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Ty3, a Position-specific Retrotransposon in Budding Yeast

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ABSTRACT Long terminal repeat (LTR) retrotransposons constitute significant fractions of many eukaryotic genomes. Two ancient families are Ty1/Copia (*Pseudoviridae*) and Ty3/Gypsy (*Metaviridae*). The Ty3/Gypsy family probably gave rise to retroviruses based on the domain order, similarity of sequences, and the envelopes encoded by some members. The Ty3 element of *Saccharomyces cerevisiae* is one of the most completely characterized elements at the molecular level. Ty3 is induced in mating cells by pheromone stimulation of the mitogen-activated protein kinase pathway as cells accumulate in G1. The two Ty3 open reading frames are translated into Gag3 and Gag3–Pol3 polyprotein precursors. In haploid mating cells Gag3 and Gag3–Pol3 are assembled together with Ty3 genomic RNA into immature virus-like particles in cellular foci containing RNA processing body proteins. Virus-like particle Gag3 is then processed by Ty3 protease into capsid, spacer, and nucleocapsid, and Gag3–Pol3 into those proteins and additionally, protease, reverse transcriptase, and integrase. After haploid cells mate and become diploid, genomic RNA is reverse transcribed into cDNA. Ty3 integration complexes interact with components of the RNA polymerase III transcription complex resulting in Ty3 integration precisely at the transcription start site. Ty3 activation during mating enables proliferation of Ty3 between genomes and has intriguing parallels with metazoan retrotransposon activation in germ cell lineages. Identification of nuclear pore, DNA replication, transcription, and repair host factors that affect retrotransposition has provided insights into how hosts and retrotransposons interact to balance genome stability and plasticity.

INTRODUCTION

Long terminal repeat (LTR) retrotransposons occur throughout eukaryotic phyla, but vary greatly in both the types of elements and the representation among species.

The majority of some genomes are composed of these elements. The LTR retrotransposons are taxonomically divided into *Pseudoviridae* (or Ty1/Copia) and *Metaviridae* (or Ty3/Gypsy) elements based on genome organization and relatedness of proteins encoded (reviewed in references 1–3) (Gypsy Database 2.0, gydb.org). The Ty3/Gypsy family has shared ancestry with retroviruses and some members encode envelope enabling intercellular transmission. The eponymous founding elements of these LTR retrotransposon families, Ty1/Copia and Ty3/Gypsy, exist in *Saccharomyces cerevisiae* (Ty1 and Ty3) and in *Drosophila* (Copia and Gypsy).

This review is focused on Ty3, a representative of the Ty3/Gypsy retrotransposon group, which has been extensively characterized at the molecular level. Ty3 LTRs flank two overlapping open reading frames (ORFs), GAG3 and POL3, which encode structural and catalytic functions, respectively. Ty3 was discovered as a polymorphism associated with tRNA genes (tDNAs) and has been extensively studied as a model of targeted integration. A combination of approaches has implicated

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numerous host factors in control of Ty3 retrotransposition. Altogether, studies of Ty3 provide for a relatively comprehensive, albeit incomplete, picture of the ongoing remodeling of a eukaryotic genome by an LTR retrotransposon.

TY3 DIVERSITY

Ty3 is the sole representative of the Ty3/Gypsy family in *S. cerevisiae*. The reference *S. cerevisiae* strain BY4741 contains two closely related, full-length Ty3 elements one of which, YGRWTy3-1, is transpositionally active. In addition to the four LTRs associated with full-length elements, BY4741 contains 41 isolated LTRs (4–6) derived from recombination of the LTRs flanking full-length elements. These isolated LTRs were named sigma elements (7, 8) before the discovery of full-length Ty3 (9). Ty1/Copia elements in *S. cerevisiae* are represented by Ty1, -2, -4, and -5. Ty1 (see Curcio, Lutz, and Lesage, Chapter 41) is the most abundant of these, with 32 full-length elements, two truncated elements with single LTRs, and 185 isolated LTRs (4–6).

Phylogenetic analysis of full-length elements and isolated LTRs has provided insight into the time of appearance and retrotransposition activity relative to host phylogeny based on occurrence of integrants shared among diverging host species and the divergence of flanking LTRs of individual full-length copies, as these are regenerated from the same template during retrotransposition (10). Comparison of Ty3 elements and LTRs in related *Saccharomyces* species showed that LTRs flanking full-length elements in the reference strain were identical and LTRs overall had > 96% identity. In comparison, Ty1, Ty2, and Ty5 LTRs display 70 to 99% identity (5, 6). Although this suggests that Ty3 appeared recently relative to other elements, analysis of a large number of related *Saccharomyces* genomes identified ancient, shared Ty3 insertions pre-dating the appearance of *S. cerevisiae* (4). However, the presence of 24 highly similar, short-branch LTRs is consistent with recent retrotransposition of elements related to the full-length *S. cerevisiae* elements (4). Fifteen highly degenerate LTRs were identified in *S. cerevisiae* that were more similar to *Saccharomyces paradoxus* Ty3p than to *S. cerevisiae* Ty3 LTRs. Because common insertions preceding the divergence of *S. paradoxus* and *S. cerevisiae* were absent, these elements were concluded to have entered the *S. cerevisiae* genome via horizontal transmission from *S. paradoxus* or from some ancestral species shared between *S. paradoxus* and *S. cerevisiae* (4). Ty3 also has a greater proportion of solo LTRs

relative to full-length elements than other Tys except Ty4. Although the reason for this is not clear, it is possible that this explains the absence of full-length copies of Ty3p from the modern *S. cerevisiae* genome (4).

TY3 OVERVIEW

Integrated, full-length copies of Ty3 are 5.4 kb in length, comprised of two LTRs of 340-bp each, flanking overlapping ORFs, *GAG3* and *POL3*. Ty3 is transcribed into a 5.2-kb genomic (g)RNA that begins and ends in the LTRs (Figure 1). Translation of this RNA yields polyprotein precursors, a 34-kDa Gag3 and a 178-kDa Gag3–Pol3 product of frameshifting between *GAG3* and *POL3*. Gag3 contains major structural protein domains CApsid (CA), SPacer (SP), and NucleoCApsid (NC). Gag3–Pol3, in addition to those structural domains, contains catalytic domains PRotease (PR), Reverse Transcriptase (RT), and INtegrase (IN), and a spacer “J” between the PR and RT domains. Gag3 and Gag3–Pol3 assemble together with gRNA into immature 44 to 53 nm diameter virus-like particles (VLPs). Assembly of these occurs in cytoplasmic foci referred to as retrosomes (11) in which Ty3 proteins and RNA are concentrated with host assembly factors. Retrosomes form over the first several hours of Ty3 expression and become generally perinuclear. Within the VLP, precursor polyproteins undergo proteolytic processing by Ty3 PR into mature species. These populations of VLPs are much more diverse in size and morphology and range from 25 to 52 nm with electron dense cores. Subsequently Ty3 RT reverse transcribes gRNA into cDNA and remodeling of the VLP accompanies nuclear entry of a preintegration complex (PIC) of unknown composition. Integration into the transcription start site (TSS) of genes transcribed by RNA polymerase III (RNAP III) completes the cycle.

The reference *S. cerevisiae* strain BY4741 contains two full-length elements, YGRWTy3-1 and YILWTy3-1 (9, 12). YGRWTy3-1 is transpositionally active. YILWTy3-1 is transcribed and translated, but is inactivated by a frameshift mutation in the IN-coding region. The active element, YGRWTy3-1, is comprised of 340-bp LTRs flanking an internal coding domain of 4671 bp for a total length of 5351 bp. Ty3 null strains have been derived by deletion of endogenous elements (13).

TY3 EXPRESSION

Mating control of Ty3 expression

Ty3 transcription initiates in the upstream LTR and terminates downstream of that point in the downstream

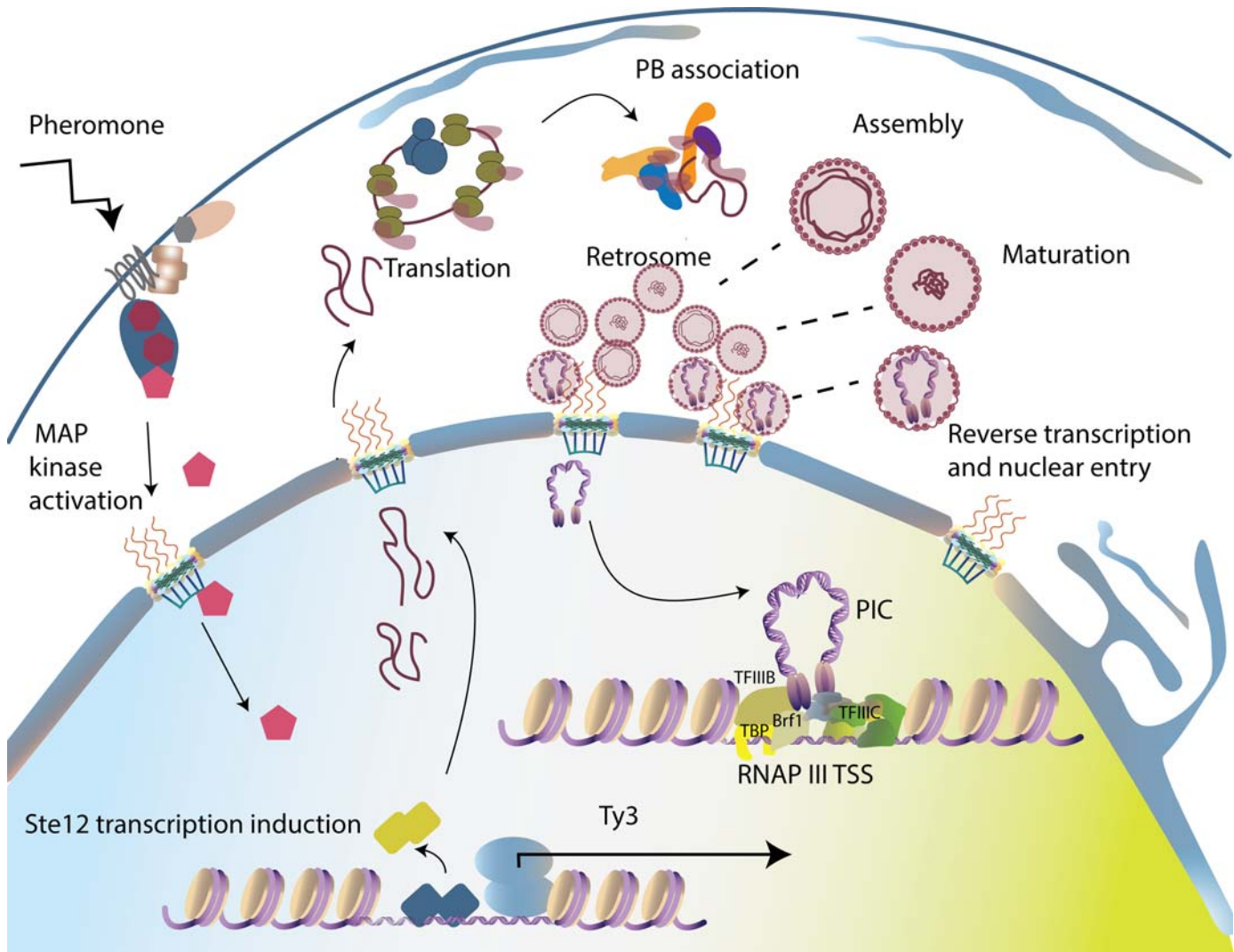


FIGURE 1 Ty3 retrotransposition. (A) Ty3 replication cycle. Pheromone binding to *MATa* or *MATa* pheromone receptors activates G protein-coupled mating signal transduction via mitogen-activated protein (MAP) kinase kinase kinase Ste7, MAP kinase kinase Ste11 and MAP kinase Fus3 (rose). Scaffold protein Ste5 (blue) supports specificity of their interaction preventing crosstalk with the filamentous growth pathway. Fus3 phosphorylates Dig1 and Dig2 negative regulators (gold) of Ste12 (dark blue), which then dissociate allowing Ste12 activation of RNA Pol II transcription of Ty3. Ty3 poly(A) RNA (maroon) is exported and translated into Gag3 and Gag3-Pol3 (tan), which then associate, together with the gRNA and RNA processing body (PB) factors, forming retrosomes within which Ty3 VLPs assemble. These foci become perinuclear over time. Assembly activates protease (PR) processing and maturation of the virus-like particles (VLPs). After cells mate (not shown) reverse transcription of the gRNA into cDNA occurs. Uncoating (dissociation of Gag3) presumably accompanies nuclear entry of the PIC. (A, B) Ty3 cDNA associates with RNAP III transcription initiation complexes composed of TFIIB (yellow) and TFIIC (green). *In vitro* TATA binding protein and Brf1 constitute the minimum target, but *in vivo* evidence suggests that TFIIC can also be present. [doi:10.1128/microbiolspec.MDNA3-0057-2014.f1](https://doi.org/10.1128/microbiolspec.MDNA3-0057-2014.f1)

LTR to yield the 5.2-kb RNA. This defines terminal RNA segments as U5 (unique to the 5' end); R (repeated); and U3 (unique to the 3' end) for a full-

length element structure of U3-R-U5-internal domain-U3-R-U5 and RNA structure of R-U5-internal domain-U3-R (Figure 2A, B). This pattern of expression is

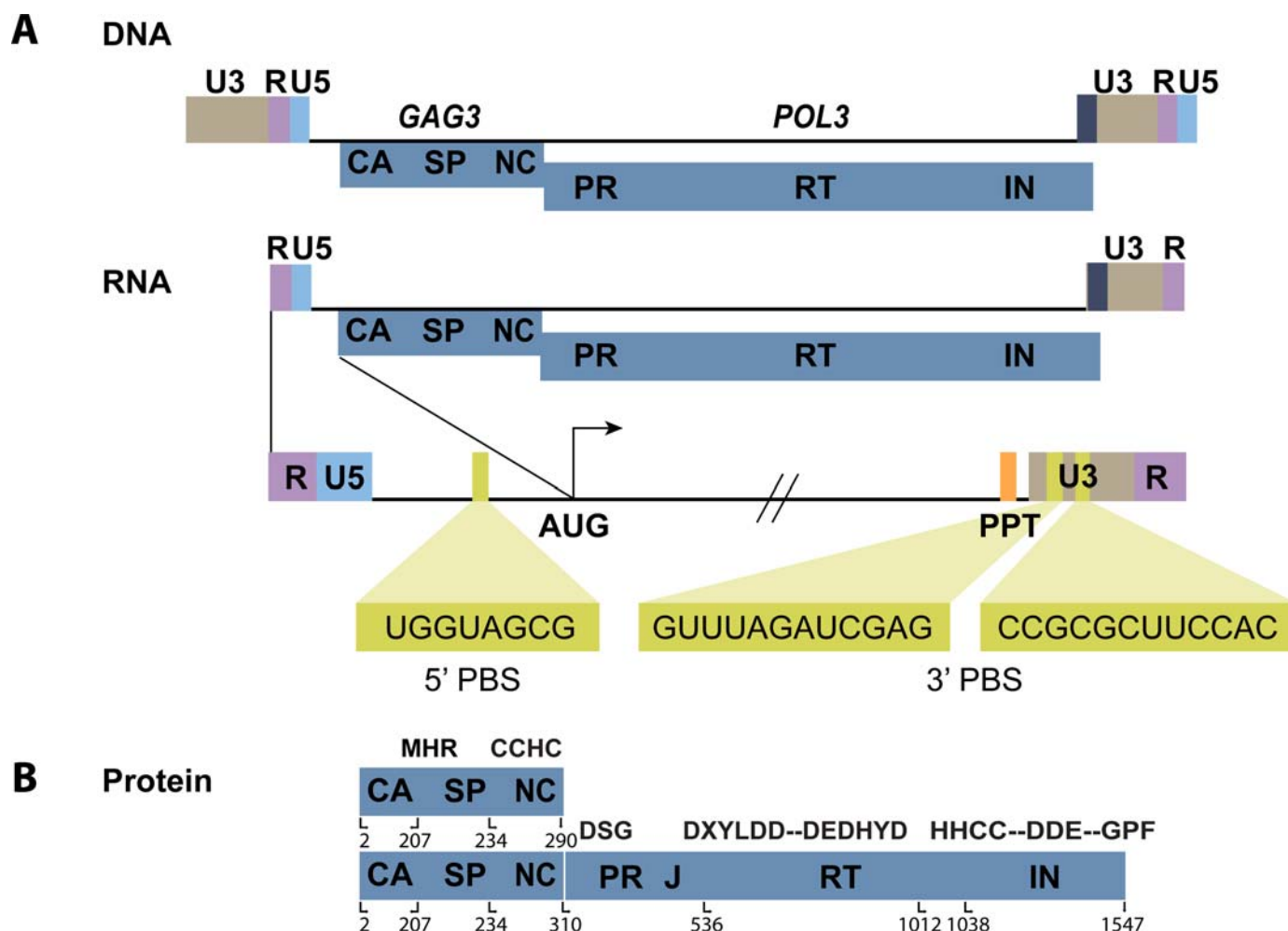


FIGURE 2 Ty3 DNA, RNA, and protein. (A) DNA and RNA. The 5.4-kbp Ty3 element is transcribed into a 5.2-kb RNA. The major 5' end of Ty3 RNA maps to 118 nucleotides (nt) inside the upstream long terminal repeat (LTR), and the 3' end to heterogeneous positions between 243 and 273 nt inside the downstream LTR, as well as beyond the downstream LTR (14). The overlap resulting from termination downstream of the position of initiation results in a sequence that is repeated ("R") and defines 5' U5 and 3' U3 sequences. The initiator AUG of the GAG3 open reading frame (ORF) occurs at nucleotides 76 to 78 inside the Ty3 internal domain for a total 5' untranslated region (UTR) of 193 nt; POL3 extends into the downstream LTR to define a 3' UTR of ~227 nt (12, 14). Candidate upstream TATA and downstream polyadenylation sites are identified, but not experimentally verified. The RNA contains a bipartite primer binding site (PBS), which anneals to initiator tRNA^{Met} in the upstream untranslated region and the downstream LTR (gold boxes). The GAG3 and POL3 ORFs encode Gag3 and Gag3–Pol3. (B) Protein. Gag3 is 290 amino acids (aa) and contains domains that mature via Ty3 PR processing into 207-aa capsid (CA), 27-aa spacer (SP), and 57-aa nucleocapsid (NC). Gag3–Pol3 contains those and additionally, protease (PR); reverse transcriptase (RT) starting at amino acids 536; and two forms of integrase (IN) domains (starting at amino acids 1012 and 1038) produced via a programmed frameshift. The POL3 ORF terminates within the downstream LTR so that the polypurine tract (PPT) plus strand primer is actually within the IN-coding region. (C) Reverse transcription of Ty3 genomic RNA. The tRNA primes synthesis of a minus-strand strong stop containing U5 and R segments, which then transfers to the 3' end and primes extension of the minus strand. The plus-strand strong stop intermediate is initiated with cleavage by RNaseH at the downstream end of the PPT just outside the downstream LTR and is extended through U3, R, and U5 and likely copied into the 3' end of the tRNA then transferred to the 5' end of the RNA and extended to form the plus strand of the cDNA. Although as described in the text minus- and plus-strand strong-stop intermediates have been identified, the overall flow described is based on the retrovirus model. An additional possibility (not shown) is that the 5' and 3' ends are transiently joined in a lariat RNA (see text). Bottom, the full-length cDNA has two extra base pairs on each end derived from a 2-nt offset between the priming sites and the LTR ends of the integrated element. Integrase (IN) processes 2 nt from each 3' end and mediates the nucleophilic attacks of the resulting hydroxyls at 5-bp staggered positions flanking the RNA polymerase III (RNAPIII) transcription initiation sites. The integration site is repaired, resulting in 5-bp direct repeats flanking the ends of the Ty3 element. doi:10.1128/microbiolspec.MDNA3-0057-2014.f2

Figure 2 continues on next page

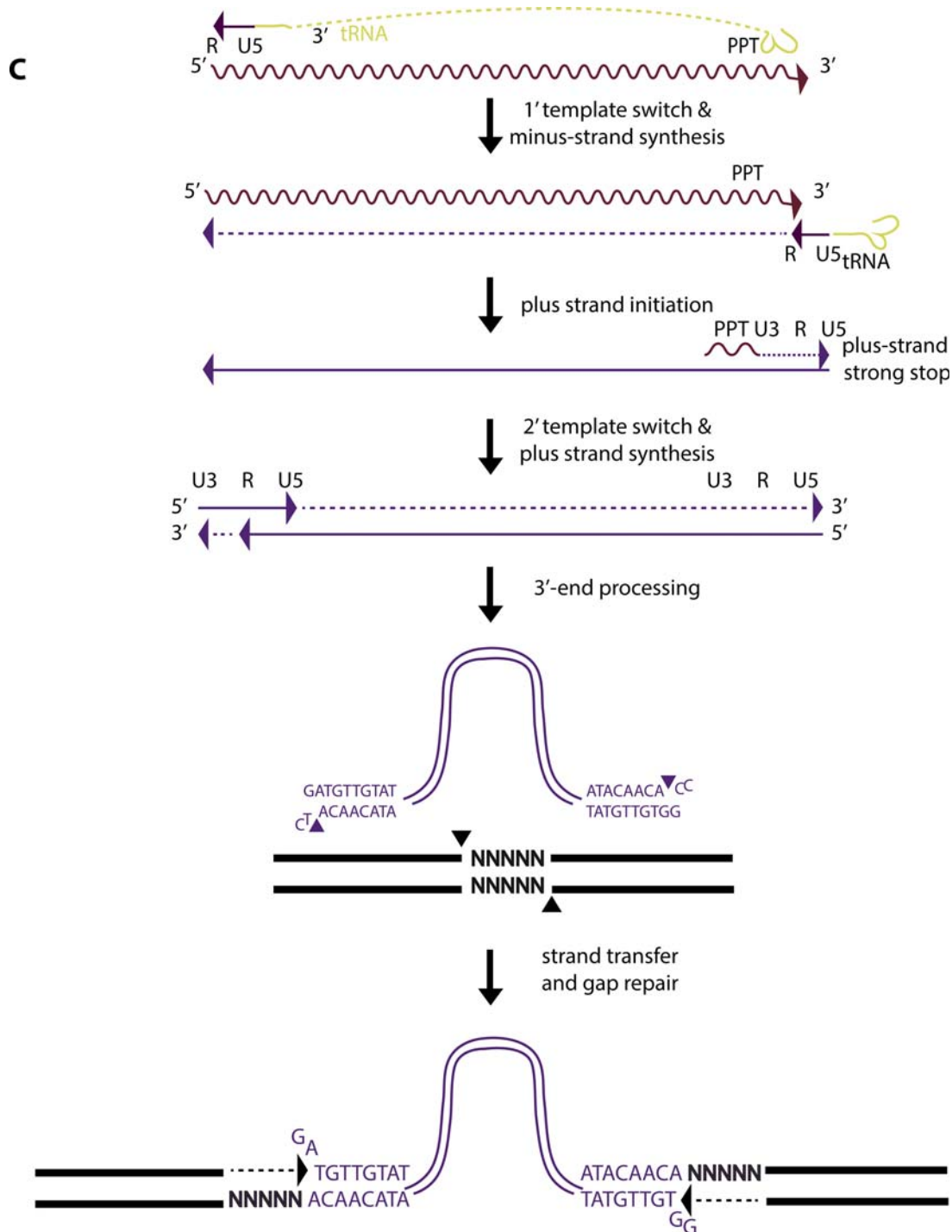


FIGURE 2 (continued)

characteristic of LTR retrotransposons and to retroviruses. The Ty3 GAG3 ORF lies downstream of R-U5, but the *POL3* ORF overlaps U3.

Saccharomyces cerevisiae exists in haploid *MATa* and *MATα* mating types that secrete mating-type-specific pheromones that bind to receptors triggering mitogen-

activated protein (MAP) kinase signaling. Ty3 transcription and retrotransposition are subject to mating type control (Figure 1A). In haploid cells, Ty3 is expressed at low levels. However, the full-length Ty3 elements (13, 14) and some LTRs are induced up to 80-fold in mating cells (15). This activates the MAP

kinase Fus3 to relieve Dig1,2 repression of positive transcription factor Ste12 bound to pheromone response elements. These pheromone response elements occur in promoters of genes mediating physiological changes in preparation for mating including agglutinin production, enhanced vesicular trafficking, polarized cell growth (shmooing), and arrest in G1 of the cell cycle (reviewed in reference 16). Deletion analysis identified two adjacent PRE sequences within the Ty3 U3.

Pheromone induction of Ty3 transcription in G1-arrested cells is accompanied by translation of Ty3 proteins, and formation and maturation of VLPs. However, reverse transcription is delayed until cells resume cycling (17). Experiments in which transposing elements were activated in one mating type, and integrations of those elements were detected into plasmids carried in the opposite mating type demonstrated that this control of cDNA production promotes invasion of Ty3 into new host genomes (18). Once cells have mated, Ty3 is repressed by the action of the MAT a1/a2 heterodimer formed from proteins expressed from the MAT loci of the two haploid mating cell types. Deletion analysis identified MAT a1/a2 repressor binding sites in the U3 region (13).

Ty3 is also regulated in response to environmental conditions. Similar to Ty1, it is induced in *bas1Δ* cells during adenylate nucleotide deprivation. *BAS1* encodes a Myb-related factor that regulates transcription of genes in purine and histidine biosynthetic pathways and meiotic recombination at specific genes (19–21). Although the basis of induction has not been explored for Ty3, Ty1 induction is inversely correlated with anti-sense transcription and depends on both the absence of Bas1 and the presence of the bHLH transcription factor Tye7 (22).

Interaction of Ty3 RNA polymerase II and flanking tDNA RNA polymerase III promoters

The unique Ty3 specificity for integration at the RNAP III TSS provides a context in which to examine interactions of RNAP II and RNAP III promoters. Investigation of the interactions between the *SUP2* tDNA RNAP III promoter and the adjacent Ty3 LTR RNAP II promoter showed that mutations that inactivated the tDNA promoter greatly enhanced pheromone induction of the LTR promoter (23). The interference by RNAP III promoters with RNAP II repression was further defined and shown to be generalizable (24) and dependent upon localization of tDNA and flanking sequences to the nucleolus (25, 26). Hence, similar to expression of chromodomain retrotransposons that

target heterochromatic regions, expression of Ty3, at least under noninducing conditions, might be attenuated by genomic context (18, 27).

As suggested in the foregoing discussion, repression of Ty3 by association with tDNAs is not absolute. In addition to Ty3 being inducible under certain conditions, integrations of genetically tagged Ty3 elements are readily selectable (28). A possible event which could relieve nucleolar repression of tDNA-associated LTR promoters is relocation of transcriptionally active DNA to Nuclear Pore Complexes (NPC) referred to as gene gating (29). Although the nucleolus is located on the nuclear envelope in yeast, genes could transiently relocate to other NPC. In addition to gene gating for RNAP II promoters such as the LTRs, transcriptionally active tDNAs have been shown to localize to NPC (30). Consistent with the latter possibility, chromatin conformation capture analysis of yeast chromosomal DNA indicated that a subpopulation of tDNAs are not associated with rDNA (31).

Ty1/Copia and Ty3/Gypsy coexistence in budding yeast

Ty1 and Ty3 share a subset of host factors and are both induced by MAP kinase signaling in response to environmental stimuli. As described above, Ty3 is induced in haploid cells by pheromone signaling through the mating MAP kinase pathway, which activates transcription factor Ste12 (13, 18). Ty1 is induced under MAP kinase activation of Ste12/Tec1 in diploid cells, in response to nutritional deprivation (32). Despite common upstream components of these pathways, cross talk between them is controlled by mating MAP kinase Fus3-mediated degradation of Tec1 in haploid cells and mating type suppression of Ty3 in diploid cells (33). Ty1 constitutes a high fraction of RNA in haploid cells (34), but Ty1 proteins are actively degraded during the mating pheromone response (35–37). Hence, despite overlapping induction pathways and shared host factors, Ty1 and Ty3 occupy discrete retrotransposition niches.

TY3 RETROTRANSPOSITION ASSAYS

Because retrotransposition is a relatively rare event, occurring in less than 1% of induced cell cultures, the sensitivity of assays used in genetic screens affects the spectrum of Ty or host mutants recovered. Genetic screens require patching naive cells, replica plating patches onto one or more types of selective medium and screening for papillation frequency. Given the rarity of

events, identification of mutants deficient in transposition becomes nontrivial. Identification of the step in Ty3 retrotransposition affected by mutations is further complicated because expression at the RNA, protein, and even cDNA level is relatively robust, but cells in which transposition occurs are infrequent. One possible explanation of this apparent disparity is that Ty3 VLPs assemble in cytoplasmic clusters. As clusters increase in size, the ability of individual VLPs to access nuclear pores may become limiting.

Ty3 can be induced under native or synthetic regulation. Induction under the native pheromone promoter allows evaluation of host factors in the physiologic context of retrotransposition, but transcription increases as cells accumulate in G1, and so is relatively asynchronous. Alternatively Ty3 has been engineered to be synchronously inducible in the presence of galactose by substitution of the *GAL1-10* upstream activating sequence for the upstream Ty3 U3 region (9, 13, 14). Galactose induces a synchronous response, but has the disadvantage that galactose metabolism is under catabolite repression, so that rapid induction requires pre-growth in a nonglucose carbon source in which the growth rate slows.

Ty3 targeting offers the possibility of monitoring retrotransposition by selecting for insertions into target traps. One such target is composed of a pair of divergent tDNAs, one of which is a suppressor tDNA (18). Expression from the target suppressor is blocked by mutual interference of the tDNA transcription complexes so that cells containing a suppressible allele in a nutritional marker are auxotrophic on selective medium. Ty3 integration between the tDNAs relieves interference and growth correlates with retrotransposition.

Genetic assays in which a marker is inserted into the retrotransposon in theory allow straightforward retrotransposition assays. However, in the case of Ty3, the *POL3* ORF, which encodes IN, overlaps important *cis*-acting sequences within and flanking the LTR so that there is no spacer into which a marker can be inserted without inactivating the element. This impediment was circumvented by introducing a short repeat of the downstream end of *POL3*, which supplies a second, non-coding, copy of the *cis*-acting polypurine tract (PPT) and LTR sequences required for replication and integration. A genetic marker, for example, *HIS3*, can be inserted between these repeats (38). This Ty3 can be expressed from a *URA3*-marked plasmid so that in a *his3 ura3* background, retrotransposed cells can be selected on medium lacking histidine and containing 5-fluoro-uracil, which selects against the *URA3*-marked plasmid (39)

but for the transposed element. Incorporation of an anti-sense intron into *HIS3*, a strategy devised for Ty1 (40), further simplifies the assay. In this strategy, the marker, which is transcribed anti-sense to the Ty, is disrupted by an artificial intron oriented so that splicing signals are read in the Ty sense. Ty3 transcription, splicing, reverse transcription, and integration generate a genomic copy of the selectable intron-free marker (Figure 3, left panel). This assay has the limitation that it superimposes a requirement for splicing into genetic screens.

Physical retrotransposition assays have the major advantages of using native elements, not requiring long periods of selective cell growth, and the ability to examine the activity of specific targets. In the case of Ty3, PCR using primers annealing inside Ty3 and within or downstream of tDNAs allows rapid and specific measurement of retrotransposition (41). In a modification of this approach suitable for next generation sequencing, a short unique sequence was inserted into the test Ty3. After expression, genomic DNA fragmentation and ligation of adapters, PCR using primers annealing to the Ty3 tag and downstream adapter allowed next generation sequencing of transposon-genome junction sites (Tpn-Seq) (Figure 3, right panel) (38) (our unpublished results).

TY3 VIRUS-LIKE PARTICLES: COMPONENTS AND ASSEMBLY

Polyprotein precursor expression and maturation

Ty3 mRNA is translated into a 290-amino-acid (aa) (34 kDa) Gag3 structural polyprotein precursor from the first ORF, *GAG3*, and a 1547-aa (178 kDa) Gag3-Pol3 fusion polyprotein precursor by a programmed plus-one frameshift between *GAG3* and *POL3* (28, 42). Gag3 contains structural domains CA, SP, and NC. The Gag3-Pol3 polyprotein precursor, in addition to Gag3 domains, contains critical catalytic domains PR, RT, and IN. These are encoded in that order, which is the same as in retroviruses. The N-termini of these proteins have been determined by protein sequencing and mass spectrometry (43, 44).

Precursor Gag3 and Gag3-Pol3 polyproteins multimerize via Gag3-Gag3 interactions into immature VLPs (44, 45). Within VLPs, PR is activated to process Gag3 and Gag3-Pol3 into protein domains of the mature VLPs (Figure 2B). Ty3 PR is a 15-kDa aspartyl protease with DSG active site residues, similar to retroviral PR, and required for polyprotein processing (43). Based on the retrovirus precedent, it is likely that dimerization of

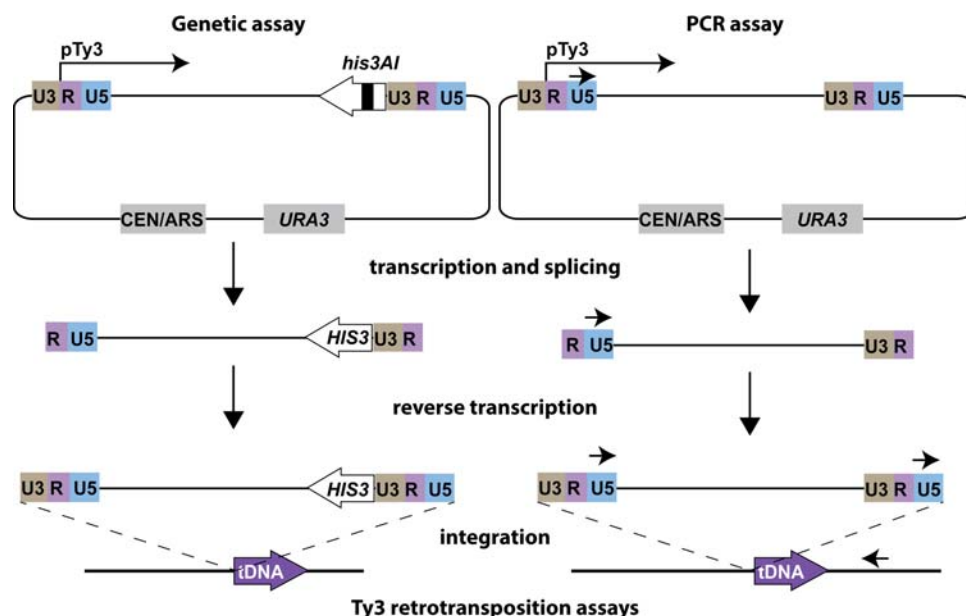


FIGURE 3 Retrotransposition assays. Ty3 retrotransposition can be assayed using the *his3AI* reporter embedded in the mobilized Ty3 (genetic) or by PCR. In the case of the Genetic Assay (left panel), Ty3 transcription is accompanied by splicing of a synthetic intron which is antisense to the *HIS3* marker, preventing *HIS3* from being productively expressed. After Ty3 transcription, splicing and reverse transcription, the intronless *HIS3* gene is expressed and cells in which transposition has occurred are selected on medium lacking histidine. Alternatively, retrotransposition of a tagged Ty3 element can be monitored by PCR assay (right panel) using one primer complementary to the Ty3 tag and one complementary to a sequence present in a tDNA family or in the unique sequence downstream of any tDNA target. [doi:10.1128/microbiolspec.MDNA3-0057-2014.f3](https://doi.org/10.1128/microbiolspec.MDNA3-0057-2014.f3)

PR within the VLP promotes its activation. In addition to PR itself, Gag3–Pol3 processing by PR generates 55-kDa Ty3 RT and 61-kDa and 58-kDa IN proteins (46). The significance of the difference in N-terminal processing between these IN forms is unknown. However, the 61-kDa IN species expressed as a recombinant protein is capable of *in vitro* strand-transfer activity (47). Within cells expressing wild-type (WT) Ty3, VLPs are heterogeneous, representing a mixture of mature and immature forms (43).

In addition to catalytic domains with known functions encoded in *POL3*, a Junction (J) domain inferred to be 10 kDa is encoded between PR- and RT-coding regions (43, 48). This includes a 26-aa motif, which occurs twice in YGRWTy3-1 and three times in YILWTy3-1. Deletion of J within boundaries chosen to reconstruct the processing site decreased Ty3 cDNA levels dramatically, but did not abrogate retrotransposition (48). Chimeras constructed between the YGRWTy3-1 and YILWTy3-1 elements showed that the J domain does not account for the inactivity of the YILWTy3-1 (12).

Fluorescence microscopy provides a powerful tool for examining the appearance and subcellular localization of

retrotransposon proteins and RNA. In cells undergoing pheromone induction, a monomeric cherry-tagged version of the Gag3 precursor polypeptide appears within about 10 min as a diffuse cytoplasmic signal and a green fluorescent protein (GFP) tagged version of the full-length Gag3–Pol3 precursor polypeptide appears within about 30 min (our unpublished results) (49). Immunoblotting shows that processed products, likely reflecting VLP formation, become more abundant than Gag3 between 2 to 4 hours (our unpublished results), and clusters of VLPs can also be detected during this time by transmission electron microscopy (our unpublished results). Anti-GFP immuno-electron microscopy shows that Ty3 proteins tagged with GFP localize to these clusters (49). Southern blotting detects cDNA within 6 hours.

Frameshifting

Greater amounts of structural than catalytic proteins are required to assemble VLPs (28). The ratio of these components is controlled by a programmed plus-one frameshift near the downstream end of *GAG3*. The relative abundance of Gag3 and the presence of this domain in Gag3–Pol3 not only drives assembly, but

insures inclusion of catalytic proteins (28, 45, 50). The key frameshifting event is mediated by a 7-nucleotide (nt) sequence (GCG AGU U) read as Ala-Ser within the GAG3 frame. Within this sequence, the Ser codon (AGU) is decoded by a rare tRNA, stimulating plus-one frameshifting into the GCG GUU frame. This sequence is translated as Ala-Val, resulting in formation of full-length Gag3–Pol3 (42). Shifting is further stimulated by a downstream enhancer structural element of 12 nt. The Ty3 recoding shift is essentially executed by the incoming amino-acyl tRNA. Hence, this programmed frameshift differs from both the retrovirus “slippery” minus-one frameshift and the plus-one Ty1 frameshift, which depend upon shifting of ribosome-bound tRNAs. The inefficiency of this process causes the Gag3–Pol3 precursor to be produced at 5% to 10% of the level of structural proteins (28, 42). Given this mechanism, it is not surprising that growth conditions affect the frequency of frameshifting and modulate retrotransposition. For example, frameshifting increases in cells grown in galactose-containing medium (51).

Virus-like particle structure

Immature VLPs contain Gag3, Gag3–Pol3, dimeric Ty3 gRNA (52), tRNAs including Ty3 primer initiator tRNA^{Met} (tRNA^{Met1}) (53) and probably other host RNAs and proteins (52, 54). Gag3 is the major structural protein of immature Ty3 VLPs, and its expression in *Escherichia coli* is sufficient to achieve formation of aberrant particles (55). Formation of VLPs correlates strongly with ability of mutants to undergo proteolytic processing and reverse transcription. The simplest interpretation is that the VLP shell not only triggers processing to activate protein domain functions, but also selectively sequesters, concentrates and protects enzymes and substrates. Ty3 PR and RT active site mutants, and WT VLP populations have been characterized by transmission electron microscopy and atomic force microscopy (44, 56–58). Immature VLPs generated from Ty3 PR active site mutants are relatively uniform, and appear roughly spherical with diameters measured by atomic force microscopy of 44 to 53 nm. Striking physical features of the VLPs, particularly evident in atomic force microscopy preparations of immature forms, are knob-like capsomeres with six-fold symmetry interspersed with knobs of five-fold symmetry suggesting a pseudo-icosahedral structure. Transmission electron microscopy of immature VLPs shows an outer layer that is presumably the Gag3 and associated RNA. Populations of VLPs in cells expressing WT Ty3 are more heterogeneous, with diameters ranging from 25

to 52 nm. Many of these have electron-dense centers, presumably reflecting NC and complexed RNA released from the shell. Smaller particles could be cores that have generated cDNA and have remodeled in some way.

Ty3 VLPs are extremely stable, and Gag3 spontaneously aggregates or forms particles (unpublished data). Ty1 particles have also been isolated and studied extensively, and must be similarly stable (59). In contrast, retroviral cores undergo maturation coincident with budding and so are enveloped in the mature form. These core particles can be stripped of envelope and readily dissociated (60). VLP stability may be a general feature of retrotransposons necessitated by intracellular maturation in the absence of envelope.

Capsid

The 290-aa Gag3 is processed into 207-aa CA (processed to remove the N-terminal Met), 26-aa SP and 57-aa NC species (43, 44). A CA-SP species is also prominent among Gag3 products of processing (56). CA is acetylated on the N-terminal Ser (unpublished data). Although retroviral Gag CA domains are not generally conserved in sequence, they are remarkably similar in structure. They share N-terminal domain (NTD) and C-terminal domain (CTD) bundles of alpha helices (61, 62) with an intervening linker. Modeling suggests that, similar to retroviral CA domains, the CA domain of Ty3 Gag3 forms alpha helical bundles with the NTD contributing the outer surface of the VLP (58). The N-terminal 20-aa of Ty3 Gag3 contains a late domain motif (YPXL). Late motifs are named after their function “late” in the retrovirus lifecycle when structural proteins interact with “Endosomal Sorting Complexes Required for Transport” (ESCRT) proteins to sequester maturing VLPs into plasma membrane budding structures (63). Intriguingly although Ty3 has not been observed budding into intracellular membranes, density equilibrium centrifugation suggests that it is transiently associated with membranes (our unpublished results), and ESCRT proteins are required for Ty3 retrotransposition (64). Although retroviral CA domains are mainly conserved at the structural level, a “Major Homology Region” (MHR) is found in Gag CA domains and also occurs in Ty3 CA from amino acids 86 to 100 (QGX₂EX₃FX₃LX₃H) (65). The Ty3 MHR occurs N-terminal in Ty3 Gag3 compared with the MHR in retroviral Gag, but mutations in the MHR cause similar defects in Ty3 and retroviral particle morphogenesis.

Analysis of Gag3 mutants generated by Ala scanning identified residues that affect assembly as evaluated by transmission electron microscopy visualization of VLPs,

RNA packaging, and concentration of Ty3 protein into foci. Assembly was particularly sensitive to disruption of the N-terminal 100 aa (58). Only a few individual mutations completely abrogated particle formation and in one case this was accompanied by appearance of extensive cytoplasmic filaments. Visualization with RFP and GFP fusions showed that these filaments contained Gag3 and at least two components of processing bodies (PB) (58). Two-hybrid analysis showed that interactions occur between the NTD and NTD, as well as between the NTD and CTD and that introduction of mutations with effects on assembly disrupted these interactions (45, 58).

Nucleocapsid

The 57-aa Ty3 NC domain of Gag3 is critical for capture of the gRNA. The NC NTD contains 17 basic aa and is followed by one copy of the conserved zinc knuckle CX₂CX₄HX₄C present in one or two copies in retroviral NC domains (11, 50). Mutations in conserved residues within and around the zinc knuckle eliminated concentration of Ty3 RNA into retrosome foci, and significantly reduced gRNA incorporation into VLPs (packaging) and retrotransposition (57). Unexpectedly, substitution of the first and second, but not the third and fourth, residues of the CCHC motif caused accumulation of Gag3 in nuclear, partially condensed particles. Because substitution of any one of the CCHC residues should disrupt zinc binding and gRNA packaging, such disparate phenotypes suggest that this subdomain has more than one role in assembly. In addition, the finding that Gag3 accumulates efficiently in the nucleus of these mutants suggests that, similar to the scenario proposed for Rous sarcoma virus gRNA packaging (66, 67), Ty3 Gag3 unbound to gRNA might at some stage invade the nucleus to scavenge for gRNA. Potentially related scenarios for Gag perinuclear capture of newly exported gRNA are proposed for human immunodeficiency virus 1 (HIV-1) (68, 69) and Ty1 (70).

Spacer

A SP domain separates Gag3 CA and NC domains, and a similar situation exists in a subset of retrovirus Gag proteins, including those of HIV-1 and Rous sarcoma virus (71, 72). These SP domains are not conserved, but are thought to participate in assembly, possibly substituting for functions performed by the CTD of CA for retroviruses lacking SP (73) (reviewed in reference 11). Ty3 SP is distinguished from retrovirus SP domains in that it is longer and more acidic. As is the case for retroviruses, removal of SP is important for

morphogenesis. Mutations at the Ty3 SP–NC junction that disrupt PR processing also block processing of CA–SP, but the converse is not the case. This situation suggests that Gag3 processing is ordered (56), similar to what occurs for HIV-1 (74). Because failure to produce free NC does not block gRNA packaging but blocks appearance of cDNA, these observations argue that the NC domain of immature Gag3 functions in packaging, but mature NC must chaperone nucleic acid interactions during reverse transcription. Disruption of CA–SP processing also blocks retrotransposition but subsequent to cDNA production. Surprisingly, deletion of the SP domain by reconstructing a hybrid processed CA–NC mutant, allowed a low level of retrotransposition, indicating that SP is not absolutely essential. In contrast, conversion of acidic SP residues to Ala disrupted VLP formation and eliminated retrotransposition. Together these observations are consistent with a model in which the Ty3 acidic SP domain could interact with the basic NTD of NC in a nonessential condensation function that promotes assembly. However, if SP is present, it is essential that it be released from CA to allow progression through some step subsequent to reverse transcription, such as VLP uncoating.

Genomic RNA

The Ty3 gRNA contains *cis*-acting sequences, enabling Gag3-mediated packaging (*psi*), priming of reverse transcription, and integration. Ty3 gRNA is dimeric in VLPs (52) and by analogy with retroviruses has roles in packaging and reverse transcription. The primer for Ty3 minus-strand reverse transcription is tRNA^{Met} (53), which anneals to complementary primer binding site (PBS) split between just downstream of U5 and within U3 (75). The primer for plus-strand reverse transcription is a PPT just upstream of U3. *Cis*-acting sequences were collectively first delimited by determining that Ty3 nucleotides 429 to 4979 of the 5.2-kb RNA were dispensable for retrotransposition in the presence of a helper Ty3 to provide proteins (28). Subsequently, an assay in which packaged RNA was protected from exonuclease digestion further delimited the Ty3 packaging sequence (*psi*) (76). These experiments showed that an abbreviated RNA with the Ty3 5' untranslated region (UTR) lacking both the upstream and downstream PBS and having a heterologous 3' UTR is sufficient to mediate packaging of a short RNA encoding Gag3 (76) (Figure 2C). At least a subset of retroviral *psi* sequences, including that of murine leukemia virus, are upstream sequences containing hairpin structures. Within these hairpin loops, short inverted repeats seed

gRNA dimer formation, and trigger annealing of the RNA sequences within the opposing gRNA hairpins. The resulting gRNA dimers expose binding sites for the NC domain of Gag (77). Although such features are not identified for Ty3, the 5' end of the gRNA does contain the region required in *cis* for protection by Gag3.

To better understand the subcellular localization of VLP assembly, Ty3 RNA was fluorescently tagged. RNA tagged with tandem hairpin binding sites for RNA MS2 bacteriophage CApsid (MS2-CA) can be visualized if expressed in cells together with MS2-CA fused to a fluorescent protein reporter (78). Ty3 gRNA tagged with the MS2-CA binding site is active for retrotransposition and was visualized with MS2 CA fused to red fluorescent protein (RFP) or GFP (49, 57, 76). Examination of cells expressing Ty3 RNA visualized with MS2-CA-GFP showed that gRNA colocalizes in foci with Gag3 identified using anti-CA antibodies (49).

Retrosome localization of virus-like particle assembly

One of the striking features of *Drosophila* (79) and *S. cerevisiae* (54) expressing Ty3/Gypsy and Ty1/Copia retrotransposons (80) is occurrence of VLP clusters or retrosomes. Several observations support the interpretation that, at least in the case of Ty3, retrosomes are associated with assembly of Ty3 protein and RNA into VLPs (Figure 1A). First, Ty3 RNA, proteins, and VLPs accumulate in clusters and individual VLPs are rarely observed outside clusters; second, mutations that disrupt assembly either abrogate formation of clusters or cause less dense clusters of poorly formed particles; third, deletion of genes encoding host proteins required for assembly also disrupts cluster formation (49, 56–58) (our unpublished results). Studies of Ty3 retrosome formation have used antibodies against individual Ty3 proteins as well as GFP, RFP, and monomeric cherry (mCherry) as *in vivo* reporters for Ty3 RNA and protein (49). Ty3 Gag3 and Gag3–Pol3 have been fused alternatively to GFP, RFP or mCherry. Within 1 hour of Ty3 induction, fluorescently tagged Ty3 gRNA and proteins progress from diffuse fluorescence to small foci, which aggregate over the next several hours into larger foci containing VLPs and representing retrosomes (49) (our unpublished results). Quantitation of the ability of Ty3 RNAs lacking various Ty3 gRNA attributes (e.g., frameshift, *POL3* ORF, PBS) or having UTRs from heterologous RNAs showed that in the presence of Gag3, RNAs localize to foci if they contain either the 5' Ty3 UTR or a second long, poorly translated ORF (76). However, *GAG3–POL3* RNA in which the Ty3 UTR are substituted with

heterologous UTRs is not efficiently packaged, consistent with the 5' UTR containing *psi*. Hence, localization to foci does not insure assembly of Ty3 RNA into particles.

Retrosome association with RNA processing body components

Screens for Ty1 and Ty3 host factors identified conserved components of RNA PB as factors required for WT levels of retrotransposition (64, 81). PB are RNA granules containing concentrations of RNA deadenylation-dependent degradation components including deadenylation enzymes, translation suppressors, decapping activators and enzymes, helicases, and exonuclease. PB were initially identified as sites of mRNA degradation (82), but this is unlikely to be their exclusive function. A subset of RNAs in PB is competent to resume polysome-associated translation (83). In addition, at least some poly(A)-dependent degradation occurs cotranslationally (84). Indeed further investigation indicated that PB and stress granule (SG) ribonucleoprotein particles, which reversibly sequester nontranslating RNAs during stress (85), share a subset of components (86). A revised interpretation is that these granules represent different ends of a spectrum of ribonucleoprotein complexes (87). PB proteins colocalizing with Ty3 retrosomes (49) include translational repressors Pat1 and Dhh1 (88–90); decapping activator Lsm1 (91); decapping protein Dcp2 (92); and 5' exonuclease Xrn1 (87, 93–95).

Galactose induction of Ty3 expression causes formation and/or enlargement of PB, suggesting that PB proteins interact directly with Ty3 proteins and/or RNA. Examination of galactose-induced Ty3 protein and RNA fluorescent reporters in cells expressing PB-GFP reporters showed that Ty3 RNA and protein and PB components colocalize (11, 49). PB formation is also observed under conditions independent of galactose induction of Ty3. These conditions include nutritional stress (86), stationary phase (96), and mating (97). Ty3 protein and PB factors also colocalize in mating cells (49).

On the surface, it is surprising that retroelement assembly is associated with factors engaged in RNA degradation. However, as discussed above, not all PB targeted RNAs are degraded and the natural sequestration of RNAs in PB away from translation could provide a mechanism for Ty3 RNA to transition from translation into assembly. The *POL3* ORF, downstream of the frameshift would have a lower density of ribosomes and could be subject to binding by PB translational repressors. Translational repression might enhance access of Gag3 to the 5' packaging site, thereby protecting

the 5' end from decapping and degradation. Greater interaction with PB or trafficking factors would then promote assembly by concentrating VLP components (76). In the absence of such enhanced concentration, Ty3 proteins and RNA might fail to assemble. PB factors are also required for Ty1 assembly (98, 99). However, Ty1 retroosomes are not completely coincident with PB or SG foci (98, 99) (see Curcio, Lutz and Lesage, Chapter 41).

REVERSE TRANSCRIPTASE AND REVERSE TRANSCRIPTION

Reverse transcription is the defining feature of the retroelement replication. Non-LTR retrotransposon RT containing solely the polymerase domain (Pol) likely preceded LTR retrotransposon RTs that contain Pol and RNaseH domains (3). The Pol catalytic activity is responsible for cDNA production from both plus-strand RNA and minus-strand cDNA templates. The RNaseH domain is responsible for degradation of template RNA in DNA–RNA heteroduplexes as well as the cleavage at PPT to create the plus-strand primer. Pol and RNaseH domains, each have conserved carboxylate triad active sites competent to chelate two Mg^{2+} or Mn^{2+} ions. However, these reactions differ in both their structural context and in the reactions they mediate: in Pol, a hydroxyl attacks a dNTP; whereas in RNaseH, H_2O is activated to hydrolyze a polynucleotide chain (see Hughes, Chapter 46) (100, 101).

Relationship of Ty3 RT to other RT enzymes

Despite conservation of RT Pol and RNaseH domains among retroviral RT, there is diversity in quaternary structure: gamma-retroviruses, such as murine leukemia virus, have monomeric RT (Pol–RNaseH); alpha-retroviruses, such as Rous sarcoma virus, use an RT/RT-IN heterodimer; and lenti-retroviruses such as HIV-1, use a Pol–RNaseH/Pol (p61/p56) to highlight a few variations (see Hughes, Chapter 46; Skalka, Chapter 48). Retroviruses likely evolved from progenitor LTR retrotransposons related to Ty3/Gypsy. Ty3 RT has about 25% identity in the Pol domain with monomeric murine leukemia virus RT (2). Ty3/Gypsy retrotransposon RNaseH domains occur immediately after the Pol domain. Retroviral RNaseH domains are not only separated from the Pol domain by a RNaseH fold, that lacks the key carboxylate triad, but are actually more closely related to RNaseH domains of cellular enzymes than RNaseH domains of LTR retrotransposons. An attractive explanation is that retroviruses acquired a

cellular RNaseH domain, but retained the progenitor retroelement RNaseH domain as a catalytically inactive connector between Pol and RNaseH (102).

Ty3 reverse transcriptase structure and active sites

In vivo, Ty3 Gag3–Pol3 is processed into 55-kDa RT and 116-kDa RT-IN fusion species (46). *In vitro* recombinant 55-kDa RT displays an ability to extend synthetic primers on an RNA template comparable to that of recombinant retroviral RT (103, 104). Inspection shows Ty3 RT amino acids D151–X₅₉–Y211–L212–D213–D214 consistent with the conserved retroviral Pol active-site motif, D–Xn–Y–L–D–D. The Ty3 protein sequence D358–E401–D426–H427–Y459–D469 is consistent with the RNaseH carboxylate triad in other members of the Ty3/Gypsy family, D–E–DH–Y–D (104). The conserved D–E–D triad is required for metal binding and activity, as are the His and Tyr residues (105). However, consistent with different origins of the RNaseH domain, the retroviral RNaseH motif, D–E–D–HD, differs from the Ty3/Gypsy motif, in that Tyr is lacking and the His residue is moved away from the carboxylate triad (e.g. HIV-1 D443–E478–D498–H539–D549).

The crystal structure of the Ty3 55-kDa RT bound to a 16-bp RNA/DNA heteroduplex PPT was the first retrotransposon RT crystal structure. It revealed a novel, substrate-induced dimer structure (106). The bound nucleic acid is intermediate between the A and B forms, a polynucleotide duplex with little structural deformation. In the Ty3 RT, subunits “A” and “B” have similar conserved subdomain folds, but these are arranged asymmetrically. Subunit A displays the characteristic Pol right-handed topology with palm subdomain containing the active site carboxylates, fingers stabilizing the RNA primer and thumb interacting with template DNA. In contrast, subunit B thumb and RNaseH subdomains are rotated by approximately 90°. This arrangement obstructs interaction between the subunit A RNaseH active site and scissile phosphate, but leaves an unobstructed path for subunit B RNaseH. Altogether the structure suggests that catalytic activities are shared between the two subunits. In support of that interpretation, RT proteins mutated in the half-dimer interface or in the RNaseH active site lack *in vitro* activity, but complement when combined *in vitro*. Hence, Ty3 and HIV-1 RT have similar conserved subdomains, but the overall structures show that subunits play different roles. In the case of Ty3 RT, substrate binding induces RT dimerization, whereas in the case of HIV-1, RT forms a heterodimer in the absence of substrate. Ty3 RT

enzymatic activity is split between Pol, contributed by subunit A, and RNaseH contributed by subunit B. In contrast, HIV-1 Pol and RNaseH activities are contained in p66 and the p51 subunit functions as a scaffold. Nonetheless, p51 of HIV RT and subunit B of Ty3 RT have similar subdomain arrangements, including the positions of the vestigial RNaseH p51 connector domain and active Ty3 subunit B RNaseH.

Mechanism of reverse transcription

The sites for initiation of reverse transcription of Ty3 minus-strand and plus-strand cDNA synthesis intermediates and full-length cDNA are identified. Overall, observations are consistent with the sequence of events proposed for replication of retrovirus gRNA (Figure 2C, see legend for detailed description) (see Skalka, Chapter 48; Hughes, Chapter 46). Ty3 minus-strand reverse transcription is primed from tRNA^{Met}_i (53) annealed to a bipartite PBS (75) and the plus-strand is primed from a downstream PPT. Overall, Ty3 RT performs an amazingly diverse set of activities: RNA- and DNA-dependent DNA synthesis, strand transfers, degradation of the RNA component of the RNA/DNA heteroduplex, and RNA endonucleolytic cleavages to create the plus-strand primer.

Ty3 cDNA primers

Ty3 minus-strand synthesis is primed by tRNA^{Met}_i. However, this tRNA rather than annealing to a 17-bp PBS as is the case for retrovirus tRNA primers, binds to a bipartite PBS, an 8-bp segment 2 nt downstream of U5 and two adjacent segments thousands of bases downstream within U3, for an additional 23 nt (75). Ty1 also uses a bipartite tRNA^{Met}_i PBS with a short upstream segment and two longer downstream segments. However, in the case of Ty1, the bipartite PBS is completely in the 5' end (107). Ty retroelements differ from retroviruses, which typically have 17–18 bases of uninterrupted complementarity to the tRNA primer just downstream of U5 (108).

Ty3, Ty1 (109) and retrovirus plus-strand primers are PPT, but have distinguishing properties. The Ty3 PPT is GAGAGAGAGGAAGA. It differs from that of retroviral PPT in being slightly shorter and lacking homopolymeric tracts. Based on the sensitivity of RNaseH activities to mutation and the crystal structure, Ty3 RT thumb residues Q290-F292-G294-N297-Y298 (corresponding to HIV-1 RT Q258-L260-G262-N265-W266) interact with the DNA backbone and position the PPT into the RNaseH active site (106, 110). Nuclear magnetic resonance of unbound Ty3 PPT heteroduplex and the crystal structure of the RT-bound PPT hetero-

duplex agree on mostly base-paired and A-form duplex lacking major structural distortions. However, nuclear magnetic resonance revealed sugar puckers at position +1 (G) of the RNA strand (where *in vitro* cleavage is between –1 and +1) from C3' endo to mixed C3'/C2' endo (111). Perturbations using locked nucleic acid analogs to constrain sugar rings and abasic tetrahydrofuran linkages, which lack base pairing, indicated that the 5' and 3' ends of the PPT are more sensitive than internal positions to substitution with novel constituents. This suggests that PPT ends contribute to RNaseH recognition (112, 113). Underscoring these apparent distinctions from retroviral PPT, Ty3 RNaseH fails to recognize at least one example of a retrovirus PPT, that of HIV (AAAAGAAAAGGGGGGA) (104, 114).

RNA–RNA interactions supporting strand transfer

Packaging into core particles and participation in strong-stop cDNA intermediates strand transfers during reverse transcription are two requirements of LTR retrotransposon and retrovirus gRNAs. Dimer formation by gRNA and direct interactions between the 5' and 3' ends of the gRNAs, respectively, have been proposed to participate in these activities. It is noteworthy that tRNA^{Met}_i is shared as the minus-strand primer by distantly related elements Ty3 (53), Ty1 (53) and Ty5 (115). In the case of Ty3, which has a bipartite PBS so that annealed tRNA^{Met}_i bridges the 5' and 3' gRNA ends, the primer is proposed to facilitate strand transfer (75). An alternative proposal is that a transient covalent structure connects 5' and 3' ends, thereby supporting transfer of intermediates. In the case of Ty1 and Ty3, the lariat debranching enzyme, Dbr1, is required for WT levels of cDNA production (116). Based on this requirement, and consistent with *in vitro* observations, it was proposed that a 5' : 3' lariat transiently links the Ty1 gRNA ends (117). Although the evidence for Ty1 is incomplete (118), experiments with HIV-1, which also requires Dbr1, appear supportive of an intermediate lariat in an alternative reverse transcription pathway (119).

Ty3 gRNA dimerization

Ty3 VLPs contain gRNA dimers (52). In the case of retroviruses, the dimerization initiation sequence maps within the *psi* packaging sequence, and this duplex structure is proposed to be a feature that identifies gRNA for packaging (reviewed in references 77 and 120). Gabus *et al.* (75) suggested that palindromic 5' ends of the two bridging tDNA^{Met}_i molecules form an interface between the two Ty3 gRNAs. In addition to

linking 5' and 3' ends, this would mediate dimerization of the gRNA. In an *in vitro* system with mini-Ty3 element substrate, minus-strand strong-stop transfer and gRNA dimer formation were dependent upon the bipartite PBS and tRNA^{Met}_i primer (75, 103). However, the bipartite Ty3 PBS is not required for Ty3 gRNA packaging, suggesting either that dimer formation is not required as it is for retroviruses, or that tRNA^{Met}_i does not provide this interface (76). Ty1, which also uses tRNA^{Met}_i to prime reverse transcription, was similarly proposed to dimerize through the tRNA interface. However, recent analysis of Ty1 VLP RNA using Selective 2' Hydroxyl Acylation Analyzed by Primer Extension (SHAPE) did not identify the proposed tRNA^{Met}_i interface, but rather a palindromic sequence within the 5' end of the gRNA similar to the retroviral dimerization initiation sequence (121).

NUCLEAR ENTRY

Ty3 accesses integration targets via the nuclear pore complex

Ty3 VLPs assemble in the cytoplasm, but must access nuclear targets. In fungi the nuclear envelope does not break down during mitosis so that NPC are the gateway for multimolecular complexes. The yeast NPC is a conserved, eight-fold symmetric outer- and inner-ring and channel structure with a nuclear basket and a central meshwork of filamentous proteins rich in Phe-Gly (FG) repeats (122–124). NPC are dynamic and participate in multiple activities including gene gating, replication, and dsDNA break repair (29, 125). Because Ty3 VLPs are generally above the ~39-nm upper limit for complexes accommodated by the pore channel (126), it is likely that cDNA transit through the NPC is accompanied by significant remodeling also known as uncoating. This requirement is shared with fungal retroelements Ty1 (127, 128) and Tf1 (129). In nondividing animal cells, those retroviruses that are infectious, including HIV-1 and avian sarcoma leukosis virus, also access chromosomal DNA through the NPC (130, 131) (see Bushman and Craigie, Chapter 45). One unifying scenario is that the NPC plays a dynamic role in promoting entry by transforming both retrotransposon and VLPs and retrovirus cores into PIC.

Nuclear pore complex components as host factors

Consistent with the idea that nuclear access of retroelements involves cooperation between retroelements

and host factors, multiple factors that facilitate (co-factors) or antagonize (restriction factors) retrotransposition have been identified. Genomewide screens for Ty3 host factors identified members of the FG filaments (Nup159 and Nup100); and outer ring Nup84 complex (Nup120, Nup133, and Nup84) as Ty3 restriction factors. Gtr1, a negative regulator of Ran; importin Kap120; inner ring adapters Nup59 and Nup157; and FG filament Nup116 (combined with Nup100 constitutes hNup98) were identified as cofactors (64, 132, 133).

Deletions of genes encoding components of the Nup84 complex increased retrotransposition frequency. The NPC outer ring Nup84-Nup120-Nup133 (hNup107-hNup160-hNup107, respectively) subcomplex participates in NPC organogenesis, and yeast mutants lacking one of these components cluster pores on the nuclear envelope (134, 135). In *nup133Δ* and *nup120Δ* mutants, the Ty3 retrovirus co-localizes with the clustered NPC. Physical co-localization between Ty3 VLP clusters and NPC is consistent with cumulative association of fluorescent Ty3 protein clusters on the periphery of DAPI-stained nuclei and with localization observed in some transmission electron microscopy images (133). Although it is attractive to think that mutations in the Nup84 complex enhance access of VLPs to NPC across a greater surface of the nuclear envelope, it is equally possible that distortion caused by loss of NPC outer ring coat proteins reduces the stringency of the NPC size filter.

Nups identified as Ty3 cofactors might mediate the VLP docking required for nuclear entry, or play a role in uncoating. Members of the GLFG subset (Nup110 and Nup116) of FG Nups were identified as Ty3 retrotransposition cofactors (64, 132). A complicating factor in examination of the role of FG Nups is the fact that individual proteins are essential and the different classes of FG repeats are redundant (122, 136). The roles of FG repeat families were dissected using mutants in which combinations of deletions of specific FG repeats have been achieved so that strains are viable, but lack certain types of FG repeats (136). These experiments highlighted a role for GLFG repeat Nups, Nup100 and Nup116 (133). *In vitro* investigation showed not only that assembly-competent immature and mature VLPs interact with GLFG repeat Nups, but that GLFG repeats specifically interact with recombinant Gag3 (133).

An attractive model is that FG hydrophobic filaments interact with the VLP surface and promote dissociation of CA. Although specific contacts for Gag3 subdomains in uncoating have not been identified, mutations in CA

NTD and SP block retrotransposition subsequent to cDNA synthesis (56, 58). This phenotype is consistent with an uncoating defect.

Redundancy of mechanisms of nuclear entry

A complicating factor in understanding nuclear entry of all retroelements is the multiplicity of potential VLP mediators of nuclear import. In the case of HIV-1, MA, Vpr, IN and CA have been implicated in nuclear entry (130, 137–139). In addition to Ty3 Gag (49) implication in nuclear entry, a bipartite basic nuclear localization sequence occurs in IN (amino acids 401–436 of IN) (140). Mutations in this motif block integration at a post-cDNA synthesis stage and also block nuclear localization of IN expressed as an independent protein. Hence, there could be redundant routes of PIC nuclear entry, or Gag3 and IN could cooperate in sequential NPC association and translocation to insure efficient and directional nuclear entry.

INTEGRASE AND INTEGRATION

Functions of integrase

Mutations in Ty3 IN block retrotransposition at multiple steps: before or during cDNA synthesis, 3' end processing, and strand transfer into the genome. The best understood of these steps is its role in targeted integration. The substrate for this activity is the ends of the Ty3 cDNA. As occurs for some retroelements including retroviruses, the Ty3 extra-chromosomal cDNA has two extra base pairs on each end and these are removed from the 3' ends by IN before strand transfer (46). Integrated Ty3 has terminal 8-bp inverted repeats, the terminal dinucleotides of which are conserved among transposable elements (5'-TGTTGTAT/ATACAACA-3'). The cDNA is joined to host DNA by IN with strand transfers staggered by 5 bp, which are filled in on each strand so that the integrated Ty3 ends are flanked by 5-bp direct repeats of host sequence (Figure 2C).

Comparison of retrotransposons and retroviruses suggests that the former benefit from integration that avoids disruption of host functions by avoiding ORFs or targeting heterochromatin or both, whereas the latter benefit from targeting expressed regions. Yeast Ty1–4 and *Dictyostelium* TRE5 and TRE3 are targeted to tDNAs (141–145); Ty5 integrates into heterochromatin (146); Maggy chromodomain element targets H3K9Me (147), and Tf1 targets intergenic regions (see Levin, Chapter 43). Of the characterized retroelements, Ty3 has the most specific pattern of integration.

Structural features of Ty3 integrase

Long terminal repeat retrotransposon and retrovirus integrases have related three-domain structures. Alignment of the Ty3 61-kDa IN with other retroelement IN proteins shows conservation of a catalytic core domain flanked by less-conserved NTD and CTD domains. The first crystal structure of a retrovirus IN, that of prototypic foamy virus IN complexed oligonucleotides representing cDNA ends and target DNA provided insight into this class of proteins (148, 149). Overall retroviral IN proteins function as a tetramer composed of dimers bound to the ends of the cDNA with the inner subunits participating in strand transfer. The NTD of each inner subunit functions in *trans* with the catalytic core domain of the other subunit. Outer subunits of each dimer are thought to have similar folds, but whether they have distinct functions in target association is not yet known (150). The crystal structure showed that prototypic foamy virus IN has an N-terminal extended domain of about 100 residues. This is not highly conserved among retroviral IN proteins but is thought to make contacts with the viral DNA backbone in the case of prototypic foamy virus IN. The IN NTD is typically defined by a conserved HX_{3–7}H_{23–32}CX₂C motif near its N terminus. The position of the Ty3 zinc-binding motif, H93-X-H95-X₅-H101-X₂₉-C131-X₂-C134, indicates that Ty3 IN also has an N-terminal extended domain. The Ty3 catalytic core domain contains approximately a dozen residues highly conserved among Ty3/Gypsy and retrovirus IN proteins. These include Ty3 residues D164-X₆₀-D225-X₃₅-E261 representing the D-Xn-D-X₃₅-E Mg²⁺ binding motif common to polynucleotidyl esterases, including DNA cut-and-paste transposable elements (151, 152). Mutations in this motif do not affect assembly or reverse transcription, but abrogate 3'-end processing and integration (46). Similarity between Ty3 IN and retroviral IN proteins is sufficient to support *in vitro* strand-transfer activity of a small subset of 27 chimeras of Ty3, prototypic foamy virus, and HIV IN proteins (153).

Just C-terminal to the catalytic core domain, is the Ty3 GPF/Y motif, G398-P399-F400, which is found in a subset of retroelement IN proteins (154). In Tf1 this motif is located in a subdomain that participates in multimerization (155). Overall, the CTD of IN is poorly conserved among retroviruses and LTR retrotransposons (154).

Functions of nonconserved integrase domains

Ala-scanning mutagenesis of the Ty3 IN NTD and CTD produced mutations in each domain that underwent

processing, but lacked cDNA (156). This pattern of IN mutant phenotypes is similar to that of class II mutants of HIV-1 IN (157). These form immature cores. Retrovirus immature cores disassemble upon proteolytic processing and reassemble as mature cores inside the envelope (158). However, class II mutants fail to correctly re-assemble and fail to make cDNA (159). The similarities of the mutant phenotypes suggest that Ty3 IN NTD and CTD have a role in post-processing rearrangement of domains required for cDNA synthesis.

The Ty3 IN CTD contains a bipartite basic nuclear localization sequences (R401-RVVKKINDNAYELDLNSHKKKHRVINVQFLKKFVYR-436) sufficient to mediate nuclear import of ectopic IN (140). As indicated above, the Ty3 nuclear localization sequence is required at some post-cDNA synthesis step, consistent with a role in nuclear import (140).

A large fraction of Ty3/Gypsy elements have IN proteins with chromodomains in the CTD and these have been taxonomically classified as *Chromoviruses*. The chromodomain is ~50 aa and mediates interactions with dimethyl and trimethyl H3K9, a signature of heterochromatin (147). This domain occurs in Ty3/Gypsy Maggy and Tf1 IN (147), but has been replaced by an alternative domain in Ty3 (154). A plausible explanation for the replacement is that the targeted dimethyl and trimethyl H3K9 modification is lacking in *S. cerevisiae* (160).

Mechanism of Ty3 targeting

Motivation for studies of Ty3 stemmed in large part from its remarkable integration specificity for the TSS of genomic RNAP III-transcribed genes including tDNAs (8, 141, 161), *SNR6* (U6 RNA), and *RDN5* genes (5S RNA) (162) (Figure 1B). The RNAP III TSS occurs in a favored context rather than an absolute consensus, and is flanked by transcription factors. This suggested early on that transcription factors might similarly position Ty3 integration. *In vivo* *S. cerevisiae* RNAP III transcription requires general factors TFIIC and TFIIIB. TFIIC associates with promoter elements boxA and boxB of about 10 bp each located 20 bp and ~20 to ~100 bp downstream of the TSS, respectively. TFIIC directs sequence independent binding of TFIIIB upstream of the TSS (163, 164). TFIIIB is comprised of subunits TATA binding protein (TBP), Brf1 and Bdp1. It is considered the initiation factor because once bound, it is sufficient *in vitro* to mediate multiple rounds of RNAP III transcription (165, 166). *SNR6* and a subset of tDNAs, have an upstream TATA element that contributes to transcription and TSS selection (167, 168).

In vitro, in a purified system with a TATA-containing template, TBP mediates binding of Brf1, and the two are sufficient for transcription (169). Genomewide chromatin immunoprecipitation–DNA microarray experiments showed that in growing cultures, yeast RNAP III genes are mostly occupied by TFIIB, TFIIC, and RNAP III (170–172).

The target requirements of Ty3 for integration are similar to requirements for transcription. Mutagenesis of targets showed that promoter elements are required for Ty3 integration *in vivo*, and underscored roles for the TATA box and boxA in copositioning the TSS and integration site (162, 173). *In vitro* Ty3 integration can be detected by PCR using primers binding within Ty3 and downstream of the target. Early experiments used VLPs as a source of cDNA and IN protein. Experiments showed that TFIIC and TFIIB are required to target integration to TATA-less tDNAs (174), but that TFIIC is dispensable at genes where the upstream TATA element directly mediates binding of TBP and Brf1 (175, 176). *In vitro* at least, RNAP III is not required and competes with the PIC for access to targets (177). A refined system uses recombinant IN and a fusion of Brf1 and TBP constructed as a structural mimetic (Brf1_{NTD}-TBP-Brf1_{CTD}) (178). In this reaction, in the presence of Mg²⁺, IN targets strand transfer of the 3' end of a DNA duplex representing the terminal 20 bp of Ty3 cDNA into the *SNR6* TSS (47). Surprisingly, in the presence of Mn²⁺, strand transfer independent of Brf1-TBP-Brf1 occurs within the plasmid target proximal to sequences resembling the Ty3 LTR ends, rather than the RNAP III TSS (47).

Genomewide identification of Ty3 targets and RNAP III factor binding sites

Next generation sequencing enables genomewide assessment of retroelement targeting and use of Ty3 as a probe for RNAP III-transcribed genes, as well as an entrée to additional features of RNAP III activation (38). Next generation sequencing analysis of genomic DNA from cells representing ~10,000 independent Ty3-*HIS3* retrotransposition events identified ~300 significant insertion sites. These included 275 tDNAs, and the eight additional types of RNAP III-transcribed genes (*iYGR033C*, *RNA170*, *RPR1*, *SCR1*, *SNR52*, *SNR6*, *ZOD1*, and *RDN5*) and 18 integrations associated with LTRs. Because of the frequent occurrence of boxA and boxB sequences in the *S. cerevisiae* genome, it was possible that integrations would associate with boxA–boxB pairs resembling RNAP III promoters. Five insertion sites were identified that were not known RNAP

III-transcribed genes. However, insertions were flanked by boxA- and boxB-like sequences. Two of the five sites were subcloned and shown to act as plasmid-borne targets. Targeting was sensitive to mutations in conserved positions of the boxB promoter element. There are boxB sites in the *S. cerevisiae* genome which are bound by “Extra TFIIC” (ETC), but little or no TFIIB (170–172). These are not targeted by Ty3, further underscoring the requirement for TFIIB of Ty3 integration.

One of the striking observations enabled by next generation sequencing of Ty3 insertion sites was that tDNA families, within which sequences are similar or identical, generated widely different numbers of sequencing reads (38). Ty3 could use identical genes at widely differing frequencies because of different chromatin contexts. RNAP III genes undergo specific types of epigenetic modifications and bind chromatin remodelers and condensins (179–190). In addition to possible differences in chromatin context, tDNAs also act as replication fork barriers (191), and subpopulations localize to the nucleolus (31, 192) and nuclear pores (30). Overall, it seems that we are just beginning to learn about the dynamic activities of RNAP III-transcribed genes. Ty3 offers a potentially exquisitely sensitive, indelible, *in vivo* probe for transient interactions between these genes and their chromatin caretakers.

Integration host factors

Although host RNAP III machinery plays a clear role in targeting, most genes encoding subunits of key complexes are essential and were not identified in host factor screens (64, 132). Exceptions were the La protein, a small-RNA chaperone (41) and Tfc1, a subunit of TFIIC (193). Despite the association of La with RNAP III-transcribed precursor RNAs (194), the *lhp1Δ* mutant had reduced Ty3 cDNA, so that it seems likely that the role of La precedes the integration step. A mutagenized strain expressing a C-terminal truncation of Tfc1 was identified in a screen for genes which affected recovery of integrations that activated expression of the suppressor tDNA in the divergent tDNA target described above. Although Ty3 cDNA integrates into this target in both orientations, Ty3 integration occurred in only one orientation in this mutant. This result indicates that the Ty3 PIC itself is asymmetric. A combination of genetic experiments, mutagenesis and *in vitro* pull-down assays indicated that the N-terminal extended domain of IN interacts directly with Tfc1 (193).

The example of fungal retrotransposons and availability of RNAi and high throughput sequencing spurred productive investigation into host factors involved in

targeting retrovirus integration. Although there are no known examples of the highly specific targeting or targeting of RNAP III genes, as observed for a subset of retrotransposons, retroviral integration is also nonrandom. Retrotransposons generally avoid coding regions and a subset target inactive chromatin via interactions between IN chromodomains and H3K9Me. In contrast, retroviruses integrate into chromatin associated with epigenetic marks of transcriptionally active chromatin (195). HIV-1 associates with chromatin by interaction between the IN catalytic core domain and LEDGF/P75. This factor interacts with an epigenetic mark of active chromatin, H3K36Me3, via the LEDGF PWWP domain (reviewed in reference 196) (see Bushman and Craigie, Chapter 45). The murine leukemia virus IN associates with targets via interaction between the IN CTD and Bromo-domain and ExtraTerminal domains proteins, which bind acetylated H3 and H4 tails (197) (see Bushman and Craigie, Chapter 45).

TY3–HOST INTERACTIONS

Genetic screens and cytological analyses have identified large numbers of candidate Ty host factor genes. Importantly, many of these cluster by function (64, 81, 132, 198–200). Overall there was about 15% overlap between the genes identified in Ty3 and Ty1 screens despite the relatively different assays used. Genes identified in Ty3 genomewide screens were grouped according to the gene ontology process (Figure 4). Cofactors assisting retrotransposition included those involved in catabolism, PB components, nucleoporins, checkpoint control, and vesicular trafficking proteins including ESCRT factors. The most striking class of restriction factors antagonizing retrotransposition were those related to DNA replication, particularly helicases, and certain RNA transporters, including Npl3 and Sac3. A small, but significant, percentage of cofactors and restriction factors that overlapped with those identified in Ty1 screens included replication factors (64, 199, 201), PB proteins (81, 198), and several RTT genes of various functions including chromatin remodelers and histone modification factors (64, 199).

Genes involved in replication and responses to DNA damage were the major class of restriction factors. Deletions of genes encoding replication factors Csm3, Rrm3, Mrc1, Rad6, Bre1, Sgs1 and Hpr5 enhanced Ty3 retrotransposition. Work in the Curcio laboratory on Ty1 showed that replication stress or DNA damage caused by the absence of a subset of DNA maintenance factors triggers increased Ty1 cDNA synthesis. This was traced

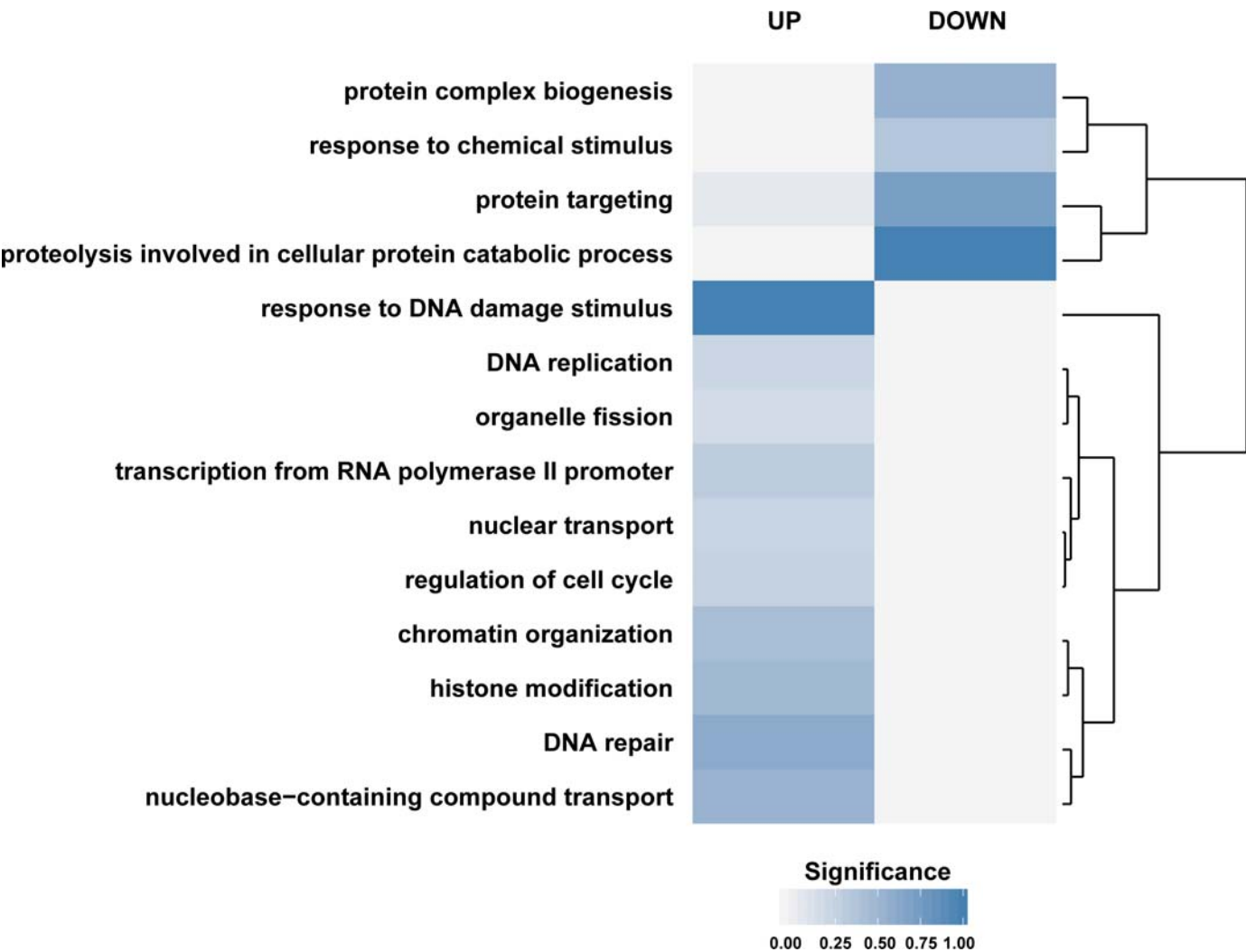


FIGURE 4 Hierarchical clustering of Ty3 cofactors and restriction factors by gene ontology (GO) groups. Gene ontology (GO) analysis was performed using the GO SLIM Biological Process mapping tool available through *Saccharomyces* Genome Database (<http://www.yeastgenome.org>). Knockout mutants identified as having either increased "Up" or decreased "Down" Ty3 retrotransposition phenotypes were analyzed for GO: Biological Process terms. Enriched categories were determined using chi-squared test. GO categories were considered enriched if two criteria were met: (i) the *P*-value was <0.05 and (ii) the number of genes in the enriched category exceeded 10% of the total number of genes in the Up or Down list. Enriched categories were converted to a heat map with hierarchical clustering using R; values represent the $[-\text{Log}(P\text{-value})]$ scaled from 0 (no significance) to 1, blue coloring reflects the intensity of significance. [doi:10.1128/microbiolspec.MDNA3-0057-2014.f4](https://doi.org/10.1128/microbiolspec.MDNA3-0057-2014.f4)

to activation of checkpoint proteins Rad24 and Rad9, as the effect was significantly reduced in backgrounds lacking one of the checkpoint genes (201). Although epistasis analysis was not conducted for Ty3, retrotransposition was decreased in the *rad24Δ* background (64) consistent with checkpoint control promoting Ty3 retrotransposition. However, tDNA targeting might also

be affected directly by mutations in replication factors. Transfer RNA genes block replication forks coming from downstream (191). If incoming replication forks disrupt targeting, fork stalling could increase integration. Alternatively, fork breakage caused by loss of key helicases could trigger localization to NPC (125), enhancing accessibility of tDNA targets to incoming integrases.

As discussed above, Ty3 retrotransposition increased in mutants with deletions of genes encoding a subset of Nup84 complex components, *nup84Δ*, *nup120Δ*, and *nup133Δ* (64). We speculate that clustering of mutant pores and retrosomes could enhance nuclear access. Alternatively, these mutations could simply make the pore more permissive for PIC translocation (133).

Among the Ty3 cofactor genes were vesicular trafficking proteins. In budding yeast, ESCRT proteins were identified as essential for the late stages of trafficking of ubiquitinated surface proteins from the plasma membrane into multivesicular bodies (202). Subsequently, these factors were shown to be critical for retrovirus budding, which is the topological equivalent of multivesicular body formation (62). ESCRT proteins identified as Ty3 cofactors include ESCRTI (Vps28), ESCRTII, (Vps25, Vps22, Vps36), and ESCRTIII (Vps20, Snf7), as well as ESCRT-related proteins Vps4 and Bro1 (64) and Vps27. Although there is no evidence of Ty3 budding, it could associate with membranes in the course of morphogenesis. An interesting alternative possibility is raised by the recent implication of ESCRT components in NPC maintenance (203).

Retrovirus genomewide host screens, with HIV-1 being a major focus, have identified large numbers of host factors, among them, many nuclear proteins (see Bushman and Craigie, Chapter 45). Metadata analysis of nine such screens concluded that ~5% of all human genes encode factors related to HIV-1 and showed low, but statistically significant, overlap (204). As in the case of the Ty elements, overlap is limited by differences among screening strategies. Ty3 host factors (64, 132) were compared with factors identified in a subset of retrovirus siRNA host factor screens (205–208) using the Overlapper tool (<http://hivsystemsbiology.org/GeneListOverlapper/app>). These results are shown in Table 1. Functional overlaps include overlaps involved in vesicular trafficking, nuclear access (porins and translocation factors), DNA repair, ubiquitin signaling, mediator complex/transcription, RNA binding, and DNA and RNA unwinding (reviewed in references 62, 209–211).

Nucleoporins and NPC translocation factors were identified as host cofactors for Ty3 and for HIV-1, which infects nondividing cells. Prominent among these are the hydrophobic Nups enriched in FG repeats (122, 123). Human HsNup98, an FG Nup (212, 213), interacts with HIV-1 Gag and is required for HIV-1 nuclear entry. Budding yeast homologs of HsNup98, ScNup116, and ScNup100 are required for WT levels of Ty3 nuclear entry and recombinant ScNup116 interacts with Ty3 VLPs and recombinant Gag3 *in vitro*. A role in for this

class of Nups in moving the uncoating VLP through the pore was proposed (133). *Schizosaccharomyces pombe* basket NUP, SpNup124, was isolated as a factor required for nuclear entry of Ty3/Gypsy element Tf1 Gag cores (129). The *S. cerevisiae* homolog, ScNup1, is essential, and so was underrepresented in Ty screens. However, HsNup153, the human homolog, is required for HIV-1 nuclear entry and mutations shift the integration bias, suggesting that basket Nups could affect NPC-proximal integration site selection (214). These observations suggest that interactions between Gag and FG Nups are a common feature of retroelement nuclear pore translocation and possibly uncoating.

Ribonucleoprotein granule components are common themes in retrotransposon and retrovirus assembly, but in seemingly distinct roles. During assembly, RNA and protein from LTR retrotransposon Tys and non-LTR retrotransposon LI LINEs, interact productively with PB and SG components (133, 215, 216). Mouse mammary tumor virus assembles intracellularly in association with YB-1-enriched ribonucleoprotein granules (217). YB-1 is a translational regulatory protein which accumulates in SG during stress, but is also found in PB. Expression of mouse mammary tumor virus Gag causes granules to increase in size, and these are distinct from SG and PB. HIV-1, which assembles into cores during extrusion into budding plasma membrane, actively antagonizes formation of SG, but associates with Staufen in cytoplasmic ribonucleoprotein particles (218). Host HIV-1 restriction factors, including apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) cytidine deaminase family members, overexpressed Mov10 helicase (219–221), and RNAi components, associate with PB (210, 222). The PB factors also restrict endogenous retroviruses (223). However, studies disagree about the positive contributions of PB factors to retrovirus infection. DEAD box helicase DDX6 (ScDhh1) is ascribed a positive role in the intracellular packaging of prototypic foamy virus RNA, but studies differ as to roles in HIV-1 core assembly (224, 225). Overall these studies suggest that individual components of ribonucleoprotein granules, including PB and SG, interact with assembling retrovirus RNA and proteins, but assembly is not associated with these granules *per se*. Although the examples are limited, they are consistent with ribonucleoprotein granules sequestering proteins to protect host RNAs from inappropriate degradation or translational suppression, while providing a source of soluble proteins to interact with specific components of viruses. These associate with specialized granule components intracellularly, or complete assembly during budding on the plasma membrane.

TABLE 1 Host factors in common between Ty3 and HIV-1

ORF	Gene name	Full name	Ty3 ΔTPN ¹	Human homolog	Retrovirus host factor ²
YBR284W	YBR284W		up, down	AMPD1, AMPD2, AMPD3	
YAR003W	SWD1	Set1c, WD40 repeat protein	up, down	RBBP5, WDR72	
YPR119W	CLB2	CycLin B	up	CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCNE1, CCNE2, CNTD2	
YPL256C	CLN2	CycLin	up	CCNB1, CCNB2, CNTD2	
YPL024W	RMI1	RecQ Mediated genome Instability	up		
YOR144C	ELG1	Enhanced Level of Genomic instability	up		
YOR039W	CKB2	Casein Kinase Beta' subunit	up	CSNK2B	
YNR052C	POP2	PGK promoter directed OverProduction	up	CNOT7, CNOT8	
YNL242W	ATG2	AuTophagy related	up	ATG2A, ATG2B	
YNL138W	SRV2	Suppressor of RasVal19	up	CAP1, CAP2	
YMR190C	SGS1	Slow Growth Suppressor	up	BLM, RECQL, RECQL5, WRN	
YMR048W	CSM3	Chromosome Segregation in Meiosis	up		
YML051W	GAL80	GALactose metabolism	up	GFOD1, GFOD2	
YLR399C	BDF1	BromoDomain Factor	up	BRD2, BRD3, BRD4, BRDT, CREBBP, EP300	(207)
YLR268W	SEC22	SECRetory	up	SEC22A, SEC22C	
YLR235C	YLR235C		up		
YLR015W	BRE2	BREfeldin A sensitivity	up	ASH2L	
YLL044W	YLL044W		up		
YLL030C	RRT7	Regulator of rDNA Transcription	up		
YLL002W	RTT109	Regulator of Ty1 Transposition	up		
YKR082W	NUP133	NUclear Pore	up	NUP133	(206)
YKL135C	APL2	clathrin Adaptor Protein complex Large chain	up	AP1B1, AP2B1, AP4B1	
YKL068W	NUP100	NUclear Pore	up	NUP98, NUPL1	(207)
YKL057C	NUP120	NUclear Pore	up		
YKL041W	VPS24	Vacuolar Protein Sorting	up	CHMP3	
YJL092W	SRS2	Suppressor of Rad Six	up		
YJL047C	RTT101	Regulator of Ty1 Transposition	up	CUL1	
YIR021W	MRS1	Mitochondrial RNA Splicing	up		
YIL090W	ICE2	Inheritance of Cortical ER	up		
YIL069C	RPS24B	Ribosomal Protein of the Small subunit	up	RPS24	
YIL015W	BAR1	BARrier to the alpha factor response	up	CTSD, CTSE, NAPSA, PGA3, PGA4, PGA5, PGC, REN	
YIL011W	TIR3	Tip1-Related	up		
YIL009C-A	EST3	Ever Shorter Telomeres	up		
YHR177W	YHR177W		up		
YHR031C	RRM3	rDNA Recombination Mutation	up	PIF1	
YHL030W	ECM29	ExtraCellular Mutant	up	KIAA0368	
YHL003C	LAG1	Longevity Assurance Gene	up	CERS1, CERS2, CERS3, CERS4, CERS5, CERS6, TRAM1, TRAM1L1, TRAM2	
YGR270W	YTA7	Yeast Tat-binding Analog	up	ATAD2, ATAD2B	
YGR056W	RSC1	Remodel the Structure of Chromatin	up	PBRM1	
YGL174W	BUD13	BUD site selection	up	BUD13	
YGL066W	SGF73	SaGa associated Factor, 73 kDa	up	ATXN7, ATXN7L1, ATXN7L2, ATXN7L3, ATXN7L3B	
YGL058W	RAD6	RADiation sensitive	up	UBE2A, UBE2B, UBE2S, UBE2W	(207)
YER155C	BEM2	Bud EMergence	up	ARHGAP21, ARHGAP23, ARHGAP35, CHN1, GMIP, HMHA1, RACGAP1, SYDE1, SYDE2	
YER060W-A	FCY22	FluoroCYtosine resistance	up		
YER057C	HMF1	Homologous Mmf1p Factor	up	HRSP12	
YEL013W	VAC8	VACuole related	up	ANKAR, ARMC3, ARMC4, CTNNB1, JUP	
YEL004W	YEA4		up	SLC35B4	
YDR448W	ADA2	transcriptional ADAPtor	up	TADA2A, TADA2B	

(continued)

TABLE 1 Host factors in common between Ty3 and HIV-1 (*continued*)

ORF	Gene name	Full name	Ty3 Δ TPN ¹	Human homolog	Retrovirus host factor ²
YDR432W	NPL3	Nuclear Protein Localization	up	CIRBP, DMKN, HNRNPA1, HNRNPA1L2, HNRNPA2B1, HNRNPA3, LOR, RBMX, RBMXL1, RBMXL2, RBMY1A1, RBMY1B, RBMY1D, RBMY1E, RBMY1F, RBMY1J, SRSF1, SRSF9	
YDR290W	YDR290W		up		
YDR289C	RTT103	Regulator of Ty1 Transposition	up	RPRD1A, RPRD1B, RPRD2	
YDR176W	NGG1		up	TADA3	
YDR159W	SAC3	Suppressor of ACTin	up	MCM3AP, SAC3D1	
YDR074W	TPS2	Trehalose-6-phosphate PhoSpha-tase	up		
YDL116W	NUP84	NUclear Pore	up	NUP107	(206)
YDL074C	BRE1	BREfeldin A sensitivity	up	RNF20, RNF40	
YDL056W	MBP1	Mlul-box Binding Protein	up		
YCR020W-B	HTL1	High-Temperature Lethal	up		
YCL061C	MRC1	Mediator of the Replication Check-point	up		
YCL037C	SRO9	Suppressor of rho3	up		
YBR277C	YBR277C		up		
YBR269C	FMP21	Found in Mitochondrial Proteome	up	C6orf57	
YBL094C	YBL094C		up		
YBL006C	LDB7	Low Dye Binding	up		
YPL084W	BRO1	BCK1-like Resistance to Osmotic shock	down	PDCD6IP, PTPN23, RHPN1, RHPN2	
YNL236W	SIN4	Switch INdependent	down		
YMR154C	RIM13	Regulator of IME2	down		
YPR173C	VPS4	Vacuolar Protein Sorting	down	KATNA1, KATNAL1, KATNAL2, VPS4A, VPS4B	(209)
YPL225W	YPL225W		down	PBDC1	
YPL150W	YPL150W		down	MELK	
YPL125W	KAP120	KAryoPherin	down	IPO11	
YPL065W	VPS28	Vacuolar Protein Sorting	down	VPS28	
YPL042C	SSN3	Suppressor of SNf1	down	CDK19, CDK8	
YPL002C	SNF8	Sucrose NonFermenting	down	SNF8	
YOR275C	RIM20	Regulator of IME2	down	PDCD6IP, PTPN23	
YOR270C	VPH1	Vacuolar pH	down	ATP6V0A1, ATP6V0A2, ATP6V0A4, TCIRG1	(206)
YOR055W	YOR055W		down		
YOR054C	VHS3	Viable in a Hal3 Sit4 background	down	PPCDC	
YOL083W	ATG34	AuTophagy related	down		
YNR059W	MNT4	MaNnosylTransferase	down		
YNR006W	VPS27	Vacuolar Protein Sorting	down	GGA1, GGA2, GGA3, HGS, TOM1, TOM1L1, TOM1L2, WDFY1, WDFY2, ZFYVE21	(209) (206)
YNL224C	SQS1	SQuelch of Splicing suppression	down	SON	
YNL136W	EAF7	Esa1-Associated Factor	down	MRGBP	
YNL059C	ARP5	Actin-Related Protein	down	ACTR5	
YMR158W-B	YMR158W-B		down		
YMR123W	PKR1	Pichia farinosa Killer toxin Resistance	down		
YMR116C	ASC1	Absence of growth Suppressor of Cyp1	down	GNB2L1, WDR31	
YMR077C	VPS20	Vacuolar Protein Sorting	down	CHMP6, CHMP7	
YMR058W	FET3	FErrous Transport	down		
YML121W	GTR1	GTp binding protein Resemblance	down	RRAGA, RRAGB	(206)
YML097C	VPS9	Vacuolar Protein Sorting	down	GAPVD1, RABGEF1, RIN1, RIN2, RIN3, RINL, VPS9D1	(206)
YML081C-A	ATP18	ATP synthase	down		
YML073C	RPL6A	Ribosomal Protein of the Large subunit	down	RPL6	
YLR423C	ATG17	AuTophagy related	down		
YLR417W	VPS36	Vacuolar Protein Sorting	down	VPS36	

(continued)

TABLE 1 Host factors in common between Ty3 and HIV-1 (continued)

ORF	Gene name	Full name	Ty3 Δ TPN ¹	Human homolog	Retrovirus host factor ²
YLR242C	ARV1	ARE2 Required for Viability	down	ARV1	
YLR025W	SNF7	Sucrose NonFermenting	down	CHMP4A, CHMP4B, CHMP4C	
YKR099W	BAS1	BASal	down	MYB, MYBL1, MYBL2, SNAPC4	
YKR020W	VPS51	Vacuolar Protein Sorting	down		
YKL149C	DBR1	DeBRanching	down	DBR1	
YJR137C	MET5	METHionine requiring	down		
YJR121W	ATP2	ATP synthase	down	ATP5A1, ATP5B	(209)
YJR120W	YJR120W		down		
YJR102C	VPS25	Vacuolar Protein Sorting	down	VPS25	
YJR032W	CPR7	Cyclosporin-sensitive Proline Rotamase	down	NKTR, PPID, PPIG	
YJL006C	CTK2	Carboxy-Terminal domain Kinase	down	CCNT1, CCNT2	(209) (206)
YIL134W	FLX1	FLavin eXchange	down	SLC25A32	
YIL115C	NUP159	NUclear Pore	down		
YIL097W	FYV10	Function required for Yeast Viability	down	MAEA	
YIL073C	SPO22	SPOrulation	down		
YHR082C	KSP1		down	CHEK2	
YHR026W	VMA16	Vacuolar Membrane Atpase	down	ATP6V0B	(208)
YHR008C	SOD2	SuperOxide Dismutase	down	SOD2	
YGR246C	BRF1	B-Related Factor	down	BRF1	
YGR188C	BUB1	Budding Uninhibited by Benzimidazole	down	BUB1, BUB1B	
YGR167W	CLC1	Clathrin Light Chain	down	CLTA, CLTB	(206)
YGL212W	VAM7	VACuolar Morphogenesis	down	STX8	
YGL173C	XRN1	eXoRiboNuclease	down	XRN1	
YGL156W	AMS1	Alpha-MannoSidase	down	MAN2C1	
YGL078C	DBP3	Dead Box Protein	down	DDX21, DDX50	(208)
YGL057C	GEP7	GENetic interactors of Prohibitins	down		
YFR019W	FAB1	Forms Aploid and Binucleate cells	down	PIKFYVE, ZFYVE21, ZFYVE28	
YFR012W	DCV1	Demands Cdc28 kinase activity for Viability	down		
YFR009W	GCN20	General Control Nonderepressible	down	ABCF1, ABCF3	
YFL007W	BLM10	BLeoMycin resistance	down	PSME4	
YFL001W	DEG1	DEpressed Growth rate	down	PUS3	
YER173W	RAD24	RADiation sensitive	down	RAD17	
YER148W	SPT15	SuPpressor of Ty	down	TBP, TBPL1, TBPL2	(209)
YER145C	FTR1	Fe TRansporter	down		
YER105C	NUP157	NUclear Pore	down	NUP155	(206)
YEL044W	IES6	Ino Eighty Subunit	down	INO80C	
YDR493W	MZM1	Mitochondrial Zinc Maintenance	down		
YDR323C	PEP7	carboxyPEPTidase Y-deficient	down	ZFYVE20	
YDR139C	RUB1	Related to UBiquitin	down	NEDD8	
YDR067C	OCA6	Oxidant-induced Cell-cycle Arrest	down		
YDL160C	DHH1	DEAD box Helicase Homolog	down	DDX6	
YDL159W	STE7	STERile	down	MAP2K1, MAP2K2, MAP2K3, SBK2, SLK, STK10, TAOK1, TAOK2, TAOK3	(206)
YDL118W	YDL118W		down		
YDL088C	ASM4		down	NUP35	
YBR293W	VBA2	Vacuolar Basic Amino acid transporter	down	SLC18A1, SLC18A2, SLC18A3, SLC18B1	
YBR263W	SHM1	Serine HydroxyMethyltransferase	down	SHMT1, SHMT2	
YBR262C	AIM5	Altered Inheritance rate of Mitochondria	down		
YBR103W	SIF2	Sir4p-Interacting Factor	down	TBL1X, TBL1XR1, TBL1Y, THOC3, WDR17	
YBR044C	TCM62	TriCarboxylic acid cycle Mutant	down	HSPD1	
YBR021W	FUR4	5-FIUoRouridine sensitivity	down		
YBL024W	NCL1	NuCLear protein	down	NSUN2	
YBL007C	SLA1	Synthetic Lethal with ABP1	down	EPS15, EPS15L1, GRAP, GRAP2, GRB2, ITSN1, ITSN2, REPS1, REPS2, SLA, SLA2	
YAL027W	SAW1	Single-strand Annealing Weakened	down		
YAL019W	FUN30	Function Unknown Now	down	SMARCAD1	

(continued)

TABLE 1 Host factors in common between Ty3 and HIV-1 (*continued*)

ORF	Gene name	Full name	Ty3 ΔTPN ¹	Human homolog	Retrovirus host factor ²
YAL013W	DEP1	Disability in regulation of Expression of genes involved in Phospholipid biosynthesis	down	BFSP2, DES, GFAP, IFLTD1, INA, KRT1, KRT10, KRT12, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT2, KRT20, KRT23, KRT24, KRT25, KRT26, KRT27, KRT28, KRT3, KRT31, KRT32, KRT33A, KRT33B, KRT34, KRT35, KRT36, KRT37, KRT38, KRT39, KRT4, KRT40, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT71, KRT72, KRT73, KRT74, KRT75, KRT76, KRT77, KRT78, KRT79, KRT8, KRT80, KRT81, KRT82, KRT83, KRT84, KRT85, KRT9, LMNA, LMNB1, LMNB2, NEFM, NES, SYNC, SYNM, VIM	(208)

¹Genes identified in genomewide screens for Ty3 host factors (64, 133).

²Retrovirus host genes identified in Ty3 screens and in retrovirus host screens (206–209).

The presence of a mixture of components in RNA granules that variously restrict and promote retroelement assembly might extend to the retroelement expression in germ cell-related lineages. In these cells, cycles of genome demethylation are accompanied by activation of retrotransposon transcription and post-transcriptional control in perinuclear granules containing PB components. Mouse, fly, nematode, and zebra fish germ cell-related lineages accumulate in these granules. When RNAi is suppressed, these cells undergo retrotransposition (95, 226–228). Based on the role of components of these granules in budding yeast retrotransposition, where RNAi is naturally absent, we speculate that in retrotransposing germ cell systems, at least a subset of these components interact with assembling retrotransposon proteins and RNAs.

PERSPECTIVES

The dawn of genomewide analysis enabled by next generation sequencing shows host genes embedded in a sea of retroelements. Together with other retrotransposons, LTR retrotransposons account for ongoing *expansion*, *adaptation*, and *remodeling* of eukaryotic genomes. Many consequences, noncoding RNAs and RNAi among them, were unexpected and raise new questions.

In the rush to apply these findings, retrotransposition itself remains largely unexplored. In animal cells, despite RNAi, LTR element retrotransposition is ongoing in germ cells, early-stage embryos, the brain, and some cancers. We know little of the mechanics of retrotransposition in these cells, and less about real-time effects of retrotransposition on those and descendant cells.

Ty3/Gypsy elements represent a major class of LTR retrotransposons, and dominate many plant and some animal genomes. Ty3, the sole representative in budding yeast, is one of the more completely understood of these elements at the molecular level. However, it is virtually a total anomaly. Classified as a Chromovirus, Ty3 lacks the defining IN chromodomain, and stands out for the specificity of its targeting. Most retrotransposons are expressed and retrotranspose in single cells, but Ty3 in the natural context of mating, retrotransposes only after diploids are formed. LTR retrotransposons are suppressed by RNAi, but due to the evolutionary loss of Dicer and Argonaute functions, this system is absent in budding yeast. Perhaps most surprisingly, Ty3 VLP assembly requires components of the RNA processing system that supposedly suppresses and degrades LTR retrotransposon RNAs in metazoans. What can we expect to learn from such an exceptional element?

Work in the Ty3 system has provided considerable information about the biochemistry of retrotransposition, much of which is proving generalizable. Three areas especially illustrate this point. First, Ty3 targeting provided the first clear evidence for interaction with host chromatin/transcription factors, and still offers the most precise tool for understanding the interaction between IN and target protein complexes and DNA. Targets in this system can be precisely predicted and manipulated to understand requirements of strand transfer. Second, Ty3/Tf1 Gypsy element retrotransposons show clear interactions with nuclear pores, and combined with targeting, can be used to explore the links between entry and integration. Known and essentially identical targets of Ty3 used at widely different frequencies, offer potential insights into unknown areas of nuclear dynamics.

Third, because Ty3 is not regulated by post transcriptional RNA interference, it offers a rare opportunity to study retrotransposition itself. It cannot be a coincidence that Ty3 retrotransposes in the yeast analog of germ cells during cell fusion, and animal retrotransposons are activated in germ cells. We know quite a bit about how retrotransposition is suppressed. Now we should think about what happens when it occurs.

At the center of all this, we have little understanding of how retrotransposition affects cells in real time. In part this is because retrotransposition occurs in a few cells per population. The recent advent of single cell biology will surely produce a wave of insights into those rare cells that are undergoing retrotransposition. Are there clusters of retrotransposition in those cells? Does retrotransposition target broken replication forks as suggested by mutant screens? One DNA break induces checkpoint arrest; do retrotransposon cDNAs have the same effect? What determines the nuclear winner between competing processes of integration and destruction of invading cDNA? Does retrotransposition encrypt an individual cell record, as theorized for neuronal tissues? Is retrotransposition a trigger in the cancers with which it is associated? We know many mechanisms through which retrotransposons are tolerated by hosts. Is there also a kind of retrotransposon race that determines how retrotransposon composition differs so dramatically among eukaryotic species? Answers to these and other questions will lead us to a better understanding of our genomes.

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