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Roles played by acidic lipids in HIV-1 Gag membrane binding

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ABSTRACT

The MA domain mediates plasma membrane (PM) targeting of HIV-1 Gag, leading to particle assembly at the PM. The interaction between MA and acidic phospholipids, in addition to N-terminal myristoyl moiety, promotes Gag binding to lipid membranes. Among acidic phospholipids, PI(4,5)P_2, a PM-specific phosphoinositide, is essential for proper HIV-1 Gag localization to the PM and efficient virus particle production. Recent studies further revealed that MA-bound RNA negatively regulates HIV-1 Gag membrane binding and that PI(4,5)P_2 is necessary to overcome this RNA-imposed block. In this review, we will summarize the current understanding of Gag-membrane interactions and discuss potential roles played by acidic phospholipids.

1. Introduction

Acidic phospholipids play an important role in recruiting cytoplasmic proteins to membrane (Lemmon, 2008; McLaughlin and Murray, 2005; McLaughlin et al., 2002). They act as binding partners for these proteins thereby functioning as membrane anchors (Lemmon, 2008). The mechanisms of protein interactions with acidic lipids fall mainly into two broad categories. One category involves structure-dependent interactions of binding motifs or domains with specific lipid headgroups. A well-studied example of this class is the pleckstrin homology (PH) domain of phospholipase C δ1 ($PH_{PLC\delta1}$). $PH_{PLC\delta1}$ is shown to specifically bind to both plasma-membrane-specific phosphoinositide PI(4,5)P2 and its soluble headgroup analogue D-myo-inositiol 1,4,5 trisphosphate (McLaughlin and Murray, 2005; McLaughlin et al., 2002; Lemmon et al., 1995; Garcia et al., 1995; Kavran et al., 1998). The second category of protein interactions with acidic lipids is mediated by unstructured clusters of basic residues that mainly mediate non-specific electrostatic interactions with charged lipids in the membrane. For example, basic residues in proteins such as K-Ras, Src and MARCKS have been shown to bind acidic lipids via non-specific electrostatic interactions (McLaughlin and Murray, 2005; McLaughlin et al., 2002).

The structural protein of HIV-1, Gag, drives the assembly process of the virus particle (Balasubramaniam and Freed, 2011; Bieniasz, 2009; Sundquist and Krausslich, 2012). Gag is synthesized as a polyprotein $Pr55^{Gag}$, which contains four major structural domains, namely matrix (MA), capsid (CA), nucleocapsid (NC), and p6, as well as spacer peptides SP1 and SP2. Each of these domains performs a specific and distinct function during the assembly process. The N-terminal MA domain targets Gag to the site of assembly and mediates membrane binding. In addition, MA is important for incorporation of viral glycoprotein Env into assembling particles. Prior to or after Gag membrane binding, Gag forms multimers. This multimerization is promoted mainly by two downstream regions of Gag. The C-terminal domain of CA mediates Gag–Gag interactions through the dimerization interface, whereas NC promotes higher order multimerization via its ability to bind RNA. The NC domain has zinc finger motifs that determine the RNA specificity of NC in genomic RNA encapsidation although non-genomic RNAs can promote Gag multimerization. The p6 domain recruits the host ESCRT machinery that helps in the scission of the nascent virus particles from the membrane.

The subcellular location at which the virus assembles is an important determinant for efficient and productive virus particle production, since it affects the efficiency of accumulation of various virus components and the fate of the progeny virions after assembly. For HIV-1, assembly takes place at the plasma membrane (PM) in most cell types including a natural host, T cells. However, in another natural host, macrophages, it was initially observed that the virus assembles in structures resembling late-endosomal multivesicular bodies (MVBs) (Pelchen-Matthews et al., 2003; Raposo et al., 2002). However, more recent studies have suggested that these compartments, termed virus containing

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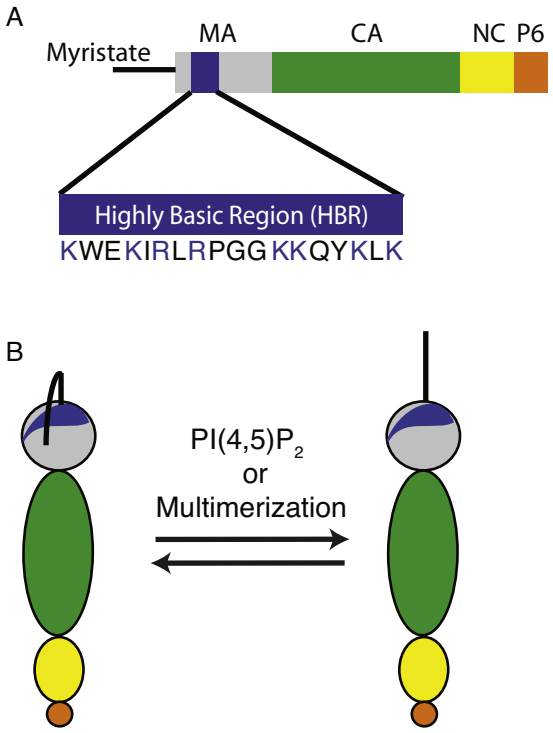

Fig. 1. (A) Bipartite signals, the myristate moiety and the highly basic region (blue), within the matrix domain (MA) (grey) are indicated. The amino acid sequence of the MA highly basic region (HBR) of HIV-1_{NL4-3} is shown (basic residues in blue). (B) HIV-1 Gag 'myristoyl-switch'. Gag exists either as myristate-sequestered (left) or myristate-exposed (right) forms. MA-PI(4,5)P_2 interaction or Gag multimerization induces exposure of the myristate moiety.

compartments (VCCs), are actually PM invaginations that extend deep into the cytoplasm (Bennett et al., 2009; Deneka et al., 2007; Welsch et al., 2007) although a population of them is not accessible to the external medium (Jouve et al., 2007).

Proper subcellular localization and membrane binding of retroviral Gag proteins are mediated by the N-terminal MA domain. In this article, we review our current understanding of the mechanisms by which Gag binds the cellular membranes with a particular emphasis on the role of acidic phospholipids.

2. Membrane binding of Gag via bipartite signals within MA

The N terminus of the MA domain is co-translationally modified by the addition of the 14-carbon acyl chain or the myristate moiety (Fig. 1A). Amino acid substitutions of either the N-terminal glycine that serves as the site for the attachment of the myristate moiety or residues that are essential for recognition by N-myristyol transferase result in defective membrane binding of Gag and virus release in cells (Freed et al., 1994; Gottlinger et al., 1989; Spearman et al., 1997; Bryant and Ratner, 1990). Consistent with these observations, using liposome flotation assay it was observed that myristoyl-deficient Gag proteins fail to bind membranes efficiently (Chukkapalli et al., 2010). Besides playing an essential role in membrane binding, myristate has also been implicated in oligomerization of HIV-1 MA (Tang et al., 2004).

Studies on many myristoylated proteins have shown that the myristate moiety can be either 'sequestered' within the protein molecule or 'exposed' to the environment around it (Fig. 1B). A transition between these states is termed 'myristoyl switch' (Tanaka et al., 1995; Nagar et al., 2003; Goldberg, 1998; Ames et al., 1997; McLaughlin and Aderem, 1995). A study that analyzed the membrane binding properties of the HIV-1 MA domain alone and full-length Gag found that the MA domain alone was not capable of binding to membranes whereas full-length Gag bound to membranes efficiently (Spearman et al., 1997; Hermida-Matsumoto and Resh, 1999; Sandefur et al., 1998; Zhou and Resh, 1996). Based on this observation it was proposed that the MA domain alone adopts a conformation different from full-length Gag and hypothesized that a conformational change might affect the exposure of myristate. Consistent with this hypothesis, some amino acid substitutions in MA impair Gag membrane binding without affecting myristoylation itself (Paillart and Gottlinger, 1999; Ono and Freed, 1999). Subsequently, NMR studies using myristoylated HIV-1 MA confirmed the existence of the myristoyl switch mechanism for HIV-1 MA (Tang et al., 2004). The study showed that the myristoyl moiety was sequestered within the hydrophobic cleft of the MA domain. Additionally, this study also found that MA exists as a mixture of a myristate-sequestered monomer and a myristate-exposed trimer in equilibrium, suggesting a link between myristoyl exposure and Gag–Gag association. Indeed, addition of the CA domain at the C terminus of MA shifted the equilibrium towards the myristate-exposed form, suggesting that promoting self-association between Gag molecules induces myristate exposure. Besides HIV-1, MA domains from other retroviruses such as HIV-2 and MPMV have also been shown to sequester myristate (Prchal et al., 2012; Saad et al., 2008). Interestingly, the study on HIV-1 MA showed that in addition to Gag multimerization, an interaction of MA with a soluble analogue of PI(4,5)P_2 triggers myristate exposure (discussed later) (Saad et al., 2006). In contrast, it was shown that PI(4,5)P_2 does not trigger myristate exposure of HIV-2 MA (Saad et al., 2008) and MPMV MA (Prchal et al., 2012). Therefore, mechanisms regulating myristate exposure might differ between different retroviral MA domains. In addition to PI(4,5)P_2 and Gag oligomerization, changes in pH and binding of calmodulin to MA have been reported to induce myristate exposure (Fledderman et al., 2010; Ghanam et al., 2010). Myristoyl switches have been shown to cause conformational changes in proteins such as ARF-1 (Goldberg, 1998), c-Abl (Nagar et al., 2003), and recoverin (Tanaka et al., 1995; Ames et al., 1997). In contrast, in the case of HIV-1, no large-scale changes in tertiary structures of the MA domain were observed regardless of whether myristate was sequestered or exposed (Tang et al., 2004). However, involvement of MA structural changes in the context of full length Gag and in the presence of lipid bilayers remains to be tested. Likewise, the requirement for various triggers remains to be examined in the presence of lipid bilayers, since spontaneous myristoyl exposure has been observed in NMR (Vlach and Saad, 2013) and in silico (Charlier et al., 2014).

Studies on other myristoylated proteins suggest that although essential, the myristate moiety alone is insufficient for stable retention of proteins on the membrane (McLaughlin and Aderem, 1995; Resh, 1996). It is thought that myristyolated proteins bind membranes reversibly and dissociate rapidly (Boman and Kahn, 1995; Peitzsch and McLaughlin, 1993). The lack of stability in membrane association mediated by myristate insertion is attributed at least partly to the length of the myristate acyl chain. The short 14-carbon myristoyl chain is thought to be inadequate in providing hydrophobic interactions sufficient for stable membrane association (Peitzsch and McLaughlin, 1993). It has been suggested that a 'secondary signal' besides the myristate moiety is necessary to achieve efficient and stable membrane binding. This is termed the 'two-signal hypothesis' (Resh, 2004). Many cytosolic proteins use the 'two-signal' mechanism for achieving stable and efficient membrane binding. For example, membrane binding of Src is mediated by the myristate moiety and the secondary signal that is provided in the form of a stretch of basic residues or 'polybasic cluster'. The polybasic cluster is known to mediate interactions with acidic phospholipids. The electrostatic interaction of the polybasic cluster with acidic lipids, along with the hydrophobic

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