proteolytically processed (arrow) into the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. gp41 is composed of an ectodomain containing the fusion peptide (FP) and heptad repeat 1 and 2 (HR1 and HR2); a membrane-spanning domain (MSD) and cytoplasmic tail (CT).

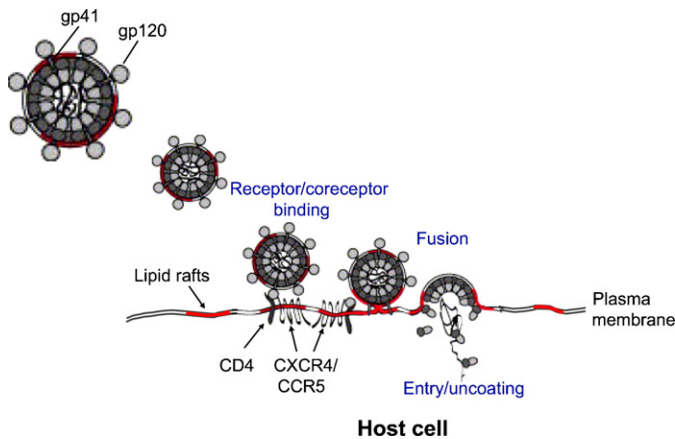


Fig. 3. HIV-1 entry. gp120 on the surface of the HIV-1 virion binds to CD4 and coreceptor (CCR5 or CXCR4) in lipid rafts (red) of the plasma membrane. Membrane fusion between viral and plasma membrane, mediated by the transmembrane Env glycoprotein gp41, results in viral entry and uncoating. Modified and reprinted with permission from Ono and Freed (2005). Copyright 2005, Elsevier Inc.

transport through the Golgi. gp160 is heavily glycosylated; indeed, more than half of its mass is composed of sugar side chains. HIV-1 entry into the host cell is a multistep process initiated by the interaction of gp120 with cell-surface receptor and coreceptor (Fig. 3). The primary receptor for HIV-1 and other primate lentiviruses is CD4; the two major coreceptors are CCR5 and CXCR4. The binding of gp120 to CD4 on the target cell surface induces conformational changes in the viral glycoprotein that induce its interaction with coreceptor (Berger et al., 1999; Doms, 2000). Coreceptor binding, in turn, triggers conformational changes in gp120 and also in the transmembrane glycoprotein gp41 that ultimately lead to the fusion of viral and host cell membrane, resulting in entry of the viral core into the target cell (Briggs et al., 2000; Deng et al., 1996; Dragic et al., 1996). Key determinants in gp41 that are directly involved in mediating membrane fusion are an N-terminal hydrophobic fusion peptide, and two heptad repeats downstream from the fusion peptide [(Freed and Martin, 2007); Fig. 2]. The heptad repeats form an antiparallel six-helix bundle after receptor and coreceptor engagement; the formation of this structure promotes the insertion of the fusion peptide into the target cell membrane, resulting in lipid mixing, fusion pore enlargement, and ultimately membrane fusion (Eckert and Kim, 2001).

There is significant evidence that the lipid composition of the target membrane affects HIV-1 Env-mediated fusion and entry, though this area has not been without controversy. Both CD4 and CCR5, but not CXCR4, are reportedly associated with lipid rafts (Kozak et al., 2002; Popik et al., 2002). It was found that a non-raft-associated CD4 mutant prevents HIV-1 infection in CD4⁺ T cells (del Real et al., 2002). However, another group observed that mutations in CD4 that prevent its association with DRM did not inhibit virus entry (Popik et al., 2002). CXCR4, although initially not associated with lipid rafts, could potentially be recruited into raft aggregates upon gp120–CD4 binding (Popik et al., 2002). Studies using beta-cyclodextrin (BCD) to remove cholesterol from the target cell membrane have demonstrated significant inhibition of HIV-1 infection (Liao et al., 2001; Manes et al., 2000; Nguyen and Taub, 2002b; Popik et al., 2002).

It is well documented that the HIV-1 lipid bilayer is enriched in cholesterol and at least some sphingolipids relative to the host cell plasma membrane (Aloia et al., 1993; Brugger et al., 2006; Chan et al., 2008). Depleting cholesterol from virions with BCD impairs HIV-1 infectivity (Campbell et al., 2002, 2004; Graham et al., 2003; Guyader et al., 2002), suggesting that virion cholesterol is important for viral entry. A highly conserved motif, LWYIK

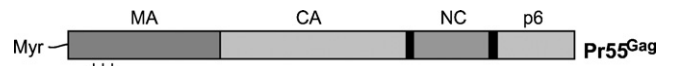


Fig. 4. Schematic representation of HIV-1 Gag proteins. The major domains in the Gag precursor (Pr55^{Gag}) – MA, CA, NC and p6 – are indicated. The N-terminal myristoylation (Myr) and the MA highly basic domain (+++) are shown.

(gp160 residues 679–683) located immediately N-terminal to the membrane-spanning domain of gp41, has been reported to specifically bind cholesterol (Li and Papadopoulos, 1998; Vincent et al., 2002). This sequence bears homology to cholesterol recognition amino acid consensus (CRAC) motifs (Epand, 2006) and mutations in this domain inhibit HIV-1 infectivity (Salzwedel et al., 1999). In addition to cholesterol, other lipids have been implicated in HIV-1 infection. SM, along with cholesterol, may promote surface aggregation of the gp41 LWYIK motif into an ordered domain, potentially enhancing gp41-mediated fusion of viral and cellular membranes (Saez-Cirion et al., 2002). PS is reportedly present at elevated levels in the outer leaflet of the HIV-1 lipid envelope and may enhance infection of macrophages by binding to a PS receptor on monocytic cells (Callahan et al., 2003).

Several GSLs on the cell membrane have been reported to serve as alternative or accessory HIV-1 receptors. These include galactosylceramide (GalCer) (Bhat et al., 1991, 1993; Fantini et al., 1997; Hammache et al., 1998a; Harouse et al., 1991), galactosyl ceramide-3-sulphate (SGalCer) (Bhat et al., 1993), and globotriaosylceramide (Gb3) (Hammache et al., 1999). It has also been reported that gangliosides GM1 and GM3 are cofactors for HIV-1 Env-mediated fusion (Hammache et al., 1998a,b, 1999; Hug et al., 2000; Puri et al., 1998; Rawat et al., 2005). GSLs may enhance HIV-1 Env-mediated fusion by serving as direct binding sites for Env on the cell surface and may promote the clustering of CD4 and coreceptors and regulate their lateral mobility in the target cell membrane (Rawat et al., 2005, 2008).

3.2. Role of lipids and lipid rafts in HIV-1 assembly/budding

HIV-1 assembly, like that of other retroviruses, is a multi-step process driven by the Gag precursor protein (Figs. 4 and 5) (Adamson and Freed, 2007; Freed, 1998; Swanstrom and Wills, 1997). Expression of the Gag precursor alone is sufficient for the

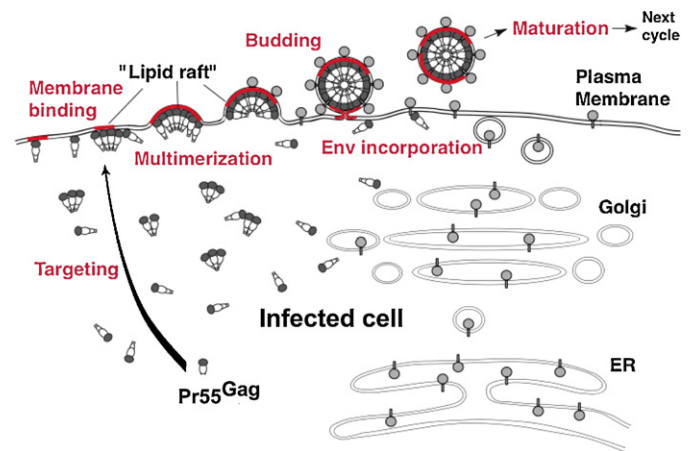


Fig. 5. Schematic representation of HIV-1 assembly and release at the plasma membrane. Pr55^{Gag} is directed post-synthesis to the plasma membrane where it associates with cholesterol- and sphingolipid-rich lipid raft microdomains (red). Gag–Gag interactions lead to the assembly of viral particles resulting in highly multimerized Gag complexes that bud from the plasma membrane. The viral Env glycoproteins are incorporated during the assembly process and virions ultimately pinch off from the cell surface. Reprinted with permission from Waheed et al. (2009). HIV protocols 2nd edition, copyright Humana press.

assembly of virus-like particles (VLPs). The viral enzymes protease (PR), reverse transcriptase (RT), and integrase (IN) are synthesized as part of the GagPol polyprotein precursor, the expression of which is the result of a ribosomal frame-shifting event during translation of the Gag precursor. During or shortly after virus particle release from the infected cell, PR cleaves both the Gag and GagPol polyprotein precursors to produce the mature Gag proteins matrix (MA), capsid (CA), nucleocapsid (NC) and p6 and the *pol*-encoded enzymes. Virus particle production requires that the Gag precursor protein, known as Pr55^{Gag} in the case of HIV-1, binds membrane, multimerizes, and engages host cell factors to promote virus pinching-off from the plasma membrane. These steps are mediated by domains sometimes referred to as the membrane binding (M), Gag–Gag interaction (I) and late (L) domains, respectively.

3.2.1. Gag

In most cell types, HIV-1 assembly occurs predominantly on the inner leaflet of the plasma membrane (Jouvenet et al., 2006; Joshi and Freed, 2007). The association of Pr55^{Gag} with membrane is mediated by a bipartite motif in the MA domain composed of a covalently attached myristic acid and a positively charged patch of amino acids (Fig. 4). The myristylation of Gag is critical for the targeting of Gag to the plasma membrane, as mutating the N-terminal glycine, to which the myristate is covalently attached, blocks Gag–membrane association and virus assembly (Bryant and Ratner, 1990; Gottlinger et al., 1989; Pal et al., 1990). The highly basic domain of MA, located between MA residues 17 and 31, forms a positively charged surface postulated to engage the negatively charged inner leaflet of the plasma membrane (Hill et al., 1996; Massiah et al., 1994; Zhou et al., 1994 see below). The role of the myristate in membrane binding is regulated by a myristyl switch mechanism; the acyl group adopts either a sequestered conformation in which it is buried within the globular core of MA or an exposed conformation in which it is able to insert directly into the inner leaflet of the host cell lipid bilayer (Ono and Freed, 1999; Paillart and Gottlinger, 1999; Spearman et al., 1997; Zhou and Resh, 1996). The myristyl switch appears to be triggered by multiple factors, including Gag multimerization (Tang et al., 2004) and phosphoinositide binding (Saad et al., 2006) (see below). Mutations in MA residues 6–8 inhibit Gag–membrane binding (Ono and Freed, 1999) apparently by disrupting the myristyl switch (Saad et al., 2007).

The concept that enveloped viruses assemble in specific membrane microdomains first arose when it was observed that the lipid composition of viral membranes differed significantly from the host cell plasma membrane from which the viral envelopes were derived (David, 1971; McSharry and Wagner, 1971; Pessin and Glaser, 1980; Quigley et al., 1971, 1972; Renkonen et al., 1971; Scheiffele et al., 1999; Slosberg and Montelaro, 1982). This difference in composition between host and viral membranes was also observed for HIV-1, and the HIV-1 lipid bilayer was found to be more ordered than the host cell plasma membrane (Aloia et al., 1992, 1993; Brugger et al., 2006; Chan et al., 2008). The molar ratio of cholesterol to total phospholipids in the HIV-1 envelope is quite high, approximately 2.5 times that of the host cell membrane, and the levels of several other raft lipids are also elevated in HIV-1 virions (Aloia et al., 1993; Brugger et al., 2006; Chan et al., 2008). In addition to lipids, some proteins are also incorporated into HIV-1 virions whereas other membrane proteins are excluded. For example, the GPI-anchored, lipid raft-associated proteins Thy-1, CD55, and CD59 are incorporated into virions (Nguyen and Hildreth, 2000; Ott, 1997; Saifuddin et al., 1995), whereas CD45, a non-raft-associated protein, is excluded (Nguyen and Hildreth, 2000). Although a number of well-established raft markers are incorporated into virions, several non-raft proteins, e.g., VSV-G and transferrin receptor, can also be detected in HIV-1 particles. While the host protein composition of HIV-1 virions can be used to support a raft origin for the

virion lipid bilayer, a variety of factors can account for the exclusion of host cell plasma membrane proteins from virions. For example, CD45 has a very long cytoplasmic domain that may sterically clash with Gag and thus exclude this protein from particles.

The hypothesis that HIV-1 assembly takes place in lipid rafts (Fig. 5) is supported by biochemical studies showing that HIV-1 Gag proteins are associated with DRM (Ding et al., 2003; Halwani et al., 2003; Holm et al., 2003; Lindwasser and Resh, 2001; Nguyen and Hildreth, 2000; Ono and Freed, 2001; Ono et al., 2007; Pickl et al., 2001). Pulse-chase analysis demonstrated that after its synthesis Gag binds very rapidly to membrane but detergent resistance is acquired more slowly (Ono and Freed, 2001). Studies with Gag truncation mutants suggest that Gag association with lipid rafts is enhanced or stabilized by Gag–Gag interactions (Lindwasser and Resh, 2001; Ono and Freed, 2001). These results are consistent with a model whereby Gag multimerization stabilizes its raft association and likely induces aggregation of small, unstable rafts in much the same way as protein–protein contacts among signaling proteins induce raft coalescence (Lingwood et al., 2008). Although Gag multimerization appears to stabilize Gag–DRM association, analysis of an assembly-deficient NC mutant revealed that lower-order multimerization was sufficient for Gag binding to membrane and acquisition of detergent resistance (Ono et al., 2005). There has been some debate in the literature about the type of membrane microdomain to which Gag localizes (Ding et al., 2003; Holm et al., 2003). Again, it should be stressed that while the DRM assay provides useful information about the lipid environment of a protein of interest, the DRMs themselves represent membrane aggregates that may not exist in the living cell. It is also worth noting that the properties of a membrane microdomain (e.g., size, stability, and detergent resistance) are heavily influenced, or even dictated, by its protein composition. Thus, microdomains that contain a highly multimerized protein like HIV-1 Gag are likely to be more dense than “classical” lipid rafts that contain monomeric minimally assembled proteins (Ding et al., 2003; Holm et al., 2003; Lindwasser and Resh, 2001). These biochemical findings are consistent with microscopic data indicating that HIV-1 Gag copatches with the known raft marker, GM1 (Fig. 6) (Holm et al., 2003; Nguyen and Hildreth, 2000; Ono et al., 2005).

The functional relevance of Gag association with lipid rafts has been investigated by using pharmacological reagents such as BCD or cholesterol biosynthesis inhibitors (statins). Treating HIV-1-expressing cells with these cholesterol-reducing agents impairs virus particle production (Ono and Freed, 2001; Pickl et al., 2001). Staging of this defect indicates that the inhibition of virus production induced by cholesterol depletion is due to reduced Gag binding to the plasma membrane and a defect in higher-order Gag multimerization (Ono et al., 2007). Treatment of Gag-expressing cells with an unsaturated myristic acid analog reduces Gag–DRM association and virus particle production, presumably as a result of substitution of the Gag N-terminal myristate with the non-raft-targeted analog (Lindwasser and Resh, 2002). Altogether, these studies argue that lipid rafts play an important role in HIV-1 assembly and release.

While Gag is the viral protein that mediates virus particle production, the efficiency of virus release can be significantly enhanced in some cell types by the small, membrane-associated HIV-1 accessory protein Vpu (Malim and Emerman, 2008). Recent studies have revealed that Vpu promotes particle release from the plasma membrane by counteracting a cellular protein, known variously as CD317, BST-2, or tetherin, that tethers budded particles at the cell surface (Neil et al., 2008; Van Damme et al., 2008). While the mechanism by which CD317/BST-2/tetherin restricts particle release remains to be defined, it is interesting to note that this host protein is itself raft-associated (Kupzig et al., 2003), again supporting the concept that assembly takes place in lipid rafts.

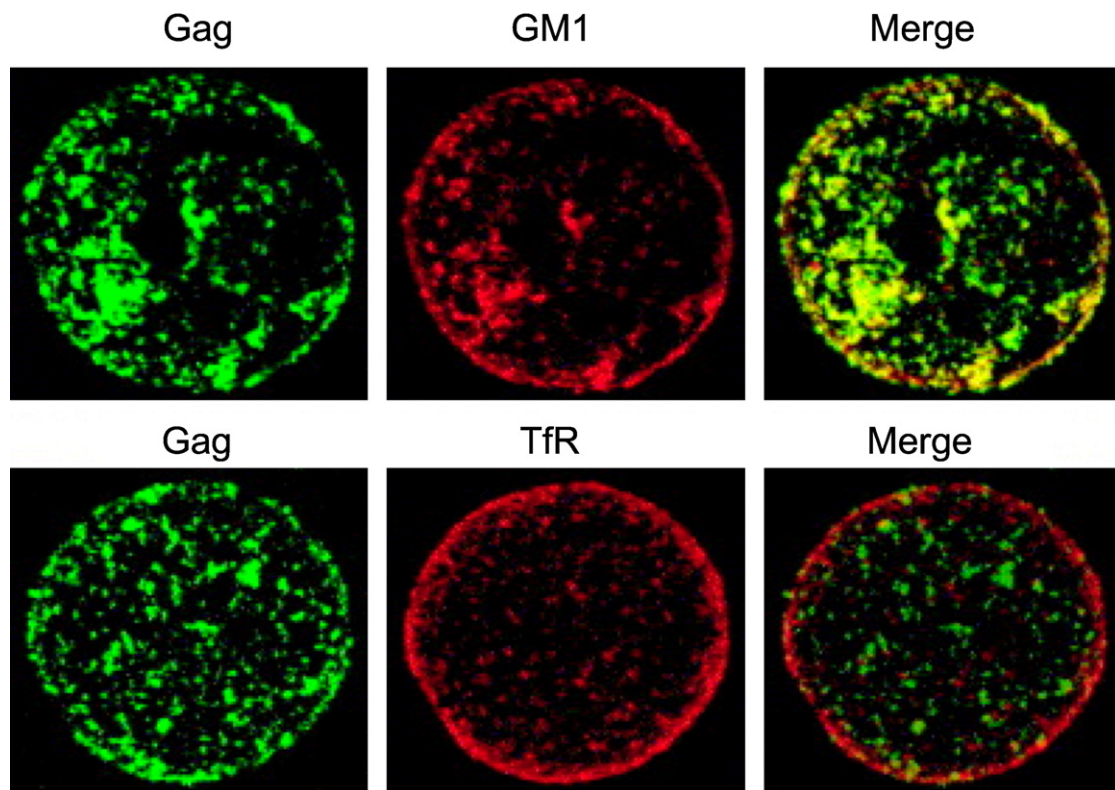


Fig. 6. HIV-1 Gag proteins copatch with the raft marker GM1 but not with the non-raft marker transferrin receptor (TfR). Jurkat cells expressing HIV-1 Gag were labeled with cholera toxin B subunit to stain GM1 or with anti-TfR antibody before fixation. Cells were then fixed and permeabilized, and incubated with anti-p17 MA antibody to detect Gag. Images collected in the z-axis using a confocal microscope were projected in 3D volume with Imaris software. Merged images of Gag and GM1 show colocalization, whereas Gag and TfR are not colocalized. Reprinted with permission from Ono and Freed (2005). Copyright 2005, Elsevier Inc.

3.2.2. Env

An essential step in the formation of infectious HIV-1 virions is the incorporation of the Env glycoproteins into virions. Although definitive proof of an Env–Gag interaction is still lacking, a large body of data supports the hypothesis that the Env complex is incorporated via an interaction between the cytoplasmic tail of gp41 and the MA domain of Gag (Freed and Martin, 2007). Interestingly, the role of the gp41 cytoplasmic tail in Env incorporation and virus replication is cell type-dependent; truncation of the gp41 tail blocks Env incorporation in a majority of T-cell lines and in primary cell types, but has little effect when virions are produced in 293T or HeLa cells (Murakami and Freed, 2000). A fraction of Env was shown to copurify with DRM (Nguyen and Hildreth, 2000; Rousso et al., 2000) and it was suggested that the palmitoylation of two Cys residues in the gp41 cytoplasmic tail is critical for Env–DRM association and Env incorporation (Rousso et al., 2000). However, Bhattacharya et al. reported that cytoplasmic tail Cys residues are required for Env targeting to DRMs but are not required for Env incorporation into virions or for virus infectivity (Bhattacharya et al., 2004). In contrast, Chen et al. reported that Cys palmitoylation is not required for either DRM association or Env incorporation and that the gp41 Cys mutants replicate with WT kinetics (Chan et al., 2005). Subsequent analysis suggested that Gag–Env interactions are required for Env association with DRM, as Env fractionated with DRM when coexpressed with Gag but not when expressed alone or with a Gag mutant that does not interact with Env (Bhattacharya et al., 2006). Cholesterol depletion with BCD completely abolished the observed DRM localization of Env (Bhattacharya et al., 2006). The hypothesis arising from this latter study is that Gag is the driving force for DRM association and that the Env glycoprotein molecules that are in lipid rafts are those that are in association with Gag. Additional support for Env–raft association was

obtained by immunostaining and copatching experiments showing that HIV-1 Env colocalizes with the raft markers Thy-1, GM1 and CD59 (Leung et al., 2008; Nguyen and Hildreth, 2000; Pickl et al., 2001).

Interestingly, a study from Nabel and co-workers observed that when HIV-1 Env and the Ebola glycoprotein (GP) are expressed with HIV-1 Gag, Env and GP localize to distinct domains with HIV-1 Gag. This segregation of HIV-1 Env and Ebola GP give rise to different virion populations, each with a single glycoprotein type, although Gag, Env and GP are all present in DRM fractions (Leung et al., 2008). These data suggest that HIV-1 Env and Ebola GP each associate with distinct lipid microdomains that are both recruited into HIV-1 particles. The mechanism of Env incorporation remains to be fully understood, and a recent study demonstrated that the recruitment of viral Env glycoproteins to sites of retrovirus budding is determined by the particular Gag that is expressed. For example, the glycoproteins of HIV-1, VSV, and MLV are actively recruited to budding sites formed by either HIV-1 or Rous sarcoma virus (RSV) Gag whereas RSV Env is recruited only to RSV budding sites (Jorgenson et al., 2009).

3.2.3. Nef

Nef, a 27-kDa accessory protein encoded by HIV-1, HIV-2 and SIV, is a major determinant for the pathogenesis of these primate lentiviruses. Nef is involved in several functions, including: (1) downregulation of cell-surface CD4 and major histocompatibility class I (MHC I) molecules, (2) modulation of signal transduction pathways required for T-cell activation, (3) enhancement of viral infectivity, and (4) regulation of cholesterol trafficking in infected cells [reviewed in Foster and Garcia, 2008]. Nef is a raft-associated protein; its membrane binding and raft association are dependent on N-terminal myristylation (Wang et al., 2000; Zheng et al., 2001). Although it is widely accepted that Nef is raft-associated, there is

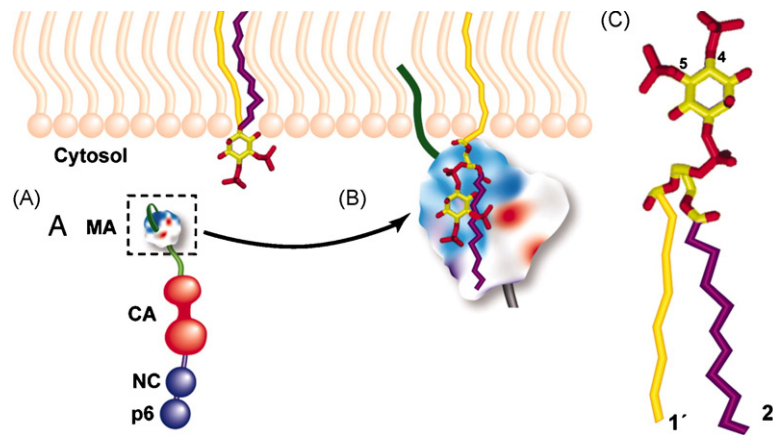


Fig. 7. Binding of the MA domain of HIV-1 Gag to PI(4,5)P₂. (A) The N-terminal myristate (dark green) is sequestered in the globular core of MA. The MA highly basic region (blue patch), CA (red), NC and p6 (dark blue) are shown. (B) Upon binding of MA to PI(4,5)P₂ in the inner leaflet of the lipid bilayer, the myristic acid (dark green) flips out from MA into the exposed conformation and inserts into the lipid bilayer. According to this model, the 2'-acyl chain (purple) of PI(4,5)P₂ is extruded from the membrane and docks into a groove in MA. (C) Structure of PI(4,5)P₂. The two phosphates (red) are attached to the inositol ring (yellow) at position 4 and 5. The 2'-acyl chain (violet) is unsaturated and kinked and the 1'-acyl chain is saturated. Reprinted with permission from Freed (2006). Copyright 2006, The National Academy of Sciences of the USA. Based on the data of Saad et al. (2006).

currently no clear consensus regarding the role of raft association in the biological functions of Nef. Several studies observed that Nef expression alters the lipid composition of HIV-1 virions, though the mechanism by which this occurs is not well defined. Zheng et al. observed that virions produced from 293T cells in the presence of Nef contain more GM1 and cholesterol and display increased infectivity compared with virions produced in the absence of Nef (Zheng et al., 2001, 2003). Mujawar and colleagues reported that Nef associates with ATP-binding cassette transporter A1 (ABCA1) in macrophages and impairs ABCA1-mediated cholesterol efflux by altering the intracellular distribution of ABCA1 (Mujawar et al., 2006). According to this study, impairment of cholesterol efflux by Nef increases the cholesterol content of virions, thereby enhancing particle infectivity (Mujawar et al., 2006). In contrast to this study, which was performed using macrophages as the virus-producer cell, Brugger and colleagues reported that when virus was produced from the MT-4 T cell line Nef increased virion SM and reduced virion PC but did not affect levels of virion cholesterol (Brugger et al., 2007). Trono and co-workers observed that Nef associates with microdomains in which Lck, Fyn and LAT kinases are enriched, and thus association of Nef with lipid rafts may serve to prime T cells for activation (Wang et al., 2000). It has been reported that Nef associates with lipid rafts to stimulate p21-activated kinase (PAK) (Krautkramer et al., 2004). Fackler and co-workers demonstrated that in addition to N-terminal myristylation, basic residues in Nef contribute to its membrane binding and raft localization. While membrane binding is generally required for all Nef activities, some Nef functions (e.g., PAK binding and infectivity enhancement) require DRM localization whereas others (e.g., CD4 downregulation) do not (Giese et al., 2006; Krautkramer et al., 2004). Consistent with this latter report, Sol-Foulon and co-workers found that DRM recruitment of Nef is not required for CD4 downregulation; however, they also observed that raft localization was not required for the ability of Nef to stimulate virus infectivity (Sol-Foulon et al., 2004). Many of the differences in the studies cited above are likely the result of different experimental systems (cell type, Nef strain of origin, etc.) as well as different experimental approaches for altering Nef-DRM localization. More work will be required to elucidate the biological implications of Nef association with lipid rafts.

3.3. PI(4,5)P₂ in HIV-1 assembly

PI(4,5)P₂ is a phosphoinositide that is concentrated primarily in the cytoplasmic leaflet of the plasma membrane (Fig. 7)

and acts as the source for two second messengers in the cell, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃) (Berridge and Irvine, 1984). PI(4,5)P₂ binds to a variety of effector molecules and regulates their function and cellular localization through its phosphorylated inositol head group. Several highly conserved PI(4,5)P₂-binding proteins have been identified that interact with the negatively charged inositol head group through a positively charged cluster of basic amino acids (McLaughlin et al., 2002). The best-characterized PI(4,5)P₂-binding motifs are the pleckstrin homology (PH) and epsin N-terminal homology (ENTH) domains (McLaughlin et al., 2002). PI(4,5)P₂ is primarily synthesized by the phosphorylation of phosphatidylinositol 4-phosphate (PI4P) by type I phosphatidylinositol 4-phosphate 5-kinase (PI4P5-K I) (Stephens et al., 1991; Whiteford et al., 1997). The activity of PI4P5-K I is regulated by a number of factors including the small G protein ADP-ribosylation factor 6 (Arf6) (Honda et al., 1999). Overexpression of a constitutively active Arf6 mutant, Arf6/Q67L, shifts cellular PI(4,5)P₂ localization to PI(4,5)P₂-enriched endosomal structures (Aikawa and Martin, 2003; Brown et al., 2001). PI(4,5)P₂ levels can also be regulated by phosphatases, including polyphosphoinositide 5-phosphatase IV (5ptaseIV), which hydrolyzes the phosphate at the D5 position and reduces plasma membrane PI(4,5)P₂ levels (Kisseleva et al., 2000).

Plasma membrane PI(4,5)P₂ plays a critical role in HIV-1 particle assembly (Chukkapalli et al., 2008; Ono et al., 2004). Overexpression of 5ptaseIV drastically reduces HIV-1 release by relocalizing Gag from the plasma membrane to CD63-positive intracellular compartments (Ono et al., 2004). Disrupting PI(4,5)P₂ levels and localization by expression of Arf6/Q67L also reduces virus release by retargeting Gag to newly formed PI(4,5)P₂-enriched endosomal vesicles (Ono et al., 2004). As mentioned previously, the plasma membrane targeting of HIV-1 Gag is regulated by a highly basic cluster of amino acids located between MA residues 17 and 31 (Freed et al., 1994; Hermida-Matsumoto and Resh, 2000; Ono et al., 1997, 2000a; Ono and Freed, 1999, 2004; Yuan et al., 1993; Zhou and Resh, 1996). The phenotype of basic domain mutants; i.e., mistargeting of Gag to a CD63⁺ late endosomal compartment (Ono and Freed, 2004), is quite similar to that observed upon PI(4,5)P₂ depletion induced by 5ptaseIV overexpression (Ono et al., 2004). This led to the suggestion that basic residues in MA interact directly with PI(4,5)P₂ (Ono et al., 2004). This prediction was borne out by studies demonstrating a direct interaction between MA and PI(4,5)P₂ (Fig. 7) (Freed, 2006; Saad et al., 2006; Shkriabai et al., 2006). Saad

and colleagues used a nuclear magnetic resonance (NMR) approach to demonstrate direct binding of the MA domain of HIV-1 Gag to a truncated, soluble PI(4,5)P₂ derivative (Saad et al., 2006) whereas Shkriabai et al. used a mass spectrometric protein footprinting analysis. While both studies observed direct binding between MA and the lipid, the two reports differed in terms of which specific residues in MA directly contacted PI(4,5)P₂. The Shkriabai et al. study identified MA residues 29 and 31 as being important for the interaction. Interestingly, these two residues have also been shown to play a critical role in Gag targeting in cells (Ono et al., 2000b; Ono and Freed, 2004; Gousset et al., 2008; Joshi et al., 2009). An important role for MA residues 29 and 31 in PI(4,5)P₂ binding was confirmed in assays demonstrating that PI(4,5)P₂ significantly enhances the efficiency of binding of full-length Gag to liposomes (Chukkapalli et al., 2008).

Several features of the NMR analysis of Summers and colleagues are noteworthy in terms of understanding the role of PI(4,5)P₂ in Gag-membrane binding (Freed, 2006; Saad et al., 2006). According to this model, PI(4,5)P₂ binds MA not only via its negatively charged inositol head group but also the unsaturated 2' fatty acyl chain of PI(4,5)P₂ binds to a hydrophobic cleft within the globular core of MA. In theory, extrusion of the unsaturated acyl chain from the lipid bilayer could promote the recruitment of Gag to lipid rafts, since the remaining acyl chain is highly saturated and would thus have an affinity for the highly saturated raft microenvironment. Another significant observation based on the NMR data is that PI(4,5)P₂ binding to MA triggers the myristyl switch, thereby increasing myristate exposure and promoting Gag-membrane binding. These data suggest that PI(4,5)P₂ acts as both a trigger for myristate exposure and a membrane anchor for HIV-1 Gag. Recent lipidomics studies suggest that HIV-1 and MLV virions are enriched in phosphatidylinositol monophosphate (PIP) and PI(4,5)P₂ along with cholesterol, ceramide and GM3 (Chan et al., 2008). The enrichment of PI(4,5)P₂ in HIV-1 virions requires the MA domain, as a Gag mutant lacking the polybasic globular head of HIV-1 MA incorporates significantly less PI(4,5)P₂ than WT (Chan et al., 2008). HIV-2 (Saad et al., 2008) and equine infectious anemia virus (EIAV) MA (Chen et al., 2008) have also been shown to bind directly to PI(4,5)P₂. All these studies suggest that HIV-1 and possibly other retroviruses bud from the lipid rafts in the PM and exploit MA-PI(4,5)P₂ interactions for virus assembly and release. Recently, Valenzuela-Fernandez and co-workers reported that PI(4,5)P₂ also plays a role in HIV-1 entry (Barrero-Villar et al., 2008) suggesting a role for this lipid in multiple steps in the HIV-1 replication cycle.

3.4. Lipid microdomains at the virological synapse

Early studies demonstrated that multiple-round HIV-1 replication takes place more efficiently via cell-cell transmission than by cell-free infection (Phillips, 1994; Sato et al., 1992). More recent studies (Arrighi et al., 2004; Fackler et al., 2007; Gousset et al., 2008; Igakura et al., 2003; Jolly et al., 2004; Jolly and Sattentau, 2004; McDonald et al., 2003; Piguet and Sattentau, 2004) demonstrated that retroviral cell-cell transmission occurs at intercellular contact sites that resemble immunological synapses (Grakoui et al., 1999; Monks et al., 1998). The virological synapse forms at the site of contact between virus-infected (effector or donor) cells and uninfected (target) cells, across which virus is transmitted (Jolly and Sattentau, 2005; Piguet and Sattentau, 2004). In the case of HIV-1, the virological synapse is formed by the recruitment of receptor (CD4) and coreceptors (CXCR4 and CCR5) from the target cell and viral Env, Gag and adhesion molecules in polarized, lipid raft-like patches on the effector cell (Fig. 8) (Jolly et al., 2004; Jolly and Sattentau, 2005). It has been reported that the formation of HIV-1 virological synapses between T cells is mediated by Env-receptor

interactions (Jolly et al., 2004) although Env expression is not required for recruitment of Gag to the macrophage-macrophage or macrophage-T-cell synapse (Gousset et al., 2008). The assembly of supramolecular synapse complexes relies on a functional actin cytoskeleton in the target cells (Jolly et al., 2004). Once Env and Gag are recruited to the cell-cell junction, virus transfer across the synapse into the target cell and rapid de novo reverse transcription take place (Davis et al., 1997; Jolly et al., 2004; Karageorgos et al., 1995). Because immunological synapses display raft-like properties (Harder et al., 2007) and HIV-1 particles bud from lipid raft microdomains, it appears likely that lipid rafts are involved in the formation and transfer of virus across the synapse. Indeed, it was reported that the raft marker GM1 concentrated with Gag and Env at the T-cell synapse and that cholesterol depletion with cyclodextrin disrupted the virological synapse (Jolly and Sattentau, 2005).

4. Inhibition of HIV-1 replication with lipid-modifying agents

4.1. Cholesterol-depleting agents

As discussed above, lipid rafts are involved in both early and late phases of the HIV-1 replication cycle. Disruption of lipid rafts with cholesterol-reducing agents interferes with both HIV-1 fusion/entry and assembly. Treating target cells or virions with BCD inhibits HIV-1 infection (Campbell et al., 2002, 2004; Graham et al., 2003; Guyader et al., 2002; Liao et al., 2001; Manes et al., 2000; Nguyen and Taub, 2002a; Popik et al., 2002) and treating virus-producing cells with BCD or statins also inhibits virus production and reduces the infectivity of the released virions (Ono and Freed, 2001; Pickl et al., 2001). The involvement of cholesterol in multiple steps of HIV-1 replication suggests that the disruption of cholesterol-rich membrane microdomains could induce antiretroviral activity. Khanna and co-workers examined this possibility by using severe combined immunodeficient (SCID) mice carrying human peripheral blood leukocytes (Hu-PBL-SCID) in which cell-associated HIV-1 was efficiently transmitted by vaginal inoculation of HIV-1-infected HuPBMCs (Khanna et al., 2002). Interestingly, application of 2-hydroxypropyl-β-cyclodextrin (2OHp-β-CD) to the vaginal mucosa prior to inoculation with HIV-1-infected cells significantly reduced virus transmission without damaging the vaginal epithelial cell membrane (Khanna et al., 2002). This study raises the possibility that cholesterol-depleting agents (e.g., the cyclodextrins) could be used clinically as chemo-preventative agents to block mucosal transmission of HIV-1.

Another approach that has been tested *in vivo* is the use of the statin drugs, which reduce cholesterol biosynthesis by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. HMG-CoA reductase produces mevalonic acid, a precursor for cholesterol biosynthesis, and isoprenoids, which can be attached to proteins posttranslationally. Statins are also known to inhibit the activity of Rho GTPase by preventing the prenylation of small G proteins by blocking HMG-CoA reductase (del Real et al., 2004). Manes and co-workers reported that lovastatin decreased HIV-1 replication in human PBMCs and reduced HIV-1 viral loads in SCID-Hu-PBMC mice and in chronically infected patients (del Real et al., 2004). The authors of this study observed that statin treatment disrupted viral entry and budding, perhaps by downregulating Rho GTPase activity (del Real et al., 2004). In contrast, several other groups failed to confirm any consistent effect of statin treatment on viral loads in patients (Moncunill et al., 2005; Probasco et al., 2008; Sklar et al., 2005). Collectively, these studies suggest that standard statin

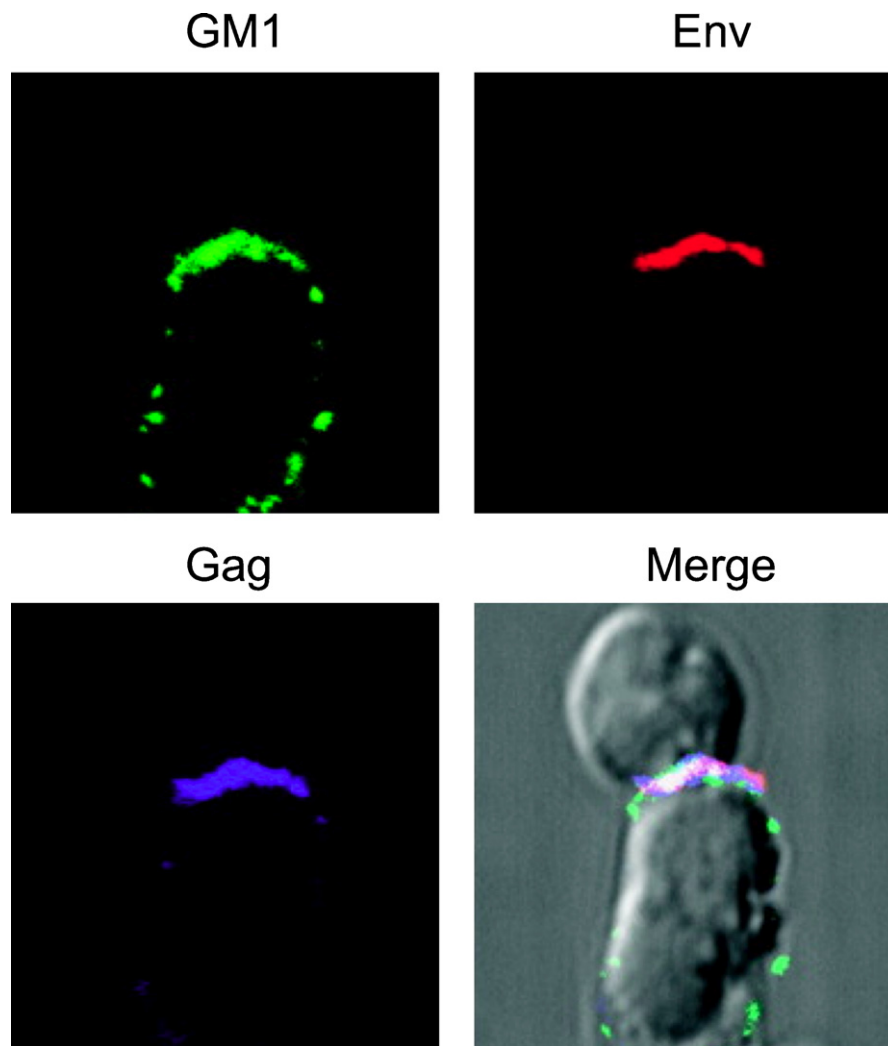


Fig. 8. HIV-1 Gag and Env concentrate in a raft-rich domain at the virological synapse. HIV-1-infected Jurkat effector cells (bottom) were incubated with primary CD4⁺ T cells (top) for 1 h at 37 °C during which the cells were stained for Env (red), fixed and stained for the raft marker GM1 with cholera toxin B subunit (green). Cells were permeabilized and labeled with anti-p17/p24 antibody (blue). Merging of three colors shows colocalization, which is superimposed in white on the corresponding Nomarski image. Reprinted with permission from Jolly and Sattentau (2005). Copyright 2005, The American Society for Microbiology.

therapy is not likely to be efficacious as an antiretroviral therapy *in vivo*.

4.2. Cholesterol-binding compounds

The above-mentioned lack of a reproducible effect of statin treatment on viral loads in patients led to the evaluation of the antiviral properties of cholesterol-binding compounds. The cholesterol-binding compound amphotericin B methyl ester (AME), a water-soluble, relatively non-cytotoxic derivative of amphotericin B, was observed to potently inhibit HIV-1 replication in culture (Hansen et al., 1990; Schaffner et al., 1986; Waheed et al., 2006). The antiviral activity of AME is due to defects in both viral entry and particle release (Waheed et al., 2006, 2007, 2008). Passaging of HIV-1 in the presence of AME led to viral resistance to this compound (Waheed et al., 2006, 2007). Mutations that conferred viral escape mapped to the cytoplasmic tail of gp41 (Waheed et al., 2006, 2007) and, remarkably, acted by creating novel PR cleavage sites in the tail, leading to truncation of gp41 after Env incorporation into virions (Waheed et al., 2007). Virus release inhibition by AME appears to result from an impaired ability of the HIV-1 accessory protein Vpu to enhance virus release (Waheed et al., 2008).

4.3. Inhibitors of sphingolipid synthesis

Sphingolipids are an important constituent of lipid rafts, and several studies suggested that modulation of sphingolipid biosynthesis has a profound effect on HIV-1 replication [reviewed in Rawat et al., 2005]. Reagents that act at various intermediate steps in this pathway have been tested for antiviral activity. L-Cycloserine, an inhibitor of serine palmitoyltransferase that inhibits the first enzyme of the sphingolipid pathway and prevents the biosynthesis of major species of sphingolipids, was reported to block HIV-1 infection and replication at least in part by downregulating CD4 expression (Mizrahi et al., 1996; Tamma et al., 1996). 1-Phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP), which inhibits glucosyl transferase activity and prevents the transfer of glucose to ceramide to form glucosylceramide, a precursor for all GSLs, has been shown to block HIV-1 entry and Env-mediated membrane fusion in various cell lines (Hug et al., 2000; Puri et al., 2004). Another inhibitor of the same pathway, N-butyldeoxynojirimycin (NB-DNJ), inhibited HIV-1 infection and syncytium formation in culture (Fischer et al., 1995, 1996), but did not demonstrate clinical efficacy in HIV-1-infected patients (Fischl et al., 1994). These studies indicate that GSL inhibitors possess anti-HIV-1 activity, though use of these inhibitors in a clinical setting will require further progress.

5. Conclusions and perspectives

It is now clear that substantial heterogeneity exists in biological membranes and that plasma membrane lipids and proteins are organized into a variety of dynamic microdomains. Many enveloped viruses, including HIV-1, have evolved to use one such microdomain, the lipid raft, during entry into and/or egress from their host cells. HIV-1 structural proteins (Gag and Env) and non-structural proteins (e.g., Nef) have been shown to associate with rafts, and host cell proteins used by HIV-1 during its replication cycle (e.g., CD4 and coreceptor) are also raft-localized. HIV-1 assembles in lipid rafts and cell–cell transmission occurs at raft-enriched virological synapses. The mechanism by which membrane microdomains promote the various steps involved in viral replication continues to be the focus of active investigation.

Although the role of lipid rafts in virus replication is well documented, many questions remain to be addressed. For example, which subsets of lipid microdomains are involved in the virus replication pathway, and what are the underlying mechanisms that direct specific raft targeting? What role do cellular factors play in targeting viral proteins to specific raft domains? Are lipid rafts a compulsory oligomerization platform and how do raft dynamics control the oligomerization and production of infectious mature virus particles? What factors regulate raft coalescence/dispersal and regulate the size of rafts under various conditions during the infection process? Perhaps most importantly, how can membrane microdomains that are critically important for a diversity of cellular processes be effectively targeted with antiviral compounds? Answering these and many additional questions will ultimately provide a more in-depth understanding of the relationship between viruses and membrane microdomains and will hopefully lead to new classes of antiretroviral therapies for treating HIV-1 infection.

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