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Beyond Plasma Membrane Targeting: Role of the MA domain of Gag in Retroviral Genome Encapsidation:

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Abstract

The MA (matrix) domain of the retroviral Gag polyprotein plays several critical roles during virus assembly. Although best known for targeting the Gag polyprotein to the inner leaflet of the plasma membrane for virus budding, more recently studies have revealed that MA also contributes to selective packaging of the genomic RNA (gRNA) into virions. In this perspective, we will summarize recent progress in understanding how MA participates in genome incorporation. We will compare the mechanisms by which the MA domains of different retroviral Gag proteins influence gRNA packaging, highlighting variations and similarities in how MA directs the subcellular trafficking of Gag, interacts with host factors, and binds to nucleic acids. A deeper understanding of how MA participates in these diverse functions at different stages in the virus assembly pathway will require more detailed information about the structure of the MA domain within the full-length Gag polyprotein. In particular, it will be necessary to understand the structural basis of the interaction of MA with gRNA, host transport factors, and membrane phospholipids. A better appreciation of the multiple roles MA plays in genome packaging and Gag localization may guide the development of novel antiviral strategies in the future.

Introduction

All infectious retroviruses contain two copies of positive-stranded genomic RNA (gRNA) packaged into virions as noncovalently linked dimers [1-4]. During the assembly of virus particles, the Gag polyprotein specifically packages gRNA, although a small amount of other viral RNAs and cellular RNAs are also found in virus particles [1, 5, 6]. What makes the process of gRNA packaging so challenging to decipher is the highly specific and selective binding of Gag to gRNA in preference to other RNAs.

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Viral RNAs undergo processing in the nucleus, just like cellular mRNAs, with the addition of a 5′ methylguanine cap and a 3′ polyA tail [7]. A portion of the viral RNA undergoes splicing to yield mRNAs that encode Env glycoproteins and accessory viral factors. The viral gRNA remains unspliced and is indistinguishable from viral mRNAs that are exported into the cytoplasm to synthesize the structural Gag and GagPol proteins. Both the viral gRNA and mRNA contain the psi (ψ) sequence, a highly structured *cis*-acting element located within the 5′-untranslated region (UTR) and/or upstream coding regions of the *gag* gene [1,2]. For some retroviruses, ψ is also present on spliced viral mRNAs, providing evidence that the ψ sequence itself is not sufficient to explain how gRNA is preferentially incorporated into virions [1,8].

Efforts to identify the *trans*-acting factors involved in gRNA packaging have centered on understanding the RNA-binding activity of the Gag polyprotein. The Gag protein possesses general nucleic acid binding activity but also specifically recognizes the ψ sequence in the context of the genome-length viral RNA. Gag is synthesized as a polyprotein precursor, and in this form Gag directs genome packaging and particle assembly within the infected cell. Once virions are released, the Gag precursor is cleaved into MA (matrix), CA (capsid), and NC (nucleocapsid) proteins plus additional peptide sequences that vary for each retrovirus [1]. A comparison of the Gag polyproteins and MA domain sequences of human immunodeficiency virus type 1 (HIV-1), Rous sarcoma virus (RSV), bovine leukemia virus (BLV), and human T cell lymphotropic/leukemia virus type 1 (HTLV-I) are depicted in Figure 1.

Despite the fact that the Gag precursor selects and packages the viral genome, many biochemical and biophysical studies to date have focused on the nucleic acid binding activities of the mature MA and NC proteins. One reason for this approach is that MA and NC are more amenable to *in vitro* biochemical methods than the full-length Gag protein due to technical challenges in purifying full-length Gag from recombinant expression systems [9-11]. Additionally, relatively pure MA and NC proteins could be readily isolated in high quantities from virions and subjected to biochemical analyses.

Studies focusing on NC demonstrate that it possesses high affinity for the ψ packaging sequence *in vitro*. Moreover, the NC domain of Gag determines whether its cognate genome is preferentially packaged over a competitor gRNA bearing the ψ sequence of a different retrovirus [2, 2-17]. However, the relatively low affinity of NC for viral RNA sequences outside of the ψ region [16-19] implies that cooperative protein-protein interactions may be stimulated by the nonspecific RNA binding of the Gag NC domain during virus particle morphogenesis. The observation that viral RNA serves a scaffolding role during virus biogenesis[5] highlights the importance of interactions between the NC domain in Gag and the viral genome in providing structural stability to the virus particle. However, it is possible that other regions of Gag, in particular the MA domain, also make contact with the gRNA during the process of particle assembly, as discussed below. Thus, despite the wealth of data pointing to NC as the critical determinant of specific genome packaging in most retroviruses, studies have revealed a complementary role for MA in specific encapsidation of gRNA.

The notion that MA might influence genome incorporation arose from early studies which reported that the mature MA proteins from different retroviruses has nucleic acids binding activity, with evidence of binding to both single stranded and double stranded RNA and DNA, although in most cases there was a lack of specificity (see Table 1)[17, 18, 20-23]. The biological relevance of the nonspecific nucleic acid binding activity of retroviral MA proteins was questioned for many years because MA is located near the lipid envelope of the virion, away from the ribonucleic acid core and crosslinking of virions did not show MA-

RNA interactions [1]. The examination of mature virions is potentially misleading, however, because gRNA packaging is mediated by the immature Gag polyprotein inside the cell, and intracellular Gag-gRNA interactions may be disrupted prior to particle release. Thus, the role of the MA domain of Gag in gRNA interactions is likely to be most crucial during immature particle formation.

In this article, we will summarize the current understanding of the role of the Gag MA domain in gRNA packaging, emphasizing the data available for HIV-1, RSV, BLV, and HTLV-I. One intriguing feature of this comparative approach is that the precise mechanisms by which the MA domains influence genome encapsidation appear to vary for different viruses. Perhaps future experiments will reveal more common themes as further information about the contribution of the Gag MA domain in selective gRNA packaging becomes available.

Direct and indirect roles of MA in genome packaging

HIV-1 MA binding to nucleic acids influences gRNA packaging and intracellular trafficking of Gag

Since the discovery of HIV-1 in 1983 [24], identifying the determinants of genome encapsidation has been the focus of intense study. Several investigators found that the NC domain binds directly to the ψ sequence by virtue of the zinc knuckle domains and basic residues [13, 25-33] and mediates Gag multimerization in conjunction with the CA domain of Gag [29, 34-37]. Genetic and imaging studies permitted the dissection of the sequence of events from Gag:gRNA binding to particle assembly, revealing that HIV-1 Gag-Gag interactions are initiated in the cytoplasm [38-41;]. Efforts to determine the subcellular location of Gag:gRNA recognition yielded different results, with data demonstrating the formation of viral ribonucleoprotein complexes near the nucleus at a pericentriolar site [42], within the cytoplasm [41], and at the plasma membrane [43]. Irrespective of where Gag:gRNA complexes form initially, they are subsequently targeted to the plasma membrane through a bipartitie signal consisting of an N-terminal myristic acid moiety and a cluster of basic residues in the Gag MA domain [44]. Studies suggest that the myristate is buried within the hydrophobic MA globular domain until Gag reaches the membrane, triggering a conformational change in MA and exposing the myristic acid for insertion into the lipid bilayer [45-47]. Binding of key basic residues in MA to the acidic phospholipid $PI(4,5)P_2$, which is enriched in the plasma membrane, may account for the specificity of Gag being targeted to the plasma membrane rather than to internal membranes [48-50].

Beyond its role in plasma membrane targeting and binding, HIV-1 MA also has nucleic acid binding activity with affinity for both RNA and DNA [51-53]. Although specific binding of HIV-1 MA to the ψ sequence has not been demonstrated, a basic-rich region of MA does bind with high affinity ($K_d = 5 \times 10^{-7}$ M) to an RNA molecule highly homologous to a segment of the *pol* sequence [54]. Viral mutants that disrupt this MA:RNA interaction exhibit a delay in replication, although the level of gRNA packaging in these mutant viruses was not examined. The region of MA that binds to this RNA was mapped to the N-terminal basic sequence, and substitution of two or more basic residues disrupted RNA binding. In support of the idea that the MA domain makes direct contact with the viral genome, basic residues in HIV-1 MA can substitute for the RNA-induced assembly functions of NC [52, 55]. *In vitro* RNA binding data suggest that Gag contains two independent RNA-binding sites, one in MA and the other in NC, that appear to contact RNA simultaneously [56].

Supporting this finding, structural studies reveal that the Gag protein adopts a U-shaped conformation in solution whereby the MA and NC domains are in close proximity [57, 58]. The conformation of Gag remains “folded over” when bound to RNA, but in the presence of

both nucleic aids and membranes a structural change is triggered, resulting in extension of the protein [58]. Thus, it appears that basic residues in both MA and NC each bind to the gRNA prior to membrane binding, although it is possible that other cellular RNAs present in HIV-1 virions may also interact with MA or NC [6]. This collection of experiments suggest that the HIV-1 Gag MA domain may directly play a role in genome encapsidation by interacting with gRNA, likely by binding to regions outside of ψ. As illustrated in Figure 2, the current model shows that the Gag MA and/or NC domains bind to gRNA in the cytoplasm, inducing dimers or small oligomers of Gag to nucleate on the viral RNA. As the Gag:gRNA complex approaches the plasma membrane, $PI(4,5)P_2$ competes with RNA for binding to MA, causing a conformational change in Gag. Gag-Gag interactions are strengthened cooperatively at the membrane, forming a dense aggregation of Gag:gRNA complexes that form an incomplete hexameric lattice that forms the immature virus particle [59-61].

RSV MA functions in nuclear localization of Gag

The alpharetrovirus RSV was discovered in 1910[62] and is the basis for many seminal discoveries, including the initial identification of the ψ RNA packaging element[63]. Further studies defined the minimal ψ packaging signal as a 160-nucleotide sequence that resides almost exclusively in the 5′ leader sequence of the genome, with its 3′ border just upstream of the splice donor site [6,64,65]. Thus, both unspliced (genome-length) and spliced viral RNAs contain the ψ site, raising the question of how the Gag protein can preferentially package the gRNA over viral mRNAs. In retroviruses like HIV-1, important portions of the packaging signal continue beyond the major 5′ splice site (several hundred bases into the *gag* coding region), making differentiation of gRNA from spliced viral mRNAs more straightforward because ψ is present only on the gRNA [11, 25, 28, 66-72]. Because of this variation in the location of ψ, it is conceivable that RSV and HIV-1 may rely on somewhat different strategies for specific, ψ-mediated gRNA selection.

The NC region of RSV Gag is the primary domain required for gRNA encapsidation. RNA interactions are mediated by basic residues flanking the two zinc-finger motifs in NC whereas residues within the zinc-fingers themselves bind directly to the minimal ψ sequence [6,64,65,73-76]. Interestingly, an RSV Gag mutant that contained the NC domain of murine leukemia virus in place of its own resulted in reduced, but not absent, packaging specificity [77]. This result, in addition to others, suggest that other regions of Gag may influence packaging efficiency in concert with NC [77,78]. In support of this possibility, mutants involving the N-terminal region of MA including deletions, basic residues substitutions, and alteration of the membrane-targeting domain are associated with defects in gRNA dimerization and selective encapsidation [79]. The decrease in selective gRNA packaging is due to changes in the protein sequence of MA rather than as a result of mutations at the RNA level that impair genome recognition or packaging. Because monomeric gRNA is packaged in these viral mutants, dimerization of the genome does not appear to be absolutely required for RSV encapsidation, unlike some other retroviruses. *In vitro* binding experiments support this finding, as the RSV NC protein binds tightly to ψ with 1:1 stoichiometry [73].

Recent studies have elucidated a mechanism by which the RSV MA domain influences gRNA packaging indirectly by virtue of its role in regulating Gag subcellular localization (Fig. 2) [80,82]. After its synthesis on cytosolic ribosomes, the RSV Gag polyprotein undergoes transient nuclear localization, a step that is required for efficient genome encapsidation [80,8]. Nuclear import occurs by virtue of nuclear localizations signals (NLS) in the MA and NC domains. The NLS in MA is a complex and nonclassical nuclear targeting signal that binds directly to importin-11, an unusual import receptor, to facilitate nuclear entry of Gag [83]. Another import factor, known as transportin SR or transportin-3, was also

identified as a mediator of MA nuclear entry, although its role in Gag nuclear targeting has not been investigated. A second NLS resides in the Gag NC domain and consists of a highly basic region that also is involved in nucleic acid binding [81]. This classical NLS binds directly to the canonical import receptor importin-α, which then recruits its cofactor importin-β to translocate Gag into the nucleus. Dissection of the molecular mechanism underlying nuclear import of Gag revealed that nucleic acids are effective competitors for binding of importin-α to NC. An RNA molecule containing the minimal ψ sequence was a much more effective competitor for binding to the NC domain of Gag compared to a nonviral RNA or DNA, demonstrating highly specific binding of ψ to the recombinant full-length RSV Gag protein *in vitro*.

RSV MA also possesses nucleic acid binding activity, with early experiments reporting an apparent disassociation constant of 1-10 nM for RSV RNA, and much lower affinity binding to ribosomal RNA (rRNA) [15,16]. Subsequent studies disputed the claim that there was any specificity of MA for viral RNA, instead finding that MA bound to viral RNA, ribosomal RNA, and DNA equally and with low affinity (Table 1) [21,84]. However, evidence for specific nucleic acid binding activity was demonstrated by the finding that RNA competes for RSV MA:importin-11 binding better than DNA does, although there is no specificity for ψ [81]. Surprisingly, the ψ sequence is a much more effective competitor than nonviral RNA for importin-11 binding to the full-length Gag protein, suggesting that the MA domain may contribute to specificity of the Gag:ψ RNA binding. This intriguing result can be explained by either (a) an indirect mechanism in which Gag NC:ψ binding induces a global structural change that disrupts MA:importin-11 interaction or (b) a direct mechanism in which Gag MA itself contacts the ψ sequence, displacing importin-11. Additional experimentation is needed to determine what direct role, if any, the specific interaction of the Gag MA domain plays in gRNA selection and packaging; however, it does seem clear that the MA and NC domains work together to spatially and temporally regulate RSV Gag nucleocytoplasmic localization, thereby influencing genome incorporation.

A combination of genetic and biochemical evidence suggest that once in the nucleus, RSV Gag recognizes and binds to the gRNA, inducing a conformational change that reveals a leucine-rich nuclear export signal (NES) within the p10 domain in Gag [80,81]. The Gag p10 NES binds directly to CRM1-RanGTP, a major exporter of RNA-binding proteins from the nucleus [81,82]. The Gag-gRNA complex is exported through the nuclear pore, shedding CRM1, and then travels to the plasma membrane where multimerization of Gag and budding occur. The nuclear localization of Gag proteins from other retroviruses (HIV-1[85], murine leukemia virus[86], Mason-Pfizer monkey virus[87] and foamy viruses [88-91]), retrotransposons (Ty1[92]), and retroelements [93,94] have also been reported (reviewed in [95]). In the case of HIV-1, the original report that the HIV-1 MA protein contains a NLS as well as a CRM1-dependent NES has been disputed [96-98]. Thus, whether HIV-1 Gag undergoes a nuclear trafficking step that is linked to gRNA packaging[85] is controversial and will require further investigation. For the human foamy virus Gag protein, nuclear localization is mediated by the RNA binding domain, leading investigators to hypothesize that foamy virus Gag picks up its gRNA in the nucleus[88]. Recently, a CRM1-dependent NES was identified and the authors again speculated that Gag:gRNA binding might occur in the nucleus, although no experiments were shown that directly test this hypothesis [89]. Thus, it is possible that directing Gag into the nucleus via the MA and NC sequences for selection of gRNA for packaging is a mechanism not unique to RSV, but may be shared by genetically diverse groups of retroviruses.

The BLV Gag MA domain plays a major role in selective gRNA packaging

Identification of the *cis* and *trans* factors required for genome packaging of the deltaretroviruses BLV led to some surprises. Rather than consisting of a concise, continuous

sequence limited to the 5′ untranslated region, the ψ sequence was found to be composed of two distinct segments of gRNA, one in the leader sequence and the other within coding sequence of *gag* [99-101]. Furthermore, although the BLV Gag NC domain contains two zinc finger domains and basic amino acids that are important for gRNA packaging[102], the mature BLV NC protein possesses only nonspecific RNA-binding activity, with no selectivity for viral RNA sequences containing ψ [22]. Instead of NC, the MA region of Gag plays a predominant role in specific selection and packaging of the genome [22,102, 103]. The BLV MA(p15) protein, which is further cleaved into MA(p10) and MA(p4) upon completion of virus maturation, binds specifically to two segments of viral RNA derived from the 5′ end of the genome: the first segment contains the viral RNA dimerization domain [22, 102, 103], while the second segment encompasses the ψ sequence, extending from the 5′ leader sequence through a portion of the *gag* gene [103]. Interestingly, the fully mature MA protein (p10) lacks specific RNA binding, implying that the immature form of MA(p15) present within the Gag precursor is involved in packaging of the gRNA by binding selectively to ψ [22]. Moreover, the MA(p15) protein, but not MA(p10), binds preferentially to a dimer of the 5′ leader viral RNA compared to the denatured viral RNA species. The MA(p15) protein:RNA complex apparently forms first, then dimerization of the 5′ viral RNA sequence ensues. Thus, binding of the MA(p15) region of BLV Gag to the 5′ leader viral RNA sequence containing both the dimerization signal and the ψ encapsidation signal may provide a mechanism to ensure that two genomes are specifically packaged into virions [22, 103].

In support of these *in vitro* studies, experiments that examined the role of the BLV MA and NC domains in gRNA encapsidation concluded that basic residues within both regions of Gag are required for optimal genome packaging[102]. The basic residues in BLV MA that are most important for gRNA packaging (K41 and H45, Fig. 1) are not involved in plasma membrane targeting of Gag. Furthermore, the codons for K41 and H45 lie outside of the ψ sequence, indicating that the deleterious effects of mutating K41 and H45 are likely mediated at the level of MA protein domain rather than due to an effect on gRNA structure or sequence [100-102]. Thus, for BLV the composite data suggest that MA and NC may both bind to ψ itself or to nearby RNA sequences that contribute to selective gRNA incorporation[102]. Alternatively, it is feasible that the MA and NC domains within a single BLV Gag molecule might bind to separate gRNA molecules as an alternative mechanism to incorporate two genomes into one virus particle (Fig. 2). Further structural studies will be needed to investigate how MA and NC act together to contribute to genome recognition and encapsidation.

Because mutants involving basic residues in the MA domain of the related deltaretrovirus HTLV-I MA [22, 102, 104] also impair infectivity but maintain plasma membrane localization, it has been suggested that HTLV-I MA [22, 102, 104] might also play a role in specific packaging of the gRNA[102]. This possibility is especially intriguing because of the functional conservation of several basic residues in the BLV and HTLV-I MA [22, 102, 104] sequences and the similarity of the three dimensional structures of the deltaretrovirus MA domains [105, 106]. While it is possible that all of the deltaretroviruses use similar mechanisms for genome encapsidation, there are no published studies to date that address this interesting possibility.

The Power of Comparative Retrovirology: Studies for the Future

Packaging of the retroviral gRNA into virus particles is essential for productive replication, hence disruption of Gag:gRNA binding is an attractive target for antiviral therapy. A critical step toward designing optimal packaging inhibitors is to understand the mechanism of encapsidation at the molecular level. The NC domain of Gag is certainly a critical determinant of in gRNA packaging, but it has become increasingly apparent that the Gag

MA region contributes to the specificity of genome recognition, influences the compartment of the cell where Gag:gRNA binding is initiated, and regulates the location and timing of the final encapsidation of gRNA into the assembling virus particle. The critical role of the Gag MA domain in genome packaging must be considered when developing antiretroviral agents that interfere with gRNA incorporation, rather than focusing solely on abrogating NC:ψ interactions [107-117].

Many of the regulatory activities of MA require the participation of host co-factors that mediate subcellular trafficking and compete with nucleic acids for binding to MA. In the case of RSV, the nuclear import factor importin-11 binds to the MA domain of Gag in the absence of nucleic acids to facilitate import of Gag into the nucleus where gRNA binding occurs [81]. Once the gRNA binds to Gag, importin-11 can no longer associate with the MA sequence, presumably to ensure that the Gag:gRNA complex has a one-way ticket out of the nucleus. It is perplexing that the RSV ψ sequence competes with MA:importin-11 binding better than nonviral RNA or DNA. Does this result imply that the RSV MA domain recognizes the ψ sequence specifically during genome binding or is the interaction due to a structural element in ψ that happens to bind to MA better than other RNAs? Further experiments will be needed to determine whether there is a significant biological role for the apparent preference of the MA domain for the ψ packaging element. In addition, the recent discovery that the foamy virus Gag protein contains an NES similar to that of RSV Gag has raised speculation that foamy virus might also select its genome genome in the nucleus[89]. Future investigations will reveal whether additional Gag proteins undergo transient nuclear trafficking for the purposes of gRNA encapsidation. If so, these Gag proteins may utilize different mechanisms for export other than CRM-1.

In contrast to RSV, the HIV-1 Gag MA domain appears to bind selectively to a sequence in the gRNA at a location other than ψ [54]. Apparently, the MA and NC domains of HIV-1 Gag both make contact with the gRNA in the cytoplasm. It is possible that the MA domain binds to a segment of the gRNA near ψ, and this interaction may enhance specificity by (a) altering the conformation of ψ, (b) by promoting genome dimerization, or (c) by inducing tighter binding of NC to ψ via an allosteric effect. Alternatively, if MA binds to a segment outside of ψ with high affinity, then Gag has two “handles” (the other being NC bound to ψ) to hold on more tightly to the gRNA. Once the HIV-1 Gag:gRNA complex approaches the periphery of the cell, the plasma membrane specific phosphoinositol $PI(4,5)P_2$ competes successfully with nucleic acids for binding to the MA domain [50,188]. The Gag MA:$PI(4,5)P_2$ association induces a conformational change in Gag that results in elongation of the protein, with the N terminus buried in the lipid bilayer of the plasma membrane and the C terminal region bound to the gRNA [57]. In this way, the MA domain regulates the timing of the extension of Gag and ensures that the switch in conformation occurs at the correct location —the plasma membrane. These carefully orchestrated events guarantee that the genome becomes encapsidated into the emerging virus particle during the budding process.

The mechanism of gRNA selection and packaging is less clear for the deltaretroviruses BLV and HTLV-I, but it appears that the MA and NC domains share in facilitating specific genome binding. Whether the MA and NC regions of BLV Gag bind to the same or different gRNA molecules is not known (Figure 2); this question is worth pursuing because it may provide a key insight into how retroviruses ensure that two genomes are incorporated into every virion. The observation that the BLV Gag MA domain has specific affinity for the ψ packaging signal also raises the question of whether MA remains associated with the gRNA within the immature and mature virus particles. Many of the details regarding the mechanism of specific RNA packaging for HTLV-I remain poorly defined, although a recent report suggests it does that HTLV-I and HIV-1 MA have fundamentally different mechanisms of interaction with both nucleic acids and membranes[119].

It is curious that different retroviruses appear to have evolved distinct mechanisms to govern genomic RNA encapsidation even though the outcome of the process is remarkably conserved throughout the retrovirus family. The Gag MA domain has either leading or supportive roles in genome selection and encapsidation depending on the virus. Although the mechanistic details may differ, comparative studies across retroviral genera have provided compelling support for the involvement of MA in genomic RNA packaging. The value of these comparative studies has elevated the importance of the observations made in each individual virus and has emphasized the need for continuing to study the properties of multiple retroviruses.

In the future, studies investigating the role of the Gag MA domain in gRNA packaging should focus on determining where in the host cell Gag initially binds the genome, how MA facilitates subcellular trafficking of the viral ribonucleoprotein complex, and the influence of MA:gRNA interactions on Gag multimerization. It is hopeful that determining the ultrastructural properties of Gag bound to the gRNA at high resolution will reveal whether MA contacts the RNA in an immature virus particle or whether this interaction is limited to the intracellular environment. Perhaps the most difficult yet enormously informative experiments will be to solve high-resolution structures of Gag (with and without viral RNA) in complex with its cellular binding partners, including protein co-factors and membrane components. Defining alternative structures of Gag may yield clues about the dynamic conformational changes induced by transient interactions with RNA, proteins, and lipids that are needed to complete the complicated journey from the ribosome to the site of assembly on the plasma membrane. Successful outcomes of these experimental approaches will be the critical next steps in elucidating common themes and uncovering distinct roles for the MA domain of retroviral Gag proteins in encapsidation of the viral RNA genome.

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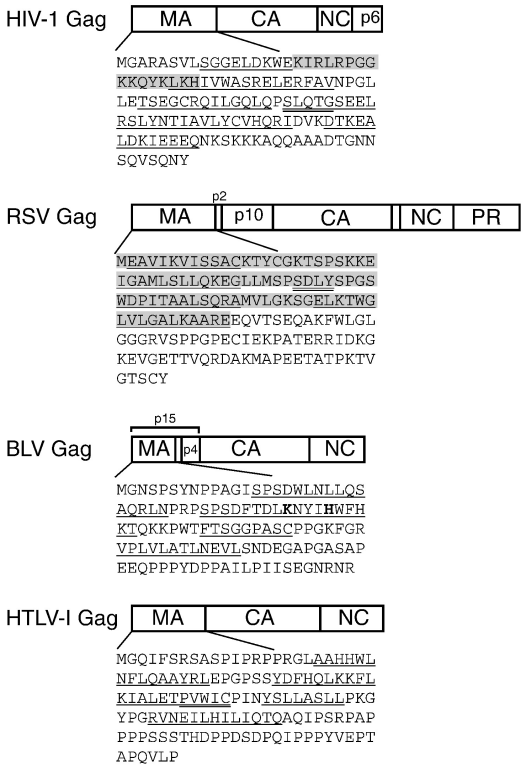

Figure. 1. Domain structure of Gag and sequences of the MA domains of RSV, HIV-1, BLV, and HTLV-1

The organization of the domains in retroviral Gag polyproteins discussed in this review (MA, matrix; CA, capsid; NC, nucleocapsid); PR (protease). The amino acid sequences of each MA domain are indicated, with the α-helices underlined and the 3_{10} helices underlined with double lines based on coordinates defined by three-dimensional structural analyses [106, 120-122]. Residues in bold-face type represent residues that are required for interactions with nucleic acids. In HIV-1 MA, the gray box denotes the highly basic region (HBR) which is involved in nucleic acid binding, $PIP(4,5)_2$ interaction, and plasma membrane targeting. For RSV MA, the gray box delineates the membrane-binding domain and the NLS in MA. The boundaries of the helices shown for HTLV-I MA are predicted from the three-dimensional structure solved for the homologous HTLV-II MA protein[106]. Residues indicated in bold-type in the BLV MA sequence have been implicated in genome encapsidation[102].

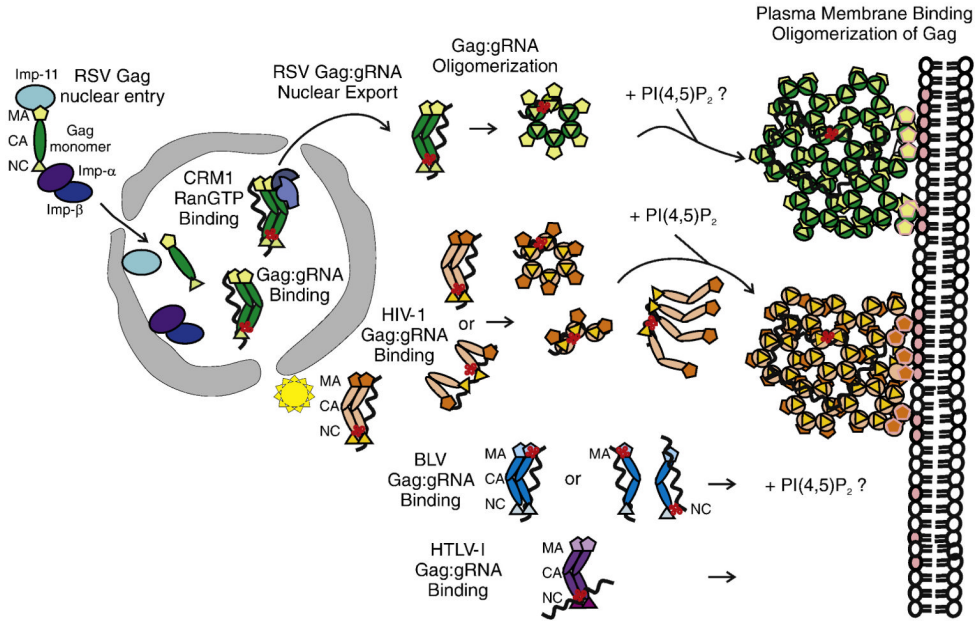

Fig. 2. Model illustrating the role of the Gag MA domain in regulating gRNA binding, subcellular trafficking, genome encapsidation and particle assembly

After synthesis in the cytoplasm, Gag proteins destined to bind the gRNA for packaging are transported to specific subcellular locations. The MA domain of Gag is represented by pentagons, CA by ovals, and NC by triangles. The gRNA is a wavy black line and the ψ sequence is depicted as a red cloverleaf. For RSV Gag (green), NLSs in the MA and NC domains interact with host import factors importin-11 and the importin-α/β complex to direct Gag into the nucleus where Gag interacts with the ψ sequence on the gRNA. RSV Gag:gRNA binding induces a conformation change in RSV Gag that promotes binding to CRM1:RanGTP, facilitating nuclear export. RSV Gag forms oligomers that are transported to the plasma membrane, possibly through an interaction with the phosphoinositol $PIP(4,5)_2$ (denoted by pink ovals in the inner leaflet of the lipid bilayer). A discontinuous hexameric lattice of RSV Gag proteins bound to gRNA assembles at the membrane, encapsidating the genome into the assembling particle. For HIV (orange), Gag interacts with its gRNA at a pericentriolar location (illustrated as a yellow star) or in the cytoplasm, inducing Gag dimer formation. The model illustrates the MA and NC domains interacting with gRNA in an extended conformation (top) or in a folded conformation (bottom), with the NC domain binding to ψ and the MA domain binding to the gRNA at a different location. It is possible that the MA domain is bound to a cellular RNA rather than to the gRNA. HIV-1 Gag:gRNA oligomers form and are transported toward the periphery of the cell. Upon binding to PIP(4,5)2, the MA domain releases the gRNA and Gag adopts an extended conformation with MA facing the membrane and NC binding to the gRNA. The hexamers of Gag associate with the plasma membrane and assemble into a hexameric lattice. For BLV (blue), the site of Gag:gRNA complex formation is not known. The MA domain of BLV Gag is shown binding to the ψ sequence, although the NC domain also contacts the gRNA through nonspecific interactions. Further details regarding the mechanism of BLV Gag assembly are not well understood. For HTLV-I, the model depicts the ψ sequence on the gRNA binding to the NC domain of Gag because it is not known whether MA plays a role in genome encapsidation. It has been shown that nucleic acid binding does not influence membrane binding, and $PIP(4,5)_2$ is not required for membrane targeting[119]

Table 1

In vitro binding affinities for retroviral MA proteins and nucleic acids

Retrovirus MA Protein	Binding Affinity K_d (M)	Nucleic acid	Reference
HIV	$3\text{-}30 \times 10^{-9}$	RNA 50mer (SELEX)	56
HIV	5×10^{-7}	RNA with homology to the *pol* region	54
HIV	1.5×10^{-5}	ssDNA 30mer	51
HIV	5.42×10^{-7}	ssDNA 20mer	50
HIV	3.25×10^{-7}	minihelix^{Lys}	50
RSV	3.6×10^{-12}	Viral RNA	17,18
RSV	1×10^{-7}*	Viral RNA	22

*Intrinsic Kd estimated at 2.9×10^{-3} to 9.1×10^{-4} M.