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Function of a retrotransposon nucleocapsid protein

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Key words: nucleocapsid, retroelement, retrotransposon, retrovirus, Ty, reverse transcription, assembly

Long terminal repeat (LTR) retrotransposons are not only the ancient predecessors of retroviruses, but they constitute significant fractions of the genomes of many eukaryotic species. Studies of their structure and function are motivated by opportunities to gain insight into common functions of retroviruses and retrotransposons, diverse mechanisms of intracellular genomic mobility and host factors that diminish or enhance retrotransposition. This review focuses on the nucleocapsid (NC) protein of a *Saccharomyces cerevisiae* LTR retrotransposon, the metavirus, Ty3. Retrovirus NC promotes genomic (g)RNA dimerization and packaging, tRNA primer annealing, reverse transcription strand transfers, and host protein interactions with gRNA. Studies of Ty3 NC have revealed key roles for Ty3 NC in formation of retroelement assembly sites (retrosomes), and in chaperoning primer tRNA to both dimerize and circularize Ty3 gRNA. We speculate that Ty3 NC, together with P-body and stress-granule proteins, plays a role in transitioning Ty3 RNA from translation template to gRNA, and that interactions between the acidic spacer domain of Ty3 Gag3 and the adjacent basic NC domain control condensation of the virus-like particle.

Introduction

Long terminal repeat (LTR) retrotransposons are ancient intracellular precursors of retroviruses.[1-3] These elements have common functions with retroviruses[4,5] in that they assemble polyprotein precursors around genomic (g)RNA to form virus-like particles (VLPs), reverse transcribe the gRNA, and integrate the complementary (c)DNA copy into host chromosomes. Similar to retroviruses, a RNA-binding domain resides within the retrotransposon major structural polyprotein precursor or Gag protein and cis-acting packaging sequences (psi) reside within the RNA. For a subset of LTR retrotransposons, the processed form of the RNA-binding domain is homologous to retrovirus nucleocapsid (NC). Retrovirus NC proteins range in MW from 7 to 15 kDa. They provide aggregation and destabilization chaperone activities to allow duplex formation and annealing reactions critical for particle formation and replication. Despite its small size, NC not only performs these functions, but more recently has been implicated in aspects of VLP assembly involving interactions with a diverse array of host factors.

Retroviruses differ from retrotransposons in that they traffic to the cell surface, require envelopment for maturation, and enter new host cells prior to uncoating, reverse transcription and integration. In contrast, retrotransposons undergo maturation, reverse transcription, uncoating and integration within a single host cell. Enveloped, immature spherical retrovirus particles undergo reorganization as a result of proteolytic maturation. In contrast, retrotransposons must achieve a similar state competent to uncoat in the absence of the enveloped microenvironment. There is a considerable amount known about retrovirus NC (reviewed in ref. 6–10); on the other hand, relatively few retrotransposon NC proteins have been investigated and much less is known. In this review we compare functions of retrovirus NC to functions of a retrotransposon NC, in order to identify common and therefore central functions, as well as different and potentially more specialized functions. The *Saccharomyces cerevisiae Metaviridae* Ty3 NC protein has been investigated in vivo and in vitro. In this review we focus primarily on Ty3 NC and compare its functions to the functions of retrovirus NC proteins, particularly those of human immunodeficiency virus (HIV)-1 NC. The genomics structures of Ty3 and HIV-1 are shown in **Figure 1**.

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Overview of Retrovirus and LTR Retrotransposon Structure

Retrovirus genome and protein structure. The integrated form of the retrovirus has LTRs (U3-R-U5) flanking the coding region (**Fig. 1B**).[4] Viral gRNA is initiated in the upstream LTR and terminated at a position downstream of the initiation site in the downstream LTR. Thus, the transcript can be described as R-U5-coding sequence-U3-R, where U5 and U3 represent sequences uniquely present on the 5' and 3' ends of the RNA, respectively, and R represents sequence repeated on both ends. The retrovirus genome is diploid and the two gRNAs dimerize over a several hundred-nt region to template production of a full-length cDNA copy (reviewed in ref. 8). Although retroviruses display considerable diversity, gRNAs have in common three open reading frames (ORFs). Overlapping *gag* and *pol* reading frames encode major structural (Gag) and structural-catalytic

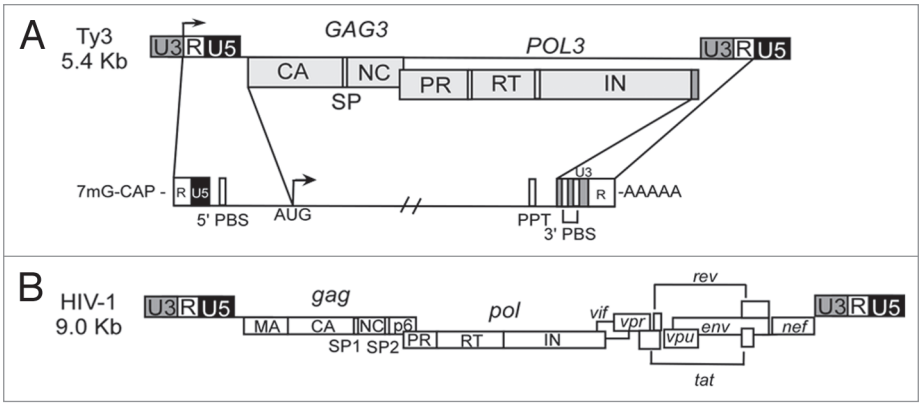

Figure 1. Ty3 and HIV-1 genome organization. (A) Ty3 genome and Ty3 RNA features. *GAG3* encodes Gag3 containing capsid (CA), spacer (SP) and nucleocapsid (NC) domains; *POL3* is translated as part of Gag3-Pol3 via a programmed frameshift and encodes protease (PR), junction (J), reverse transcriptase (RT) and integrase (IN) domains. Domains are processed into mature proteins by Ty3 PR. Ty3 RNA is capped, polyadenylated, and contains a bipartite reverse transcription primer $tRNA_{i\text{-}Met}$ binding site (PBS) in the 5′ leader sequence and in the U3 region of the transcript. Boxes at the ends of the genes indicate regions represented uniquely in the 5′ (U5), and 3′ (U3) ends of the RNA. The repeated (R) sequence is present at both ends. (B) HIV-1 genome. *Gag* encodes matrix (MA), CA, SP1, NC, SP2 and p6; *pol* encodes PR, RT and IN domains; a spliced RNA contains env which encodes envelope (Env); and vif, vpr, vpu, nef and tat are HIV-specific proteins encoded by spliced RNAs. Domains within retroviral Gag and Gag-Pol are processed by PR into mature species. U5, U3 and R are as in (A).

polyproteins (Gag-Pol). Gag-Pol is most typically expressed via programmed frameshifting which insures that it is produced at lower levels than Gag. The third ORF encodes the envelope protein (Env) expressed from a spliced RNA.[11]

Despite commonality of structure and function, retroviral Gag proteins are not highly conserved in sequence. Gag domains processed into mature proteins common to most retroviruses include matrix (MA), capsid (CA) and a RNA binding domain (reviewed in ref. 12–14). Gag-Pol is processed during maturation into Gag-derived species as well as protease (PR), reverse transcriptase (RT) and integrase (IN). For foamy viruses, the RNA-binding domain has an arginine-rich RNA-binding motif;[15] for alpha-, beta- and gamma-oncoretroviruses and lentiretroviruses the RNA-binding domain is NC. It contains one or two $\mathbf{C}X_2\mathbf{C}X_4\mathbf{H}X_2\mathbf{C}$ zinc finger (ZF) motifs embedded in a region enriched for charged basic and Asn and Gln residues (reviewed in ref. 8–10). These domains are discussed in more detail below.

Retrovirus VLP assembly can be intracellular, as in the cases of betaretroviruses and foamy virus, or can occur on the plasma membrane, as in the cases of alpha- and gammaretroviruses and lentiviruses. VLPs or multimeric precursors, respectively, are translocated to the plasma membrane where they bud in areas enriched in lipid rafts associated with Env protein (reviewed in ref. 16 and 17). Within the context of the enveloped core, PR is activated and cleaves Gag and Gag-Pol polyprotein precursors into mature MA, CA, NC, PR, RT and IN and other virus-specific proteins, a process which is associated with rearrangement of the spherical immature core into its characteristic mature form. With the exception of foamy viruses,[18] cDNA synthesis prior to infection of the new host cells is limited to a single-stranded R-U5 complementary (c)DNA known as minus-strand strong-stop (-SSS). Within the new host cell, cDNA synthesis continues within a reverse transcription complex that is translocated along the microtubule network toward the nucleus. Finally, a pre-integration complex is translocated into the nucleus and integration of the cDNA completes the cycle (reviewed in ref. 19).

LTR retrotransposon genome organization. LTR retrotransposons have a similar genomic structure to that described above for retroviruses and encode major structural and catalytic proteins (**Fig. 1A**).[1-3] As used in this review, the term retroelement will refer to retroviruses and retrotransposons. Retrotransposons are typically expressed, similarly to retroviruses, from overlapping ORFs. Because the exemplary retrotransposons in this review will be mainly budding yeast *S. cerevisiae* Ty elements (reviewed in ref. 20 and 21), we use the terms *GAG* and *POL* which follow the budding yeast convention of italicized and capitalized "gene" names to refer to the structural and catalytic protein ORFs, respectively. Ty1 *GAG* and *POL* are also sometimes referred to as *TYA* and *TYB*, respectively. Some LTR retrotransposons, including gypsy in the Metaviridae family, encode Env proteins that mediate an optional extra-cellular replication cycle.[22] Thus, the classification of a retroelement as a retrotransposon may be more related to its ability to replicate intra-cellularly and its relatedness to other retrotransposons than to its inability to infect new host cells. One clear reflection of the intracellular lifecycle of retrotransposons compared to retroviruses is the simpler domain structure of Gag, which can be comprised simply of CA and RNA-binding domains.

Five major classes of LTR retrotransposons are cataloged by the International Committee on Viral Taxonomy (ictvonline.org). Of these elements, the Pseudoviridae (previously copia-Ty1) and Metaviridae (previously gypsy-Ty3) families are most similar to retroviruses according to alignments within the Pol domain.[1] *POL* ORF organization and sequence of Metaviruses (PR-RT-IN) are more similar to those of retroviruses than are those of Pseudoviruses (PR-IN-RT). There is surprising variability within families with respect to the type of RNA-binding domain. The Metaviridae family of LTR retrotransposons includes Errantiviruses (gypsy and ZAM); Metaviruses (Ty3, Tf1, SURL and Sushi); and Semtoviruses (Tas and Bel). The Pseudoviridae family includes Pseudoviruses (Ty1, 2 and 4); Hemiviruses (Ty5 and copia); and Sireviruses (SIRE1). However, the RNA-binding domains have diverged in complex ways. For example, Metavirus Tf1 and the Pseudovirus Ty1 lack the $\mathbf{C}X_2\mathbf{C}X_4\mathbf{H}X_2\mathbf{C}$ ZF, while the Pseudoviruses copia and Ty5 and the Metavirus Ty3 contain versions of this domain. Where it occurs, the motif is not as conserved among retrotransposons as it is among retroviruses (reviewed in ref. 23).

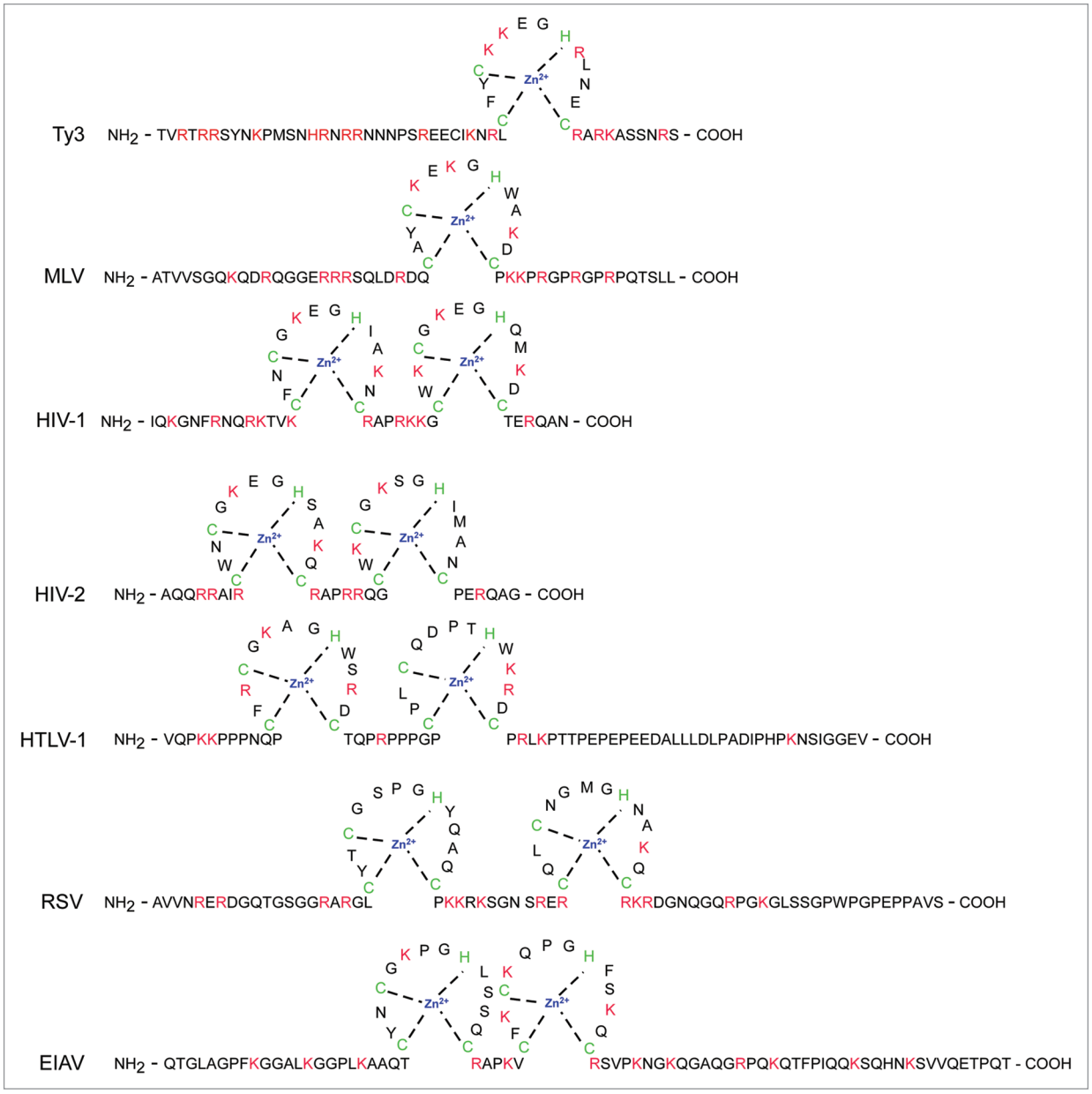

Figure 2. Ty3 and retroviral nucleocapsid proteins. Full-length sequences of NC proteins containing one or two conserved ZF motifs from the retrotransposon Ty3 and several retroviruses. Red, basic aa residues; green, conserved residues within ZF motifs depicted coordinating Zn^{2+}. Sequence accession numbers: Ty3 AAA98434.2; MLV, J02255; HIV-1, AF324493; HIV-2, AAA76840.1; HTLV-1, AAB20767.1; RSV, AAC82560.1; and EIAV, AAA43011.1.

The TY3 Model

Ty3 genome and proteins. The Ty3 Metavirus adheres to a subset of the general properties of retroviruses described above. It is 5.4 kb in length, comprised of 340-bp LTRs and overlapping *GAG3* and *POL3* ORFs (**Fig. 1A**). It is transcribed into a R-U5-*GAG3*-*POL3*-U3-R polyadenylated RNA in which *POL3* slightly overlaps the downstream U3 region. *GAG3* and *POL3* are translated into Gag3 and Gag3-Pol3 polyproteins that assemble into VLPs (reviewed in ref. 21). A high Gag3:Gag3-Pol3 ratio is maintained by programmed frameshifting.[24,25] Similar to some retroviruses, including HIV-1 (**Fig. 1**),[26] and Rous sarcoma virus (RSV),[27] CA and NC are separated by a short and essential spacer (SP). The 290-aa Gag3 is processed into mature 206-aa CA, 26-aa SP and 57-aa NC.[28,29] Gag3-Pol3 is processed into those proteins and PR, junction (J),[30] RT and IN.[29,31] Because NC is encoded at the downstream end of *GAG3*, the coding region actually spans the frameshifting site so that a 76-aa NC protein is generated by processing of Gag3-Pol3 at SP-NC and at the amino-terminus of PR.[32] Ty3 NC is similar in size and charge to retroviral NC species (**Fig. 2**). Thus, Ty3 provides a relevant model in *S. cerevisiae* for studying aspects of NC function that might be conserved or divergent depending upon requirements for viral and retrotransposon lifecycles.

Experimental control of Ty3 expression and localization of RNA and protein products. Because retrotransposons are endogenous they can accumulate in multiple copies in host genomes and confound studies with single experimentally-manipulated elements. In the case of Ty3 there relatively few native elements in common laboratory strains. In addition, strains were developed from which endogenous elements were deleted so that experimental Ty3 elements provide the sole source of Ty3 protein and gRNA.[33] Expression is naturally induced by exposure of cells to pheromone during mating.[33,34] For some experimental applications, Ty3 elements are used in which the *GAL1-10* upstream activating sequence is substituted for the upstream U3 region so that transcription can be artificially upregulated upon exposure to galactose as a carbon source.[35]

Ty3 proteins are monitored in living cells by fusing the green or red fluorescent protein (G/RFP)-coding regions in-frame to the downstream end of *GAG3* or *POL3* or in fixed cells using antibodies against Ty3 CA, NC and IN and fluorescent secondary antibodies (reviewed in ref. 31 and 36). Intracellular localization of Ty3 RNA has been monitored by tagging individual RNAs with the RNA-binding site for the MS2 phage capsid protein and co-expressing MS2 fused to RFP or GFP[36] or by fluorescence in situ hybridization (FISH) (unpublished data). Examination of cells expressing Ty3 using microscopic imaging of fluorescently-tagged Ty3 protein or RNA shows that the majority of cells form clusters of Ty3 RNA and proteins. Immuno- and transmission electron microscopy (TEM) showed that these foci also contain clusters of VLPs. These clusters are associated with RNA processing (P)-body[36] and stress-granule proteins (Sandmeyer SB and Bilanchone V, unpublished data) and have been proposed to represent sites of intracellular assembly.[36] We refer to retrotransposon assembly foci as "retrosomes".

Retroviral and TY3 NC Proteins

Retroviral NC function. The structure and functions of retroviral NC have been the subject of several recent excellent reviews.[6-10] The retroviral Gag NC domain or the mature NC engage in multiple aspects of the replication cycle. Activities include gRNA dimerization and packaging, Gag nuclear translocation, Gag multimerization, primer tRNA annealing, cDNA strand transfers, nonspecific reverse transcription suppression and integration enhancement. In addition to direct involvement of NC in these steps, NC mediates viral interactions with a remarkable assortment of host factors. The gamma retrovirus Moloney murine leukemia virus (MoMLV) NC has one ZF, but most other retrovirus NC domains have two copies (**Fig. 2**). The first of these is typically closer to the overall consensus: $\mathbf{C}_1\phi_2\mathrm{X}\mathbf{C}_4\mathrm{G}_5\mathrm{XXG}_8\mathbf{H}_9\mathrm{XXXD/E}_{14}\mathbf{C}_{15}$. MoMLV also has a slight variation of the consensus: $\mathbf{C}_1\mathrm{A}_2\mathrm{Y}_3\mathbf{C}_4\mathrm{K}_5\mathrm{XXG}_8\mathbf{H}_9\mathrm{XXXD}_{14}\mathbf{C}_{15}$.

Ty3 NC proteins. Mature Ty3 NC is processed from Gag3 (CA-SP-NC) and Gag3-Pol3. The mature 57 aa NC protein produced from Gag3 is similar to most retroviral NC proteins in size (MW = 6924.82) and charge (pI = 11.15). Processing of Ty3 Gag3-Pol3 produces an additional minor 76-aa species. However, there is no evidence that NC derived from Gag3-Pol3 has a unique function. Experiments in which the two Ty3 NC forms were provided from elements that were independently mutated showed that either source of NC was sufficient to support transposition, although relative stoichiometry of the assembled species was unrestricted in the experimental design.[32] The 57-aa NC is basic due to the presence of seventeen charged basic residues and nine Asn residues. It contains a single ZF motif. The Ty3 version is $\mathbf{C}_1\mathrm{F}_2\mathrm{Y}_3\mathbf{C}_4\mathrm{K}_5\mathrm{XXG}_8\mathbf{H}_9\mathrm{XXXE}_{14}\mathbf{C}_{15}$, which is similar to the retrovirus ZF consensus, but has aromatic residues at both the second and third positions and lacks the highly-conserved G_5. Interestingly, The aromatic residue at position 3 and the absence of G_5 in the MoMLV NC ZF are similar to the Ty3 variation. This review will focus on the functions of NC related to stages of the retrotransposon replication cycle having to do with RNA.

Initiation of Assembly

Gag assembly domains. Three major domains have been implicated in retroviral particle assembly: first, the MA M domain, including an amino-terminal acylation site and concentration of basic residues, both of which promote membrane association; second, a domain at the carboxyl-terminal end of CA, in the case of HIV-1 including SP1, which participates in Gag-Gag interactions; and third, the NC domain which mediates interaction with the RNA scaffold and thereby indirectly promotes multimerization.[37-40] Of these, in the case of HIV-1, the CA-SP1 domain contributes to assembly primarily through inter-Gag interactions.[41] Retrovirus Gag also contains one or more late (L) motifs of several amino acids that provide for interactions with components of the cellular endosomal sorting complexes required for transport (ESCRT). These interactions are required for envelopment of the assembled particle during budding (reviewed in ref. 16).

Ty3 CA modeling suggests that, similar to retroviral CA, it is composed of an amino-terminal domain (NTD) bundle of alpha helices (residues 2–139) and smaller carboxyl-terminal domain (CTD) bundle (residues 148–207).[42] Two-hybrid assays showed that the NTD interacts both with itself and with the CTD[43] and particle formation is sensitive to mutations in the first 100 residues of the NTD.[42] Mutations in some but not all residues in the conserved ZF profoundly disrupt VLP formation,[44] consistent with the model that specific Gag3 binding to RNA is important for the correct particle structure. Ty3 particles visualized by AFM are roughly spherical and have broadly-distributed diameters of approximately 42 nM. The distribution of surface capsomeres show elements of five-fold and six-fold symmetry consistent with an icosahedral-like structure.[45]

Cytoplasmic trafficking and collection of retroviral VLP components. The natural overlap of retroelement transcription, translation and assembly complicates clear definition temporally and spatially of the point at which assembly initiates. Nonetheless, evidence supports the general view that components concentrate at subcellular locations as part of the assembly process.[46] Retrovirus assembly progresses through translation of the RNA to produce Gag and Gag-Pol; commitment of RNA to assembly; concentration of gRNA, Gag and Gag-Pol at the pre-assembly or

assembly site; and assembly and budding (reviewed in ref. 6, 16, 46 and 47).

Multiple mechanisms cooperate to result in assembly of retroviral gRNA into particles.[48] Because retroviral RNAs templating Gag and Gag-Pol synthesis or those destined to become gRNAs are unspliced, they require specialized nuclear export pathways.[49] This role can be fulfilled by either retroviral proteins, such as HIV-1 Rev, that bind to specific sequences in the gRNA and act as adapters for cellular export proteins (reviewed in ref. 50 and 51), or by a viral constitutive transport element (CTE), such as that of MPMV, in the gRNA that is recognized directly by cellular export factors.[52] There could also be designation of gRNA in the nucleus. The gammaretrovirus MoMLV gRNA is derived from a pool of newly-made transcripts separate from translating RNAs.[53] Consistent with that, these gRNAs are more likely to be co-packaged with RNAs transcribed from nearby loci, suggesting that dimerization could be initiated in the nucleus.[54] In the case of RSV, Gag shuttles through the nucleus and interruption of this cycle decreases gRNA packaging. This Gag nuclear localization is mediated by MA domain interactions with importin-11 and NC domain interactions with importin-alpha/beta.[55,56] For other retroviruses, including HIV-1, the translating pool of viral RNAs contributes to gRNA.[48] However, for HIV-1, redirecting nuclear export from one pathway to another can disrupt packaging.[57] These findings suggest that even retroviral RNAs that are within the translating pool could be designated as available for packaging soon after transcription. Because there is not a requirement for packaging in cis, untranslated gRNA could be packaged in principle and Gag must be able to bind gRNA in trans with specificity. In vitro experiments demonstrated that HIV-1 Gag can negatively autoregulate translation, offering an elegant mechanism through which retrovirus RNAs could transition from translation template to gRNA.[58] These examples do not exclude the possibility of a temporal transition in gRNA designation. For example, if in the course of an infection accumulation of unbound Gag resulted in Gag nuclear entry and gRNA capture.

As alluded to above, psi sequences in the gRNA mediate selective Gag binding. When ZFs of retroviral NC proteins were swapped, the source of the ZF determined gRNA binding specificity and mutations in the ZF disrupted gRNA packaging.[59,60] For most retroviruses for which psi is defined, it consists of several hundred-nt containing a few stem-loops near or within the gRNA 5' UTR (reviewed in ref. 61). Psi sequences for which function is understood contain a dimerization initiation site (DIS), which can nucleate dimer formation via a "kissing loop" interaction. NC promotes an extension of this interaction by chaperoning re-pairing of the strands in the monomeric gRNA stems to form mature intermolecular duplexes in the dimeric gRNA. NMR studies have characterized the MoMLV and HIV-1 NC ZF complexed with psi stem loop regions (reviewed in ref. 62 and 63). In the case of MoMLV, high-affinity NC binding sites become accessible in the extended structure, suggesting the basis for stabilization of the dimer. Evidence that NC loss-of-function mutations reduced particle formation and that such mutations were rescued by substitution of a leucine zipper domain for the ZF support a model in which the ZF in mediating Gag binding to a RNA scaffold, stabilizes weaker inter-Gag interactions.[39,64]

Examination of cells over the course of a retrovirus infection shows that assembly is initiated prior to delivery of Gag to the plasma membrane. Fluorescence resonance energy transfer (FRET) experiments visualize intermolecular Gag interactions within small cytoplasmic foci.[65,66] In addition, pulse labeling and examination of multimeric species over a time course shows formation of Gag multimers prior to membrane delivery.[67-69] Intracellular Gag trafficking appears to be dependent upon gRNA, as deletion of psi in the case of MoMLV[70] and HIV-1,[71] resulted in loss of trafficking to the cell surface and to the perinuclear microtubule organizing center (MTOC), respectively. As would be predicted if interaction depended upon the ZF for specificity, mutations in HIV-1 ZF also resulted in a diffuse pattern of Gag.[72] Together these experiments indicate that specific binding by the NC domain of Gag is required for formation of cytoplasmic assembly intermediates.

There is evidence that HIV-1 protein and RNA assembly intermediates collect at the perinuclear MTOC. Fluorescence and pulse chase experiments demonstrate collection of Gag multimers and gRNA at this site.[67,71,73] Treatment of cells with latrunculin disrupts this localization, implicating the actin cytoskeleton in formation of pre-assembly foci.[71] Other data indicate that actin associates with HIV-1 Gag via the NC domain.[74-77] Localization likely promotes further multimerization. In addition, chaperonins are associated with the MTOC. In the case of betaretroviruses, which assemble into VLPs in a peri-MTOC compartment prior to endocytic trafficking to the cell surface, Gag has been shown to interact with chaperonins normally associated with tubulin assembly.[78] Although HIV-1 does not fully assemble at the MTOC, chaperonins might modulate multimerization. The assembly factor ABCE1 associates at early stages of multimerization with Gag and this interaction is also mediated by NC.[69,79] HIV-1 RNA contains binding sites for the ribonucleoprotein (RNP) component, hnRNP A2. Knockdown of hnRNP A2 in HIV-1 producing cells causes accumulation of HIV-1 RNA in the nucleus and in the vicinity of the MTOC.[73] Thus, accumulation of assembling complexes might also result in recruitment of newly-transcribed RNA for assembly. Finally, proximity to the MTOC offers access to the microtubule network and endocytic trafficking to the cell surface where assembly and/or budding occur (reviewed in ref. 49).

Collection of Ty VLP proteins in retrosomes. Retrotransposons assemble through a contracted version of the retrovirus pathway: translation of the RNA to produce Gag and Gag-Pol; commitment of RNA to assembly; and concentration and assembly of gRNA, Gag and Gag-Pol. Similar to retroviruses, the Ty3 retrotransposon translates template RNA into Gag and Gag-Pol which are observed in multiple foci shortly after induction.[31,36] However, in contrast to retroviruses, these Ty3 foci coalesce within a few hours into one or two large foci or retrosomes per cell. VLPs are primarily but not exclusively observed in the foci, and Gag assembly mutants show defects in focus formation.[42] Therefore foci are presumed to represent Ty3 assembly

sites. Ty1 retrotransposons form similar concentrations of Ty RNA, protein and VLPs.[80-82] Ty1 and Ty3,[36] elements appear to share certain "host" components of these clusters, but not others, indicating that Ty1 and Ty3 clusters are likely distinct. However, because representatives of both major classes of LTR retrotransposons in *S. cerevisiae* assemble particles within foci of concentrated components, we refer to both classes of retrotransposons assembly clusters as retrosomes. In yeast the spindle pole body acts as the microtubule organizing center. Despite a perinuclear position in many cells, Ty3 retrosomes do not consistently localize in the vicinity of the spindle pole body (Sandmeyer SB and Beliakova-Bethell N, unpublished data). Thus, the retrosome is probably functionally distinct from the peri-MTOC concentration of assembling betaretroviruses or lentivirus intermediates.

Collection of Ty3 RNA in retrosomes. Concentration of Ty3 RNA in retrosomes requires both cis-acting and trans-acting functions. Although psi has not been mapped for Ty3, analysis of cells expressing helper Ty3 elements and a *HIS3*-tagged donor Ty3 showed that the donor 5' Ty3 UTR and downstream PPT-U3-R sequences were sufficient to allow retrotransposition of the *HIS3* marker. Thus, these Ty3 sequences are sufficient for packaging, reverse transcription and integration.[24] Expression of Ty3 RNA results in rapid concentration of Ty3 protein and RNA into retrosomes[36] and protection of the RNA from nuclease digestion, indicating that it is packaged. In contrast, similarly expressed control RNAs do not form such clusters and are not protected from digestion, even in cells expressing Gag3. It was anticipated that sequences required for retrotransposition would include those required for localization to retrosomes and for Gag3-mediated assembly. When chimeric RNAs were tested, either the Ty3 5' UTR and 3' PPT-U3-R or *GAG3-POL3,* flanked by non-Ty3 UTRs (but not *GAG3*, flanked by non-Ty3 UTRs), was sufficient for RNA localization to retrosomes in cells expressing Gag3 (SBS, Beliakova-Bethell N, KAC, Bilanchone V, manuscript in preparation). However, sequence flanked by the Ty3 5'UTR and 3'PPT-U3-R was packaged, whereas the *GAG3-POL3* RNA flanked by non-Ty3 UTRs was not. Thus, Ty3 RNA promotes assembly at two stages. In the first stage, association is specific, but is mediated by the 5' UTR and 3' PPT-U3-R or *POL3* and allows for cytoplasmic coalescence of Gag3 and RNA with retrosome components. In the second stage, sequences within the Ty3 5' UTR and PPT-U3-R are recognized by Gag3 and gRNA is assembled into VLPs. Retrosome localization does not insure RNA assembly into Ty3 VLPs.

Ty3 retrosomes contain P-body and stress granule components. The dramatic formation of retrosomes observed in cells expressing Ty3 prompted investigation of the cytoplasmic components with which they are associated. Because P-body proteins had been implicated in retrotransposition by genome-wide screens of mutants defective in Ty1,[83] and Ty3,[84] the association of a subset of these with Ty3 components was explored. In *S. cerevisiae*, proteins identified in P-body complexes include proteins implicated in translation repression (Dhh1, Pat1 and Ded1), decapping (Lsm1, Edc1, Dcp2), RNA unwinding (Dhh1 and Ded1), 5' to 3' RNA degradation (Xrn1/Kem1), and nonsense-mediated decay (NMD) (Upf1-3).[85,86] Dhh1, Pat1, Edc3, Dcp2 and Xrn1/Kem1 GFP reporter fusions co-localize with fluorescently-tagged Ty3 protein and Lsm1, Dhh1 and Xrn1 are essential for wt levels of retrotransposition.[36,84] Sequestration of assembling particles with RNA degradation factors is counter-intuitive. However, in yeast P bodies are necessary for formation of stress granules in which RNA can be sequestered during stress and then returned to polysomes,[85,87,88] and recent work shows that decapping-dependent RNA degradation occurs co-translationally.[89] In addition, mass spectrometry analysis of proteins co-purifying with Gag3 complexes identified a subset of stress-granule proteins that have been localized to retrosomes by fluorescence microscopy (Bilanchone V, unpublished results). Thus, P-body and stress-granule proteins could cooperate with Gag3 to create an assembly environment sequestered from translation and degradation. The association of retrotransposon assembly with RNA granules would make considerable sense. P-body translation repression functions would promote transition from translation to assembly while stress-granule functions would include proteins involved in stabilizing or folding other proteins, binding RNA and initiating translation.[90,91] Such factors could provide chaperones for assembling Ty3 proteins as well as concentrating initiation factors to recruit primer initiator $tRNA^{Met}$ ($tRNA_i^{Met}$), to the retrosome.

Several results indicate that there is likely to be physical association between Ty3 proteins and RNA and P-body and stress-granule proteins. Mutations in Ty3 CA that disrupt Ty3 particle formation have effects on P-body protein patterns. Two CA mutations that profoundly disrupted Ty3 assembly dissociated Ty3 Gag-Pol-RFP and Dhh1-GFP fluorescence. A mutation in CA that caused formation of fibrillar Gag3-Pol3-RFP structures throughout cells caused a similar reorganization of Dhh1-GFP and Xrn1/Kem1-GFP reporters.[42] The DEAD-box helicase Dhh1 tagged with GST co-precipitates with Gag3, although it is not known if this represents a direct or indirect interaction (Beliakova-Bethell N, Clemens K, unpublished data).

The role of retrosomes in retrotransposition is not likely to be simple; in spite of clear evidence that P-body and stress-granule components contribute in positive ways to retrotransposition, other data point to a role for retrosomes in limiting transposition. For example, a Gag3 amino-terminal Ala substitution mutation decreased cluster size, but increased transposition dramatically (Sandmeyer SB, Zhang M, Bilanchone V, unpublished data).

Model for Ty3 transition from template to genomic RNA. An intriguing problem in the case of translated retroviral genomes is how the transition of the RNA from translation template to gRNA is controlled. A mutation in the Ty3 translation initiation AUG of Ty3 elements marked with a *HIS3* insert essentially eliminates retrotransposition in cells expressing helper Ty3 (Sandemeyer SB, Beliakova-Bethell N, unpublished data). Although the mutant Ty3 RNA level is low, if gRNA were delivered directly from the nucleus without translation, this mutation would not alter assembly into particles. The reduction in transposition suggests that at least some amount of Ty3 gRNA is derived from the translated pool. The 5' and 3' ends of translating RNAs are bound by translation initiation factors and poly(A) binding

proteins, respectively and are bridged by eIF4G (reviewed in ref. 92). Circularization is proposed to protect the ends of the translating RNA and promote delivery of the reinitiating 60S subunit to the initiation site. However, retroelement RNA, which represents both the template and the genome, is subject to special constraints. Because programmed frameshifting releases ribosomes from retroelement template RNAs upstream of the 3' ORF, retroelement gRNAs have relatively low downstream ribosomal occupancy. This could attenuate translation reinitiation on retroelement RNA and make it susceptible to P-body protein mediated translational repression and aggregation. Three observations make this particularly relevant to yeast LTR retrotransposons. First, $tRNA_i^{Met}$ is the reverse transcription primer used by Ty1, Ty3 and Ty5 yeast LTR retrotransposons and is observed in the vicinity of Ty3 retrosomes (Bilanchone V, unpublished results). Second, Ty3[36] retrosomes include P-body translational repressors (e.g., Dhh1 and Lsm1).[85,87,88,93,94] Third, Ty3 and Ty1 require subsets of P body-proteins for retrotransposition.[81,82] We speculate that the Gag3 binds to psi sequences in the retroelement gRNA and interferes with translation initiation, increasing their susceptibility to binding by P-body translation repressors and thereby nucleating retrosome coalescence.

Effects of substitution mutations in conserved residues of the Ty3 ZF. On the basis of effects of mutations in the MoMLV,[95] RSV[96] and HIV-1,[97] ZF, mutations in Ty3 ZF might have been expected to leave particle assembly intact and result in nonspecific RNA packaging. However, substitution of three conserved Cys residues and one His residue in the single ZF of Ty3 NC showed an unexpected range of phenotypes[44] and dramatically decreased assembly. A model in which the ZF acts as a modular structure, predicts that substitution of any Cys or the single His residue within the Ty3 ZF would similarly disrupt zinc chelation and therefore function. Surprisingly, substitution of Ala for conserved ZF residues in Ty3 NC (C_1, C_4, H_9 and C_{15}) produced graduated effects, progressing from most severe disruption of assembly at the amino-terminal end (C_1A) to least severe at the carboxyl-terminal end (C_{15}A). C_1A mutant Gag3 localized to the nucleus where it formed electron-dense aggregates and loose spheres. In contrast, H_9A and C_{15}A mutants formed electron-dense "balls" of Gag3 with significant numbers of extra-nuclear particles resembling VLPs. Similarly, Ty3 RNA foci were more severely diminished in cells expressing the C_1A mutant than in cells expressing the C_{15}A mutant. The effects of these mutations argued that a region overlapping the ZF plays an important role, not only in assembly, but in cytoplasmic retention of Gag3. Mutant Gag3-Pol3 tagged with GFP did not localize to the nucleus and in the less severe H_9A and C_{15}A mutants, some particles were observed both inside and outside of the nucleus. In cells expressing wt Ty3, nuclear Gag3 is not detectable. However, an interesting possibility raised by these results is that in the absence of RNA binding, wt Gag3 might traffic into the nucleus to recruit newly-made RNA for packaging.

Role of the basic NTD of Ty3 NC. Retrovirus NC function as a RNA chaperone depends upon a positive-charge bias.[8-10] Ty3 NC is similarly positively charged with 17 basic residues. A series of Ala-scanning mutants in which two or three charged residues within a window of five aa were changed to Ala was examined to determine the contributions of basic residues to VLP morphogenesis.[44] None of the double mutations affected transposition and only two of the four triple mutations resulted in partial transposition defects. The stronger of these changed charged residues within the ZF to Ala and resulted in a 50% decrease in cDNA production. Overall, the effects of substituting local sets of charged residues in the Ty3 NC protein indicates that they do not individually perform essential functions in retrotransposition.

In order to test for roles of the basic NTD and NC itself, a mutant having a deletion of the basic NTD (ΔNTD, residues 236 to 265 including 10 basic residues), and a mutant having a NC domain deletion (ΔNC aa 237 to 281) were tested for effects on retrotransposition.[44] ΔNTD and even ΔNC mutations permitted CA-SP processing, formation of Gag3 matrices, NC function is not required for some level of particle assembly and maturation. However, ΔNTD or ΔNC severely disrupted transposition and abrogated cDNA synthesis. These mutants were evaluated for localization with P-body proteins, VLP assembly and RNA packaging. Overall, deletion of the entire NC domain had similar, but more dramatic effects than deletion of the basic NTD. These reduced, but did not eliminate Gag3-Pol3-RFP foci, presumably based on CA domain interactions. These foci had reduced association with P-body components. Similar to the ZF mutants, ΔNTD and ΔNC Gag3 partitioned into the nucleus. TEM analysis showed that within the nucleus, ΔNC Gag formed a striking, tightly-packed matrix array of particles without electron-dense centers. However, ΔNTD formed electron-dense, fuzzy balls that were larger than normal VLPs. Both classes of mutations eliminated gRNA protection, consistent with a central role for the basic domain of Ty3 NC in RNA association.

Gag3 nuclear localization activity in Ty3 NC. The results of experiments described above indicated that Gag3 remains predominantly cytoplasmic due to an activity that maps to a region overlapping the ZF. In addition, they suggest either that Gag3 not engaged with RNA can diffuse or that it can be actively translocated into the nucleus. In order to map domains of Gag3 with NLS activity, Gag3 and CA, CA-SP, CA CTD, CA NTD, SP-NC and NC were expressed fused to tandem repeats of the GFP-coding sequence (2xGFP).[98] NC-2xGFP concentrated in the nucleus and SP-NC-2xGFP showed diffuse fluorescence in the cytoplasm and nucleus. Other fusions formed foci some of which appear nuclear or perinuclear.[98] As described above even ΔNTD and ΔNC mutant Gag was found in large nuclear aggregates, although localization of those deletion mutants could have occurred by diffusion and retention of aggregates.[44] RSV Gag is actively transported into the nucleus and exported.[55,99,100] Interruption of this pathway does not block protein expression, but causes decreased gRNA packaging,[100] supporting the model that RSV Gag captures gRNA in the nucleus. Although wt Ty3 Gag3 does not accumulate in the nucleus to detectable levels, evidence that Gag3 contains NLS activity and that mutations that interfere with Gag3 binding to Ty3 RNA result in Gag3 nuclear accumulation would support a two-phase model. In the first phase, Gag3 associates with Ty3 gRNA in the cytoplasm, but in

the second phase, unbound Gag3 accumulates and then translocates into the nucleus where it associates with newly-transcribed Ty3 RNA. This model would require nuclear export of Ty3 Gag which has not yet been demonstrated.

Interactions of NC with Related Host Factors during Assembly

Given the size and structural constraints of the retroviral NC domain, it participates in an amazing spectrum of assembly and replication functions. Interacting proteins include ones with functions upon which the virus is dependent, as well as at least one host restriction factor. Examples of interactions occur throughout retrovirus assembly. As described above, the NC domain mediates RSV Gag interactions with importins alplha/beta.[55] Evidence suggests that the NC domain could also play a role in formation of assembly intermediate foci for HIV-1. It has been known for some time that actin interacts with HIV-1 Gag[76,101,102] and recent experiments showed that the actin cytoskeleton is required for localization of Gag and gRNA to the MTOC preassembly site.[71] The NC domain also mediates interactions with assembly factors. The contribution of staufen required for gRNA packaging depends upon the NC domain.[103,104] The HIV-1 assembly factor ABCE1 which promotes early multimerization of Gag interacts with Gag via residues close to the ZF motif.[79] Nucleolin, a ribosomal assembly factor interacts with the CA-NC domain junction and mutations at this junction disrupt MLV assembly.[105] ESCRT complex budding factors associate with Gag and this interaction depends upon interactions within Gag late domains—so called because disruption results in a late cycle budding defect. The host factor ALIX/AIP1 mediates a subset of those interactions.[106] Results from several groups have recently demonstrated that host late functions are redundant with some activities of NC. For example, overexpression of late-acting ESCRT components known to interact with ALIX/AIP1,[107] or substitution of NC with the leucine zipper domain[108] suppress a subset of effects of certain NC mutations on budding. Finally, HIV-1 host restriction factor ABPOBEC3G, assembles into particles via RNA-dependent NC interaction.[109-111] Thus, NC is a multifunctional assembly domain that exploits interactions with host factors and is in turn targeted by host restriction.

An intact NC domain in Gag3 is required for development of Ty3 retrosomes which are potentially associated with P-body proteins. Although certain P-body functions clearly have positive effects on retrotransposition, certain Ty3 mutant phenotypes suggest that large retrosomes are repressive to Ty3 transposition. Thus for Ty3, P-body functions are associated with positive and negative effects. At least some P-body functions are also implicated in HIV-1 assembly. For example, staufen, is associated with P bodies.[112] HIV-1 infectivity is sensitive to non-physiological levels of Mov1, a RNA helicase associated with P bodies.[113] Finally, the restriction factor APOBEC3G[81,114] and miRNA antagonists of HIV-1,[115,116] localize with P-body components. Thus, both Ty3 and HIV-1 interact with subsets of P-body proteins. Because of its chaperone activity, NC is likely to play an important role in mediating access of these factors to the gRNA.

Processing and Activities of Mature NC in Reverse Transcription

SP-NC context and maturation. In spite of the conservation of NC ZF sequence and overall basic composition, the context of NC varies among retroviruses. In some retroviruses, including RSV, HIV-1 and equine immunodeficiency virus, CA and NC are separated by a short SP domain. In other retroviruses, including MoMLV, CA and NC are adjacent domains. Modeling based on electron cryomicroscopy of immature HIV-1 particles predicts formation of a bundle of SP alpha helices underlying the CA hexamers. This configuration is proposed to promote capsomere formation.[41] In the case of HIV-1, cleavages occur in the order NC-P6, MA-CA, SP-NC and CA-SP.[12,117] Thus, release of NC precedes separation of CA from the underlying SP bundles. Disruption of the SP-NC cleavage blocks condensation of NC and gRNA into an electron-dense particle core.[26]

The Ty3 NC domain, similar to the HIV-1 NC domain, in the polyprotein precursor is flanked by a short SP domain. However, in contrast to HIV-1, Ty3 SP is highly acidic. Mutants in which Ty3 SP-NC processing is blocked, similar to HIV-1 mutants for which processing is blocked, fail to form electron-dense cores (Sandemeyer SB, Clemens KA, in press). In contrast, Ty3 SP deletion mutants form particles and make cDNA, but fail to retrotranspose. The strong negative charge in SP might allow it to interact with the basic domain of NC during assembly. A mutant in which acidic residues were substituted with Ala failed to form retrosomes and assemble. TEM analysis showed a limited number of cells with partially-formed, VLP-like structures that lacked the tight packing observed in wt or PR- mutant retrosomes. These results suggest that Ty3 SP is not absolutely required for assembly, but if present, must be negatively charged. In the case of human T-cell lymphotrophic virus-type 1 (HTLV-1) acidic residues located in the carboxyl-terminal domain of NC are associated with lower rates of NC dissociation from nucleic acid substrates and therefore less effective chaperone activity than some other retroviral NC proteins.[118,119] It was proposed that intermolecular actions of the acidic and basic domains of HTLV-1 NC promote packing on the RNA. In the case of Ty3 Gag3, SP acidic residues appear to be required for proper assembly, but intermolecular interactions between SP and NC domains might similarly promote stable assembly of Gag3 onto the gRNA.

Ty primer annealing and gRNA circularization. Retroviral Gag exhibits higher nucleic acid binding and aggregating activity than processed NC, consistent with its role in packaging RNA into the VLP.[120] However, subsequent to proteolytic maturation, NC performs important chaperone functions throughout reverse transcription. Because of the critical role of NC in assembly, much of what we know about its activities in reverse transcription is based upon in vitro experimentation. NC plays supporting or key roles in RNP complex formation, suppression of premature reverse transcription in producer cells, melting of internal RNA structures and annealing of primer tRNA and cDNA intermediates, (reviewed in ref. 8–10). Less work has been performed with Ty3 NC than retroviral NC. However, in vitro assays indicate that it functions in at least a subset of these roles.

One of the most fascinating aspects of LTR retrotransposon replication is the diversity of reverse transcription priming strategies even within retrotransposon families. The Metavirus Ty3 as well as the Pseudoviruses copia, Ty1,[121,122] and Ty5,[123] use $tRNA_i^{Met}$ as the primer tRNA. However, Ty5 and copia prime from the 3' end of a $tRNA_i^{Met}$ internal cleavage product.[121,122] The Metavirus, Tf1, self-primes from the 3' end of an internal cleavage product of Tf1 gRNA.[124] Copia, Ty5 and Ty3 have ZF-containing NC proteins, but Ty1 and Tf1 do not. A basic peptide from the carboxyl-terminal domain of Ty1 Gag has RNA chaperone activity. This peptide and Ty3 NC can anneal primer $tRNA_i^{Met}$ to the respective retrotransposon gRNAs in vitro.[125] Thus, the presence of ZF-containing NC is not correlated with the identity of the primer species. This convergence suggests that for retrotransposons the choice of the translation initiator tRNA as primer is driven by some specific functional benefit.

Although Ty3 NC and HIV-1 NC both have primer-annealing activity, there are interesting differences in the products of these reactions and the resulting reverse transcription templates. Early studies of Ty3 identified an 8-nt sequence 2 nt downstream of U5 that is complementary to $tRNA_i^{Met}$.[126] Despite the modest complementarity, effects of mutations in $tRNA_i^{Met}$ argued convincingly that it was the primer.[122] Further inspection revealed additional complementarity to the primer within downstream U3.[127] The bipartite complementary sequence was required for $tRNA_i^{Met}$ priming of -SSS cDNA synthesis by HIV-1 or Ty3 RT in vitro.[127,128] Similarly, the Ty1 primer building site (PBS) initially identified as a 10 nt sequence complementary to the 3' end of primer $tRNA_i^{Met}$ was shown to have additional short segments of complementarity required for -SSS production. In contrast to Ty3, these were contained within the 5' end of the gRNA.[129,130] Remarkably, $tRNA_i^{Met}$ has a 12 nt GC-rich palindromic sequence at the 5' end which is unpaired when the tRNA is annealed to the Ty gRNAs. This strand of the $tRNA_i^{Met}$ stem is necessary for NC-mediated formation of Ty3 dimers in vitro.[127]

Ty3 NC promotes formation of Ty3 RT ribonucleoprotein complexes,[128] primer $tRNA_i^{Met}$ annealing to Ty1[125,127] and Ty3 template RNAs,[127] and $tRNA_i^{Met}$-primed reverse transcription of mini-Ty3 RNA to produce -SSS cDNA intermediates.[127] In addition to performing these functions on mini-templates, Ty3 NC anneals HIV-1 primer $tRNA^{Lys3}$ to HIV-1 RNA[127] and can promote dimerization of heterologous 5' HIV-1 RNA[127] and Ty1 RNAs in vitro.[125] Deletion mutation analysis indicates that the basic domain is more important for primer annealing, gRNA dimer and -SSS synthesis than is the ZF.[128]

A key step assisted by NC in reverse transcription of retroelements is the transfer of the nascent -SSS cDNA template from the 5' end of the gRNA to the 3' end to allow extension of the minus-strand cDNA. Re-pairing of the R region of the -SSS chaperoned by NC has been proposed to mediate this transfer for retroviruses.[5] Most Ty3 transcripts would have a R sequence of about 50 nts. This fits within the range of retroviral R (e.g., RSV = 21 nts; MLV = 68 nts; HIV-1 = 98 nts). Nonetheless, because experiments showed that the Ty3 bipartite (5' and 3') PBS was required for -SSS, it seems likely that $tRNA_i^{Met}$ bridges the 5' and 3' ends of the gRNA and so promotes strand transfers[127](**Fig. 3A**). Despite the very strong appeal of this model, direct testing in vivo of the requirement for a bipartite primer linker for strand transfer is complicated by the epistatic requirement of even Ty3-SSS synthesis for bipartite primer annealing. This would not be the case for Ty1 where the multipartite PBS is in the 5' end (**Fig. 3B**). Efforts to develop an in vitro reverse transcription system for that element, however, identified a region downstream of the Ty1 PBS with 14 nt of complementarity to a sequence in the U3 that was required for efficient initiation of -SSS DNA.[131] Thus, at least two mechanisms to circularize the genome in addition to the R sequence overlap are represented in budding yeast retrotransposons.

Two interesting discoveries suggest that long-range interactions between the ends of gRNA in addition to those mediated by the R region may extend beyond the yeast retroelements. First, structural analysis of HIV-1 identified a phylogenetically-conserved complementarity within a 600–700 nt region within Gag and a region of 123 nt at the downstream end of the HIV-1 gRNA. Interaction of these sequences was also proposed to enhance strand transfers[132] (**Fig. 3C**). Interestingly, a region of U3-R that contains extensive complementarity to HIV-1 primer $tRNA^{Lys3}$ has also been shown to promote strand transfer.[133,134] Finally, there is a possibility of covalent links between the 5' and 3' ends of retroelements that contribute to replication. Ty1,[135] Ty3 elements[84] and later HIV-1,[136] were shown to require the lariat debranching enzyme (yDBR1) for replication. Evidence in both retrotransposon and retrovirus systems has suggested the existence of lariat forms in which gRNA would be circularized.[137,138] However, conclusive demonstration of the role of such a branch point has been elusive.[139] Considering the ongoing function-structure analyses, as well as possibilities for high definition insights into in vivo RNA structures[140] coupled with approaches to studying host gene interactions with NC, insights into the dynamics of the strand-transfer process are likely to be forthcoming..

Summary and Perspectives

The NC domain, both in the context of Gag and as a mature protein, contributes in critical ways to retroelement replication. Retrovirus NC has long been known to participate in gRNA recognition, dimer stabilization, packaging, primer annealing and cDNA strand transfers. New functions continue to be discovered including roles in multimerization, suppression of premature reverse transcription and mediation of localization and other complex interactions with the host cellular environment. Studies in the Ty3 Metavirus retrotransposon system have shown that retrotransposon NC is likely to perform versions of these functions tailored to the intracellular environment of the retrotransposon lifecycle. These include targeting gRNA and protein to retrosome assembly sites in association with P-body and stress-granule proteins, controlling cytoplasmic versus nuclear localization of Gag3 and annealing a multipartite tRNA primer that both circularizes and dimerizes the genome. Key aspects of retrovirus and retrotransposon NC functions remain to be resolved. Continued investigation is motivated by the potential of retrotransposons to

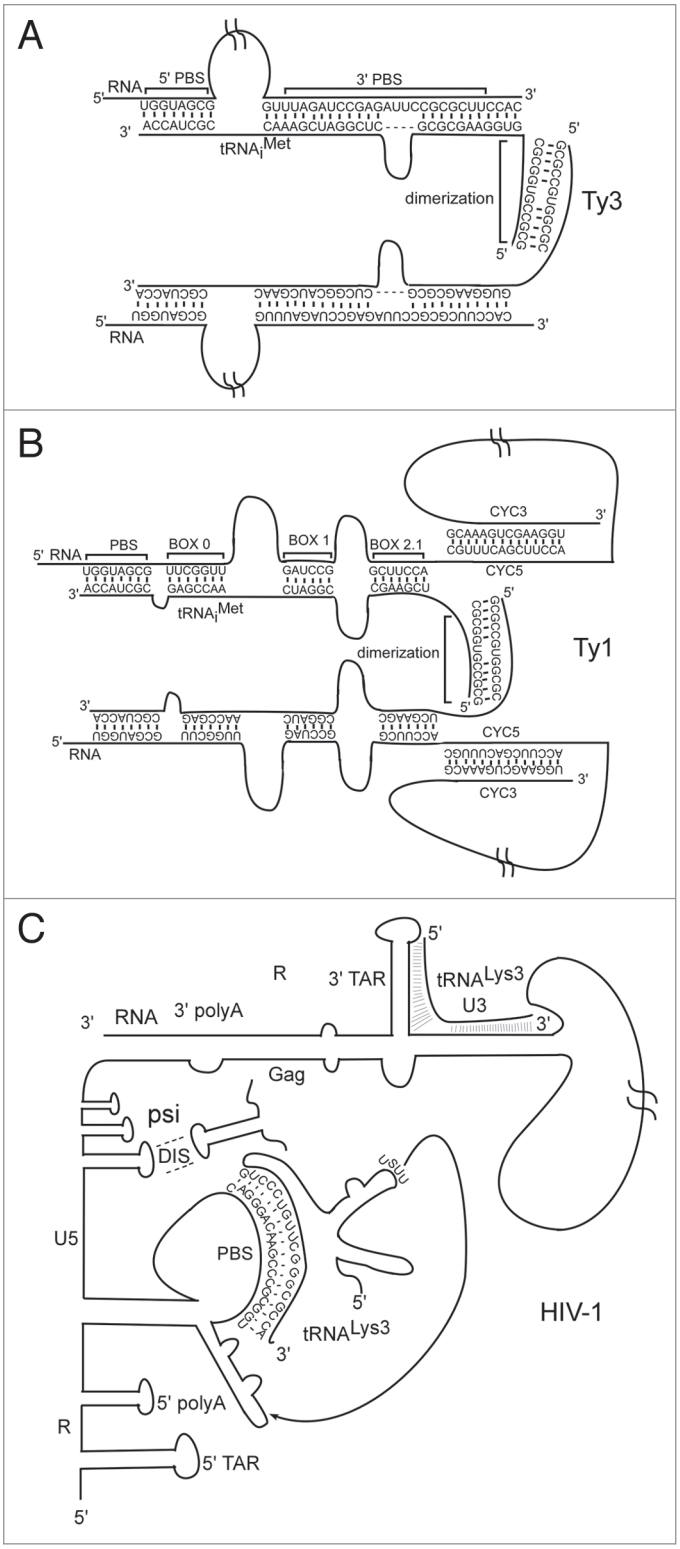

Figure 3. Circularized genomic RNA structures proposed for retroelement genomes with annealed primer tRNAs. (A) Ty3 RNA annealed to tRNA$_i^{Met}$. Ty3 RNA contains a bipartite primer binding site (PBS), comprised of regions in the 5′ leader and U3 of Ty3 RNA complementary to sequences in the 3′ acceptor end, TΨC arm and D arm of primer tRNA$_i^{Met}$.[127] Circularization is proposed to promote -SSS transfer. In vitro NC mediates dimerization of Ty3 gRNA dependent upon annealed tRNA$_i^{Met}$ molecules paired via palindromic sequences at the 5′ ends of each tRNA. (B) Ty1 tRNA$_i^{Met}$ dimerization. Ty1 RNA contains a multipartite tRNA$_i^{Met}$ PBS in the upstream end that includes the originally-described PBS, Box 0, Box 1 and Box 2.1.[130] In vitro Ty1 dimerization is mediated by Ty1 RNA-binding protein via the palindromic sequences in the primer as described in (A).[125,127] Circularization is dependent upon palindromic sequences in the 5′ (CYC5) and 3′ (CYC3) regions.[131] (C) HIV-1 RNA dimerization and circularization. The 5′ UTR PBS region is shown annealed at the PBS to the complementary primer tRNA^{Lys3}. The dimerization initiation site (DIS) promotes HIV-1 dimerization by forming a "kissing loop" interaction with the DIS of another HIV-1 RNA which transitions to an extended dimer palindromic interface (not shown). Psi, packaging sequence; polyA, polyadenylation signal; and TAR, transactivation response element. U3-R contains complementary sequence to primer tRNA^{Lys3}.[133] Complementary regions in Gag and portions of U3-R are proposed to pair and promote -SSS transfer.[132]

impact the function of eukaryotic genomes and the potential of NC as an anti-retroviral therapeutic target. Questions posed by current models will continue to stimulate future research regarding this fascinating multi-functional protein. Is retrosome gRNA capture mediated by P-body translational repressors loading onto the translating RNA? Is a flanking acidic domain that modulates basic NC domain activity a common feature of retroelement replication? Is nuclear entry and gRNA capture a default pathway for unbound Gag? Is the genome circularization observed for Ty and Tf1 elements and HIV-1 a widespread feature of retroelements? Finally, and perhaps most interestingly, what are the triggers for transitions among these functions and what controls the dynamic interactions of NC with host proteins?

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