Interaction of Mason-Pfizer monkey virus matrix protein with plasma membrane

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Budding is the final step of the late phase of retroviral life cycle. It begins with the interaction of Gag precursor with plasma membrane (PM) through its N-terminal domain, the matrix protein (MA). However, single genera of Retroviridae family differ in the way how they interact with PM. While in case of Lentiviruses (e.g., human immunodeficiency virus) the structural polyprotein precursor Gag interacts with cellular membrane prior to the assembly, Betaretroviruses (Mason-Pfizer monkey virus (M-PMV)) first assemble their virus-like particles (VLPs) in the pericentriolar region of the infected cell and therefore, already assembled particles interact with the membrane. Although both these types of retroviruses use similar mechanism of the interaction of Gag with the membrane, the difference in the site of assembly leads to some differences in the mechanism of the interaction. Here we describe the interaction of M-PMV MA with PM with emphasis on the structural aspects of the interaction with single phospholipids.

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and causes myristoyl to be released from the protein and ready for binding. The interaction of HIV-1 MA with PI(4,5)P2 composed of shorter fatty-acid chains (4 and 8 carbons in length), was experimentally proved as suitable for solution nuclear magnetic resonance (NMR) measurements because these soluble PI(4,5)P2 bind in a cleft between the second and fifth helix. The binding has also been confirmed for phosphatidylinositol phosphates (PIPs) containing natural fatty-acid residues (C18 and C20) either by interaction of MA with artificial liposomes mimicking PM or by blocking PI(4,5)P2 synthesis leading to the HIV-1 virus particles to be unable to assemble on PM (Chukkapalli et al., 2008).

The interaction of PIP was also proved for other retroviruses: HIV-2, moloney murine leukemia virus (MoMuLV) and equine infectious anemia virus (EIAV). HIV-2 MA interacts with PIP in a similar way as HIV-1 MA, but it was reported that the interaction with neither C4 nor C8 PI(4,5)P2 leads to the release of the myristate (Saad et al., 2008). The authors concluded that the reason was a weaker affinity of PI(4,5)P2 to the HIV-2 MA and further speculated that the rationale behind this phenomenon might be that HIV-2 is less infectious than HIV-1. Both HIV-1 and HIV-2 show stronger preference for PI(4,5)P2 compared to the other, differently phosphorylated PIPs. EIAV MA is naturally non-myristoylated, so its interaction is fully dependent on the interaction of basic amino-acid residues with membrane phospholipids (Chen et al., 2008). Chen has reported that PI(4,5)P2 specifically interacts with EIAV MA and also induces its oligomerization, which promotes the assembly of virus particle. MoMuLV MA also interacts with PIPs, but without any discrimination of PI(4,5)P2. However, in the presence of phosphatidylerine, it exhibits stronger and more specific interaction over other differently phosphorylated PIPs (Hamard-Peron et al., 2010). Similar behavior, i.e., preferential and stronger binding of a chosen phosphoinositide in the presence of other phospholipids, mostly in the form of micelles was also described for proteins bearing pleckstrin homology domain (Sugiki et al., 2012). An important role of different phospholipids for the interaction of HIV-1 MA with the PM has been proposed recently by Vlach and Saad (2013). They found that phosphatidylerine, phosphatidylcholine, and phosphatidylethanolamine bound to HIV-1 MA, however, to a different binding site than PI(4,5)P2 and that the interaction was weaker. The authors concluded that this interaction further stabilizes the binding of MA to the membrane.

The first evidence of the interaction of M-PMV MA with PI(4,5)P2 was reported by Stansell et al. (2007). She observed that depletion of PI(4,5)P2 from PM by overexpression of active form of PI-5-phosphatase IV led to 90% decrease of particles release from M-PMV infected cells. Direct evidence of the interaction of M-PMV MA with PI(4,5)P2 was then confirmed by Prchal et al. (2012).

Similarly to HIV-1 and HIV-2 MAs, the interaction of M-PMV MA with PI(4,5)P2 was studied using NMR spectroscopy and soluble forms of PI(4,5)P2 with 4 and 8 carbon fatty-acids. While dibutanol PI(4,5)P2 did not interact, dioctanoyl PI(4,5)P2 interacted specifically with Kd of about 100 μM, which is a comparable affinity as that of the interaction of HIV-1 MA with C8-PI(4,5)P2 (Saad et al., 2006). Similarly as for HIV-2, the interaction did not trigger the myristoyl switch.

The M-PMV MA molecule contains one PIP binding site located between the first, second and fourth helices (Figure 1). Comparison of the structures of the myristoylated and non-myristoylated M-PMV MAs showed that this binding site is present only on the surface of the myristoylated protein. Due to a slightly different orientation of the helices in the structure of the non-myristoylated MA, the proper binding pocket is inaccessible for PIP.

The PIP binding site follows the canonical shape of epitopes for binding phosphoinositides, i.e., it is composed of a hydrophobic pocket formed by all four helices and a patch of basic residues on the surface (Roth, 2004). This pocket is connected with the cavity where the myristoyl is sequestered. One of PIP’s fatty-acid chains is buried inside this hydrophobic pocket while the phosphates interact with positively charged amino acids forming the basic patch. The structure has been solved only for the complex of MA with C8-PIP, so it might be expected that one of the naturally long PIP’s fatty-acid chains will somehow interfere with the myristoyl which might lead to its exposure from the cavity. The PI(4,5)P2 molecule is sequestered deeper in the protein core, compared to HIV-1 MA, where PI(4,5)P2 remains on the surface of MA (Figure 2). The surface part of the interaction site is formed mainly by lysines and arginines from the loop between the first and second helices and terminal parts of the first, second and fourth helices. The electrostatic interaction between positively charged lysine residues (K16, K25, K27, K33, and K74) and negatively charged inositol phosphate groups is important for the interaction of M-PMV MA with the membrane, as it was proven by mutation studies (Stansell et al., 2007). Stansell found that mutations of basic residues in the proximity of PIP binding site influenced both the transport of immature viral particles and their binding to PM. Virus-like particles (VLPs) bearing mutations K16A or K20A budded into intracellular vesicles. This may indicate that the mutations disrupted the recognition
of the target membrane, likely by changing the affinity of MA for differently phosphorylated PIPs than PI(4,5)P₂. VLPs bearing R10A, R22A, K27A, K33A, or K39A mutations were accumulated near the PM, indicating that the mutations prevented the interaction of MA with PI(4,5)P₂, or other phospholipids in the membrane. The mutation K25A disrupted some early stages of VLP transport, since they were randomly distributed in the cytoplasm.

Accumulation of VLPs near PM can also be caused by mutations of non-basic amino-acid residues in M-PMV MA. Double mutations T41I/T78I, Y11F/Y28F, and Y28F/Y67F blocked the release of VLPs from the host cell, while single mutations only slowed down the release of VLPs, but failed to fully arrest it (except of T41I mutation, that showed wt-like virus release; Rhee and Hunter, 1991; Stansell et al., 2004). Since all these mutations introduce more hydrophobic amino acids, Stansell speculated that they created a pocket capable of stronger hydrophobic interactions of mutated residues with the myristoyl and thus block its release from the protein core and therefore, prevents the interaction with PM.

In summary, the MA interaction with the PM is an essential step of retroviral life cycle that allows virus release. A firm contact of Gag with the PM is mediated by the bimpartite signal, where the key player is the interaction of MA with PI(4,5)P₂. This ensures the selectivity for the PM over the membranes of cellular organelles.

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