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The Ty1 LTR-retrotransposon of budding yeast, *Saccharomyces cerevisiae*

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Summary

Long-terminal repeat (LTR)-retrotransposons generate a copy of their DNA (cDNA) by reverse transcription of their RNA genome in cytoplasmic nucleocapsids. They are widespread in the eukaryotic kingdom and are the evolutionary progenitors of retroviruses [1]. The Ty1 element of the budding yeast *Saccharomyces cerevisiae* was the first LTR-retrotransposon demonstrated to mobilize through an RNA intermediate, and not surprisingly, is the best studied. The depth of our knowledge of Ty1 biology stems not only from the predominance of active Ty1 elements in the *S. cerevisiae* genome but also the ease and breadth of genomic, biochemical and cell biology approaches available to study cellular processes in yeast. This review describes the basic structure of Ty1 and its gene products, the replication cycle, the rapidly expanding compendium of host co-factors known to influence retrotransposition and the nature of Ty1's elaborate symbiosis with its host. Our goal is to illuminate the value of Ty1 as a paradigm to explore the biology of LTR-retrotransposons in multicellular organisms, where the low frequency of retrotransposition events presents a formidable barrier to investigations of retrotransposon biology.

I. Why is Ty1 a great model system?

A. Ty1 structure and replication

Organization of the Ty1 genome—The structure of Ty1 is analogous to that of retroviral proviruses (Figure 1). The most highly characterized Ty1 element is Ty1-H3, which was isolated following its retrotransposition into plasmid DNA [2]. Nucleotide coordinates provided in this review specifically refer to Ty1-H3, unless otherwise noted. Ty1 is 5918 base pairs (bp) in length with 334 bp direct repeats, or LTRs, at each end. Ty1 LTRs, like that of most LTR-retrotransposons and retroviruses, have the dinucleotide inverted repeat, 5′-TG…CA-3′ at their termini, and are composed of three distinct domains-U3, R and U5. These domains are defined by their position in the major sense-strand

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transcript expressed from Ty1 DNA. The 38-nucleotide U5 region and 240-nucleotide U3 region are unique to the 5′ and 3′ end of the Ty1 RNA, respectively, while the R region of 56 nucleotides is repeated at both ends of the processed transcript. Functional Ty1 elements encode two partially overlapping open reading frames: *GAG* (historically known as *TYA1*) and *POL* (*TYB1*). The last 3 nucleotides of the R region of the 5′ LTR encode the first codon of *GAG*. The *GAG* ORF encodes a single functional protein with capsid and nucleic acid chaperone functions. The *POL* ORF is in the +1 frame relative to *GAG* and overlaps the last 38 base pairs of *GAG*. *POL* encodes three proteins with catalytic activity: protease (PR), integrase (IN), and reverse transcriptase/RNase H (RT/RH). Ty1 does not contain an equivalent of the retroviral *ENV* gene or any remnant of one.

The Ty1 replication cycle—The process of retrotransposition is replicative, resulting in the parental retrotransposon and a copy of the element in the genome. The major steps in Ty1 replication are analogous to those in retroviral replication, except that Ty1 replication is entirely intracellular (Figure 2). Ty1 elements are transcribed by RNA Polymerase II (Pol II), resulting in capped and polyadenylated transcripts that are exported to the cytoplasm. Translation of Ty1 RNA produces two primary gene products, p49-Gag and p199-Gag-Pol, the latter a product of a programmed translational frameshift from the *GAG* ORF to the *POL* ORF [3]. Gag, Gag-Pol and Ty1 RNA assemble into nucleocapsids known as virus-like particles (VLPs). Within the VLP, PR is autocatalytically cleaved from p199-Gag-Pol and catalyzes all additional cleavages of the Gag and Gag-Pol precursors to yield p45-Gag, p20-PR, p71-IN and p63-RT/RH. Following maturation of Ty1 proteins, Ty1 RNA is reverse transcribed into a linear, double-stranded DNA. The resulting cDNA, presumably in association with p71-IN, is imported into the nucleus. IN interacts with host proteins to target Ty1 cDNA integration to specific regions of the host genome. The cDNA is integrated into chromosomal DNA by a non-homologous strand transfer process (reviewed in [4]). Potentially, Ty1 cDNA is also an excellent substrate for gene conversion of Ty1 elements and non-degenerate solo LTRs in the genome; nonetheless, Ty1 cDNA rarely enters the genome by gene conversion of endogenous Ty1 sequences [5,6], unless integration is blocked by mutations in IN or cDNA terminal motifs that are bound by IN [7], or cells are grown at temperatures above 30°C [8]. Together, the processes of IN-mediated retrotransposition of cDNA and insertion of cDNA by homologous recombination are known as retromobility.

B. The toolbox for studying retromobility

Detection of RNA-mediated mobility events—Repression of retromobility by the host cell is a nearly universal feature of retrotransposon biology. In laboratory strains, Ty1 retromobility occurs at a rate of 10^{-5} to 10^{-7} per element per generation [9], which is too infrequently to differentiate retromobility from DNA-based recombination events. Therefore, assays to detect retromobility have focused on increasing the frequency of retrotransposition or improving the detection of RNA-mediated events versus DNA-mediated events. Beginning with the innovation that led to the discovery of LTR-retrotransposon mobility through an RNA intermediate nearly 30 years ago, an array of tools have been developed in *S. cerevisiae* to facilitate characterization of retromobility processes. In 1985, Boeke, Garfinkel, Styles and Fink isolated the functional element, Ty1-H3 and

expressed it from the galactose-inducible *GAL1* promoter on a high-copy plasmid (pGTy1). Expression of pGTy1 resulted in such a high level of retrotransposition that new genomic copies of Ty1 could be identified in cells chosen at random [2]. To demonstrate that Ty1 was mobilized via an RNA intermediate, an intron-bearing gene fragment was inserted downstream of the *POL* ORF in pGTy1. Precise intron excision in transposed elements provided unambiguous evidence that a spliced Ty1 RNA was the template for synthesis of retrotransposed DNA. Next generation pGTy1 elements bearing selectable markers were used to detect newly integrated chromosomal Ty1 elements following segregation of the plasmid copy [10]. Expression of Ty1 RNA from *GAL1* or other heterologous promoters also stimulates VLP formation, which facilitates their purification and functional and structural characterization [11,12].

Precise splicing of an intron inserted into Ty1 provided the basis for development of retrotranscript indicator genes (RIGs). RIGs are synthetic genes constructed by inserting an intron into the ORF of a selectable marker gene in an antisense and thus unspliceable orientation (Figure 3). When a RIG is inserted into a Ty1 element in the 3′ untranslated region such that Ty1 and the intron-bearing marker gene are in opposing transcriptional orientations, the intron is in a spliceable orientation in the Ty1 transcript. After the intron is spliced from the Ty1 transcript, reverse transcription of the spliced Ty1 transcript and integration of the resulting cDNA create a functional chromosomal copy of the marker gene lacking the intron. The recreated marker gene allows phenotypic detection of cells that sustain a transposition event. The RIG is activated only in newly mobilized Ty1 copies and thus can be used to detect retromobility of Ty1 in the presence of the marked parental element. Retromobility at a frequency as low as 1×10^{-9} has been detected in a simple petri plate-based assay (Figure 3)[13]. First developed to detect retrotransposition of endogenous Ty1 elements, RIGs have been adapted to study a breadth of RNA-mediated mobility events such as group II intron retrohoming in bacteria and non-LTR-retrotransposon mobility in human cells and in mice [14-17].

Separation of Ty1 RNA and protein function—LTR-retrotransposons encode an RNA that has binary functions as an mRNA and as the genomic RNA (gRNA) of VLPs. In instances in which LTR-retrotransposon proteins bind their encoding mRNA during or immediately after translation and promote its encapsidation into the VLP, the proteins are said to be *cis*-acting. A major consequence of preferential *cis* action is that only autonomous elements that encode functional proteins retrotranspose efficiently. In contrast, *trans*-acting retrotransposon proteins do not show a preference for encapsidating the RNA from which they are translated and therefore can encapsidate a gRNA from any element regardless of whether it serves as an mRNA. Ty1 proteins are *trans*-acting, resulting in the retrotransposition of both autonomous elements and non-autonomous elements that do not encode functional proteins [18]. This feature allows the function of the Ty1 RNA as a protein-coding template to be separated from its function as a template for reverse transcription. The *trans*-action of Ty1 proteins has been exploited to develop helper-donor assays. A retrotransposition-defective “helper” Ty1 element encodes functional Gag and Gag-Pol proteins required for retrotransposition but carries mutations that prevent its function as a template for reverse transcription (Figure 4). “Donor” Ty1s are non-

autonomous elements containing the minimal sequences necessary for the transcript to be recognized for encapsidation in VLPs, reverse transcription and integration. In addition, the donor Ty1 is marked with a selectable marker gene or RIG to detect its retrotransposition into the host genome. The helper-donor assay has been employed to define the role of Ty1 RNA sequences and secondary structures that are required for packaging and reverse transcription [19-21] and to study Ty1 RNA dynamics [22].

C. An element poised for action

Compared to the genomes of most other eukaryotic organisms, a relatively small proportion of the genomes of *S. cerevisiae* strains, ranging from about 1.3 to 3.4%, consists of transposon sequences [23]. Five families of LTR-retrotransposons, Ty1-Ty5 are the only transposable elements in the *S. cerevisiae* nuclear genome. In the laboratory reference strain S288C, Ty1 and Ty2 are the most and next most prevalent in the genome, respectively. Ty1 and Ty2 are closely related elements distinguished by a 1-nucleotide deletion in the Ty2 LTR, a divergent *GAG* ORF and a ∼300 nucleotide region of sequence divergence in *POL* [24]. Ty2 elements were introduced into a recent ancestor of *S. cerevisiae* from a related species, *Saccharomyces mikitae*, as a result of horizontal transfer [23,25]. There are 313 copies of Ty1 dispersed throughout the *S. cerevisiae* S288C genome, including 279 solo LTRs, 2 other truncated elements and 32 full-length elements [23,24]. The Ty2 family consists of 46 elements, of which 13 are full-length elements, 31 are solo LTRs and 2 are other truncations. Population genomic and phylogenetic analyses of S288C and other *S. cerevisiae* strains sequenced in the Saccharomyces Genome Resequencing Project [25] indicate that all full-length Ty1 and Ty2 elements, as well as all Ty2 solo LTRs in S288C have recently transposed [23,26]. In contrast, the majority of Ty1 solo LTRs result from ancient transposition events, and many are degenerate in sequence. Full-length Ty1 elements comprise three active lineages: Ty1, Ty1/Ty2 hybrids and Ty1′, with Ty1 likely being the ancestral lineage. In Ty1/Ty2 hybrid elements, the U3 domain of both LTRs is derived from Ty2 elements, while the R-U5 domains and coding regions are derived from Ty1 [27]. Ty1′ elements are distinguished by a high degree of sequence divergence in the *GAG* ORF [24,27].

Consistent with the recent transposition of Ty1 and Ty2 elements, the coding regions of members of each family are very homogeneous in sequence, with 86% and 96% invariant amino acids in Ty1 and Ty2 ORFs, respectively [24,26]. Furthermore, Ty1 and Ty2 elements in laboratory strains S288C and GRF167 are predominantly autonomous elements that encode functional gRNA and proteins capable of promoting retrotransposition in the absence of other elements [18,24]. Remarkably, the coding regions of both element families appear to be evolving under purifying selection [26,28]. The predominance of autonomous elements is puzzling, given that Ty1 proteins are *trans*-acting and display no apparent preference for mobilizing autonomous elements [18]. The mechanism of selection for autonomous Ty1 and Ty2 elements is not well understood, but the robust conversion of full-length Ty elements to solo LTRs by homologous recombination between the 5′ LTR and 3′ LTR of individual elements is likely to be a significant factor in cleansing the genome of defective elements [23,26,29]. In the diploid stage of the yeast life cycle, the predominant stage in the wild, Ty1 elements are removed with no footprint by loss of heterozygosity, a

process in which the Ty1 containing allele undergoes gene conversion by the unoccupied allele on the homolog. In addition, a significant rate of ectopic gene conversion among non-allelic Ty1 elements is likely to homogenize Ty1 sequences and could be involved in eliminating defective copies [6]. Finally, the low rate of retrotransposition in yeast limits the spread of defective copies [6,30]. The transpositional dormancy of Ty2 elements is, at least in part, a consequence of the low level of Ty2 RNA [31]. Ty1 RNA is approximately twenty times more abundant than Ty2 RNA, but retromobility is repressed at a post-transcriptional level by a mechanism known as copy number control [32,33]. This mechanism, discussed in detail below, balances the rate of gain of Ty1 elements by retrotransposition with the rate of loss by solo LTR formation and loss of heterozygosity. Nonetheless, transpositional dormancy imposed by copy number control can be modulated by a variety of extrinsic and intrinsic parameters, including cell type, activation state of MAPK and DNA damage signaling pathways, and environmental signals including temperature and nutrient availability [34-41]. Thus, the retrotransposon landscape is rife with sleeping elements, held in check but poised to mobilize when triggered by environmental or cellular cues.

II. Ty1 RNA and protein expression, localization and turnover

A. Ty1 expression

Ty1 transcript synthesis, abundance and stability—The major Ty1 transcript starts precisely at position 241 of Ty1-H3, corresponding to the first nucleotide of the 5′ LTR-R region [42], and ends at the last nucleotide of the 3′-LTR-R region [43,44], yielding a 5640 nt RNA with redundant termini (Figure 5A). Two TATA boxes (T_1 and T_2) located upstream of the transcription start site contribute to transcription; their simultaneous mutation nearly abolishes transcription [45]. The 5′ LTR has weak transcriptional activity, per se [46]. The presence of transcription factor binding sites required for full expression define the Ty1 promoter as a one kilobase region extending both upstream and downstream of the TATA boxes and including the 5′ LTR and a large portion of the *GAG* coding region (Figure 5B). Transcription termination occurs by endonucleolytic cleavage and polyadenylation of Ty1 mRNA. Two sequences, TS_1 and TS_2, located in the U3 and R portions of the 3′ LTR, respectively, contribute equally to 3′ end formation [47].

Ty1 transcription is modulated by at least nine transcription factors that bind to the Ty1 promoter (i.e. Gcr1, Ste12, Tec1, Mcm1, Tea1/Ibf1, Rap1, Gcn4, Mot3 and Tye7) and three chromatin-remodeling complexes (Swi/Snf, SAGA and ISWI) [34,48-59]. Deletion of *STE12*, *TEC1* or genes encoding subunits of the SWI/SNF chromatin remodeling complex or the SAGA (Spt/Ada/Gcn5) histone acetyltransferase complex, such as *SPT3*, leads to a severe decrease in Ty1 transcription [34,54,57,60,61]. The other transcription factors have mild or synergistic effects on Ty1 transcription or act under specific growth conditions. For example, the Gcr1 transcriptional activator of genes involved in glycolysis activates Ty1 transcription in the presence of lactate and glycerol [62]. Gcn4 is required for Ty1 transcription under amino acid starvation [56], and Tye7 activates Ty1 transcription under adenylic nucleotide stress [58].

Ty1 RNA accounts for 0.1 to 0.8% of total cellular RNA and 10% of polyadenylated mRNA [31,63]. This abundance is probably the consequence of the unusually long half-life of Ty1

RNA-- higher than the average half-life of yeast mRNAs and ~ 5 hours when expressed from pGTy1 [20,64]--rather than robust transcriptional activity [56]. Only about 15% of total cellular Ty1 RNA is polyadenylated [65], suggesting that deadenylated Ty1 RNA, unlike most deadenylated mRNAs [66], is not rapidly degraded.

Expression of individual endogenous Ty1 elements—The relative transcription level of endogenous Ty1 elements of S288C strain was resolved by tagging 31 of the 32 Ty1 elements with a *lacZ* reporter gene. Expression of individual Ty elements varied by up to 50-fold (Figure 6) [56]. Eleven highly expressed elements account for 75% of total Ty1 expression, and eight of these are Ty1/Ty2 hybrid elements. Ty1/Ty2 hybrids contain five potential binding sites for the Gcn4 transcription activator in their 5′ LTR, whereas weakly expressed elements generally have fewer Gcn4 binding sites [67]. Overproduction of Gcn4 increases Ty1 mRNA levels and stimulates the transcription of Ty1/Ty2 hybrids by a Swi/Snf and SAGA-dependent mechanism. In contrast, the transcription of Ty1 elements lacking some or all Gcn4 binding sites is low and independent of Swi/Snf. Based on these observations, it was proposed that Gcn4 recruits Swi/Snf and SAGA, which in turn renders the chromatin accessible to transcriptional activators such as Tec1 and Ste12 [56].

Antisense Ty1 RNA transcription—Two major species of antisense non-coding RNA known as Ty1-RTL or Ty1AS RNA are transcribed from Ty1 elements in *S. cerevisiae* strain S288C (Figure 5A). These transcripts have heterogenous 5′ and 3′ termini with several transcription start sites in *GAG* and two termination positions in the 5′ LTR. Ty1AS RNA is transcribed by Pol II, capped and polyadenylated [68,69]. The level of Ty1AS RNA is increased in cells lacking the SAGA subunit Spt3, indicating that transcription of sense-strand Ty1 RNA and Ty1AS RNA occurs by independent mechanisms [70,71]. Ty1AS RNA is a cryptic unstable transcript (CUT) whose stability is enhanced by deletion of genes required for 5′ to 3′ mRNA degradation, including *DCP1*, *DCP2* and *XRN1* [68,70,71]. Proposed roles for Ty1AS RNA in the regulation of Ty1 retrotransposition are discussed below.

Translation of Ty1 RNA—Little was known about the regulation of Ty1 RNA translation until recently. It was previously assumed that Ty1 RNA was translated inefficiently, because of the apparent difference in abundance between Ty1 RNA and Ty1 proteins and VLPs [12,31,63,72]. Furthermore, a computational model of the 5′ end of Ty1 RNA, bolstered by mutational analyses, suggested that there is significant secondary structure in the 53-nucleotide 5′ UTR [19], which is unusual among yeast mRNAs and is potentially inhibitory to translation initiation [73]. Recent secondary structure models of Ty1 RNA both *in vitro* and *in vivo*, which were developed using selective 2′-hydroxyl acylation of RNA analyzed by primer extension (SHAPE) data, indicate that nucleotides 1-7, 39-46 and 55-59 are involved in long-range pairing with downstream sequences, and nucleotides 15-29 form a stable stem-loop (Figure 7) [20,74]. Moreover, most Ty1 RNA molecules migrate in sucrose gradients as very high molecular weight complexes that are stable when ribosomes are dissociated with EDTA; thus, it was inferred that these Ty1 RNPs were non-polysomal and translationally inactive [71]. However, the view that Ty1 RNA is translationally inert is contradicted by a few recent findings that are consistent with active translation of Ty1 RNA.

When a premature termination codon is introduced into the *GAG* ORF, Ty1 RNA is subject to nonsense-mediated decay, a translation-coupled process that targets defective mRNA for 5′ to 3′ degradation [22]. In addition, Ty1 RNA co-purifies with ribosome-nascent chain complexes [75,76]. Finally, the global translation efficiency of genomic Ty1 elements can be estimated using data from deep sequencing of ribosome-protected mRNA fragments in a ribosomal profiling experiment [77]. Analysis of these data indicate that the translational efficiency of *GAG* (i.e., the ratio of the percentage of ribosome-protected mRNA-seq reads that align to any *GAG* ORF in the genome to the percentage of poly(A)$^+$ mRNA-seq reads that align to any *GAG* ORF) is substantially higher than the median translation efficiency of all yeast ORFs (R. J. Palumbo and M. J. Curcio, unpublished results). While this calculation does not consider the large pool of deadenylated Ty1 RNA in cells, the fact remains that at least a fraction of Ty1 RNA is actively translated.

Two non-essential ribosome biogenesis factors that promote 18S ribosomal RNA maturation and small ribosomal subunit formation, Bud21 and Bud22, are necessary for efficient translation of Ty1 RNA [76,78,79]. In a *bud22*Δ mutant, the level of Gag but not Ty1 RNA is strongly reduced, and Ty1 RNA is shifted from polysomes containing six to eight ribosomes to polysomes of three to eight ribosomes [78]. This reduction in the number of ribosomes per Ty1 RNA is consistent with initiation of Ty1 RNA translation being stalled by the limited availability of 40S rRNA subunits; however, this finding must be interpreted with caution, since the migration of Ty1 RNA in dense sucrose gradient fractions is not due solely to its translation on polysomes [71]. In a *bud21*Δ mutant, newly synthesized Gag is reduced to about one quarter of the wild-type level [76].

The role of signal recognition particle in Gag translation and stability—A conserved pathway that targets membrane and secretory proteins to the endoplasmic reticulum (ER) was recently shown to be essential for the stability of Gag [76]. Analysis of mutants in this pathway led to the unanticipated discovery that Gag is guided to the ER during translation by signal recognition particle (SRP), a universally conserved ribonucleoprotein complex that functions as a co-translational chaperone (reviewed in [80]). SRP binding to ribosome-nascent chain complexes transiently pauses translation and targets translation complexes to the SRP receptor on the ER membrane, where the nascent peptide is translocated through the membrane to the ER lumen. Ty1 RNA is associated with affinity purified SRP-ribosome-nascent chain complexes, demonstrating that SRP recognizes ribosomes synthesizing Gag, but the sequences within nascent Gag that interact with SRP have not yet been identified [75,76]. Once Ty1 RNA-ribosome-nascent Gag complexes are targeted to the ER membrane, nascent Gag is translocated to the ER lumen. The half-life of newly synthesized Gag is markedly reduced when proteins required for ER translocation are depleted, including subunits of SRP, the SRP receptor or the ER translocon. This finding suggests that Gag is rapidly degraded when it is synthesized in the cytoplasm [76].

Because translocation of Gag to the ER lumen is required for its stability, there must be a mechanism by which Gag is retrotranslocated to the cytoplasm to associate with Ty1 RNA to form VLPs. Virtually nothing is known about this mechanism, as Ty1 Gag is the first cytoplasmic yeast protein described that traverses the ER [76]. In mammalian cells, several viral proteins are retrotranslocated from the ER lumen via the ER-associated degradation

(ERAD) pathway, including the ORF2 protein of Hepatitis E virus, the precore protein Hepatitis B virus and REM protein of the betaretrovirus, MMTV [81-83]. The ERAD pathway normally retrotranslocates resident ER and membrane proteins to the cytoplasm for degradation by the proteosome [84], but these cytoplasmic viral substrates escape degradation [81,82]. Much remains to be learned about retrotranslocation of cytosolic proteins from the ER, and Ty1 can serve as a simple model system to explore this process. An important consequence of the co-translational translocation of nascent Gag to the ER and retrotranslocation of Gag to the cytoplasm is that this trafficking sequesters nascent Gag from its encoding Ty1 RNA, and thereby enhances the interaction of Gag with Ty1 RNA in *trans* [76].

Expression of *POL* by translational frameshifting—The Ty1 *POL* ORF is expressed only as a Gag-Pol fusion protein that forms when translation "slips" forward one nucleotide from the *GAG* ORF to the *POL* ORF. Programmed ribosomal frameshifting occurs at a heptanucleotide sequence, CUU-AGG-C, in the 38-nucleotide overlap between the *GAG* and *POL* ORF. Both the CUU codon and the UUA codon in the +1 frame encode leucine and can be decoded by $tRNA^{UAG}$ [85]. When $tRNA^{UAG}$ is in the P site, it can slip from the CUU codon to the +1 UUA codon [86]. Slippage of $tRNA^{UAG}$ is enhanced by pausing of the ribosome with the CUU codon in the P site and the AGG codon in the A site. Pausing at this position is a consequence of the low abundance of the AGG-decoding $tRNA^{CCU}$, which is encoded by the single copy *HSX1* gene [87,88].

Translational frameshifting is a common mechanism of optimizing the ratio of viral structural proteins to catalytic proteins for assembly of replication-competent viral particles [89]. Indeed, expression of Ty1 Gag and Pol proteins at a specific ratio is critical for proteolytic processing of Ty1 proteins and retrotransposition [88,90,91]. The amount of p199-Gag-Pol synthesized appears to be primarily a function of the efficiency of ribosomal frameshifting, which is 3-13%, depending on the assay method and experimental conditions used to measure Ty1 frameshifting efficiency [88,92]. The efficiency of Ty1 frameshifting genome-wide can be estimated from deep sequencing data generated from a ribosomal profiling experiment [77]. Our compilation of mRNA-seq reads of ribosome-protected mRNA footprints that map to Ty1 *POL* versus *GAG* reveals an overall frameshifting efficiency of 7% for genomic Ty1 elements (R. J. Palumbo and M. J. Curcio, unpublished results). While ribosomal frameshifting is a potentially important control point for Ty1 retrotransposition, few host factors that influence frameshifting other than rRNA and the tRNAs that decode the heptanucleotide sequence have been characterized. Host factors required for biosynthesis of spermine and spermidine, Spe1 and Spe2, suppress frameshifting and are essential for efficient retrotransposition [93,94]. In addition, the 40S rRNA biogenesis factor Bud22 enhances Ty1 frameshifting, but is also necessary for efficient synthesis of Gag [78].

B. Biogenesis of the VLP assembly site

Localization of Ty1 RNA and Gag protein to retrosomes—The components of retroviral particles are typically concentrated in subcellular domains known as nucleocapsid assembly sites or "virus factories". As the evolutionary progenitors of retroviruses, LTR-

retrotransposons might also be expected to have cytoplasmic domains where gRNA and Gag and Gag-Pol proteins are concentrated to promote assembly of VLPs. However, little was known about the site of VLP assembly until the serendipitous discovery of microscopically distinct cytoplasmic foci, known as T-bodies or retrosomes, where Ty1 RNA and Gag proteins co-localize [65]. In strain S288C, retrosomes are detected in 30% to 50% of cells at 20°C, a permissive temperature for retrotransposition (Figure 8)[65,70,76]. Notably, retrosomes are not detected in GRF167, the strain in which Ty1 retrotransposition was initially characterized (S. Lutz and M. J. Curcio, unpublished results). Ty1 retrosomes are not simply clusters of pre-formed VLPs, as VLP formation is very inefficient in S288C. Nonetheless, VLPs are thought to assemble within retrosomes, since clusters of VLPs are associated with Gag foci when VLP formation is induced by expressing a pGTy1 element [12,70].

The trafficking of retroviral and LTR-retrotransposon RNAs and proteins to their sites of assembly has recently begun to be unraveled [95]. HIV-1 and Rous sarcoma virus gRNAs are recruited to the presumptive assembly site by Gag, which interacts with gRNA in the cytoplasm or nucleus, respectively [96,97]. The scenario for nucleation of Ty1 assembly sites appears to be distinct, in that Ty1 RNA is targeted to the retrosome prior to or during translation [76]. Translation of Ty1 RNA in retrosomes is supported by the finding that glucose deprivation, a global inhibitor of translational initiation, cause retrosomes to disperse [65,70]. Moreover, the abundance of retrosomes is increased by hypomorphic mutations in SRP subunits Srp54 and Srp72, which reduce the rate of co-translational transport of nascent Gag to the ER. Suppression of these mild translocation defects by compensatory reductions in the rate of translational elongation rapidly reverses the increase in retrosomes [76]. This modulation of retrosome abundance in response to small changes in the rate of translocation relative to translation indicates that the Ty1 retrosome is a dynamic ribonucleoprotein complex containing Ty1 RNA translation complexes awaiting translocation of nascent Gag to the ER.

SRP-associated Ty1 RNA translation complexes are bound by Gag, presumably via a direct interaction with Ty1 RNA [76]. It is likely that multimerization of Gag bound to Ty1 RNA translation complexes is necessary for the stability of the retrosome, since retrosomes fail to appear when Gag levels are very low. For example, retrosomes are not visible in a *bud21* mutant, in which Gag is inefficiently synthesized, or in an *srp68-DAmP* mutant, in which newly synthesized Gag is rapidly degraded [76]. Retrosomes are also not detected when Gag lacking the C-terminal region required for RNA binding and VLP formation is expressed in a strain lacking endogenous Ty1 expression [22,98].

One study suggests that Gag facilitates export of Ty1 RNA from the nucleus to cytoplasmic retrosomes [22]. When expressed in the absence of endogenous Ty1 RNA, a Ty1 RNA harboring a stop codon directly downstream of the *GAG* start codon accumulates in the nucleus. Expression of functional Ty1 RNA and Gag in *trans* restores the localization of this untranslatable Ty1 RNA to cytoplasmic retrosomes. At least a fraction of Gag is located at the nuclear membrane, the outer membrane of which is continuous with the ER. Further studies are necessary to determine whether the retrosome is associated with the nuclear

membrane, or whether Gag's transit through the ER plays a role in export of Ty1 RNA from the nucleus to the retrosome.

A role for Ty1 RNA subcellular localization in retrosome formation—A cohort of host co-factors that are associated with mRNA processing bodies (P-bodies) is necessary for the localization of Ty1 RNA to retrosomes. Interestingly, these host-factors have little effect on the expression of sense-strand Ty1 RNA or the formation of VLPs. P-bodies are cytoplasmic RNA granules containing translationally repressed mRNAs and 5′ to 3′ mRNA decay factors [99]. Lsm1 and Pat1 are associated with deadenylated RNA and activate decapping and decay of mRNAs [100-103]. Xrn1 (Kem1) is the cytoplasmic 5′ to 3′ exoribonuclease that degrades mRNA [104]. In *lsm1*Δ and *pat1*Δ mutants, Ty1 RNA is dispersed throughout the cytoplasm, while in an *xrn1*Δ mutant, Ty1 RNA and Gag are in diffuse aggregates [70].

Based on the co-localization of Ty3 retrosomes with P bodies (see Chapter 42 by S. Sandmeyer), it was initially thought that partial co-localization of Ty1 Gag with P body proteins might have functional significance in the formation of the retrosome [71]. However, unlike Ty3 retrosomes, Ty1 retrosomes form under different conditions than those that promote P body formation, which reflects their divergent populations of actively translated and translationally repressed RNAs, respectively [65,70,71]. Despite the fact that Ty1 Gag and RNA are not concentrated in P bodies, P-body-associated deadenylation-dependent mRNA decay and nonsense-mediated decay factors are required for Ty1 cDNA accumulation and retrotransposition [70,71]. In *xrn1*Δ, *lsm1*Δ and *pat1*Δ mutants, there are only minor reductions in Ty1 RNA and Gag protein, and proteolytic processing of Gag, carried out by Ty1 PR in the VLP, is efficient, indicating that these mRNA decay factors are not essential for VLP formation. However, VLPs tend to be dispersed throughout the cytoplasm in *xrn1*Δ and *lsm1*Δ mutants. (A deletion of *PAT1* did not have this effect). The levels of processed p63-RT and p71-IN were also reduced in *xrn1*Δ and *lsm1*Δ mutants [70]. Deletion of *XRN1* abolished Ty1 RNA packaging in VLPs, as measured by the lack of protection of Ty1 RNA from degradation by benzonase [71]. Thus, the lack of retrosomes in these mutants is associated with formation of replication-incompetent VLPs with defects in Ty1 RNA packaging, stability of VLP-associated Pol proteins and cDNA accumulation.

It remains to be determined whether Xrn1, Lsm1 and Pat1 interact directly with Ty1 components to promote retrotransposition, or whether they act indirectly via their general effects on RNA metabolism. No direct interaction between Ty1 Gag and Xrn1, Lsm1 or Pat1 or between Ty1 RNA and Lsm1 has been detected [71,76]. Several models have been proposed to explain the lack of Ty1 retrosomes and retrotransposition in 5′ to 3′ mRNA decay factor mutants. In the first model, accumulation of cellular mRNA in the absence of 5′ to 3′ mRNA decay may interfere with the specific recognition of Ty1 RNA by Gag and result in a high level of non-specific RNA packaging [71]. The second model, based on the observation that Ty1AS RNA is stabilized in *lsm1*Δ, *pat1*Δ and *xrn1*Δ mutants, posits that elevated levels of Ty1AS RNA suppress retrosome formation and retrotransposition [33,70]. A third possibility is that mRNA decay factors are required for the localization of Ty1 RNA to a specific subcellular domain of the cell prior to translation. The tethering of Ty1 RNA translation complexes to the ER by SRP and translocation of nascent Gag cannot, in and of

itself, explain how Ty1 RNA and Gag are localized to a single address in the cell [76]. Perhaps Ty1 RNA localization, like the localization of many mRNAs, requires translational repression and active transport [105], and P body factors Xrn1, Lsm1 and Pat1 may be regulators of this fate [70]. Support for the idea that host factors traffic Ty1 RNA to the presumptive retrosome prior to translation comes from the analysis of a mutant lacking *RPL7A*, one of two paralogous genes that encode ribosomal protein L7. In an *rpl7a*Δ mutant, Ty1 RNA is translated in association with SRP, but Gag does not interact with Ty1 RNA translation complexes, and retrosomes are not formed [76]. Therefore, Rpl7a and possibly Xrn1, Lsm1 and Pat1 may direct Ty1 RNA to a specific subcellular location prior to Gag synthesis in order for newly synthesized Gag that is exiting the ER to interact with Ty1 RNA to form the retrosome.

III. Post-translational steps in retrotransposition

A. VLP formation

Gag assembly and VLP structure—Ty1 VLPs are nucleocapsid particles formed by assembly of Gag and Gag-Pol precursors into particles that encapsidate Ty1 RNA. Gag is the only Ty1 protein domain necessary for the formation of VLPs, which assemble even when Gag is expressed in *E. coli* [106,107]. The smallest fragments of the 440 amino acid-Gag protein that can assemble into particles span amino acids 41-346 or 31-363 [107-109]. Within this minimal Gag fragment, several regions have been shown to be important in assembly, including amino acids 41 to 62, 114 to 147, 223 to 287 and 330 to 346 (reviewed in [110]). Remarkably, one or two amino acid substitutions can completely block assembly or increase the size of VLPs as much as 8-fold [109]. The N-terminus of Gag is on the surface of the VLP, while the C-terminus of both mature and immature forms of Gag is buried in the core [108]. This alignment ensures that the RNA binding domain at the C-terminus of Gag and the Pol domain of p199-Gag-Pol is within the capsid shell.

Ty1 VLPs are ∼14 MDa in size and are composed of a spikey, symmetrical electron dense shell of ∼30-80 nm in diameter, surrounding a luminal core. The shell is porous and allows globular proteins ∼13 kDa, e.g. RNaseA, but not larger ∼30 kDa, e.g. benzonase, to access the gRNA packaged within the VLP [111,112]. A remarkable feature of Ty1 particles is their variability in size [107,112,113]. Expression of either unprocessed p49-Gag or mature p45-Gag, which is not subject to further proteolytic processing, results in particles of two to three different size classes. The p49-Gag precursor yields particles with icosahedral T numbers (subunits) of T=7 and T=9. C-terminal truncation of Gag results in polydisperse VLPs with a smaller average size than those formed by p49-Gag, but proteolytic processing of p49-Gag does not cause a reduction in the size of Ty1 VLPs; ergo, the heterogeneity in particle size is not a reflection of incomplete Gag processing, but rather manifests an extraordinarily flexible assembly process. The finding that VLPs formed in the presence and absence of Ty1 PR-mediated processing have the same size range provides direct experimental evidence that Gag is processed after VLPs assemble [111].

Formation of VLPs is a key step in the replication cycle of Ty1. The VLP protects Ty1 gRNA from attack by nucleases and concentrates factors that copy Ty1 gRNA into cDNA. In addition to Gag, *POL*-encoded PR, IN and RT/RH, Ty1 gRNA, the host-coded $tRNA_i^{Met}$

and deoxynucleotide triphosphates are necessary for the production of cDNA within the VLP. An exogenously expressed human antiretroviral protein, APOBEC3G, is the only non Ty1-encoded protein that has been shown to co-fractionate with Ty1 VLPs [114]. APOBEC3G, a potent inhibitor of Ty1 retrotransposition, gains access to the VLP via its RNA-dependent interaction with Ty1 Gag [114,115].

RNA packaging—By analogy with retroviruses, Ty1 RNA is bound by the nucleocapsid domain of Gag and packaged into the VLP. Ty1 RNA sequences required for Gag binding and packaging have not been precisely defined. Xu and Boeke [21] demonstrated that an internally deleted mini-Ty1 RNA containing 380-nucleotides of the 5′ terminus and 357 nucleotides of the 3′ terminus of Ty1 RNA was mobilized when Gag and Pol were expressed in *trans* from a helper-Ty1 element (Figure 4). Deletion of nucleotides 237-380 abolished retrotransposition and co-purification of mini-Ty1 RNA with VLPs, suggesting that *cis*-acting sequences required for Ty1 RNA packaging reside in this domain.

A single nucleotide resolution secondary structure model of the 5′ terminus of Ty1 gRNA within VLPs was derived using SHAPE reactivities (Figure 7). In this model, nucleotides 1-325 of Ty1 RNA extracted from VLPs (referred to as "*in virio*") form a long-range pseudoknot. The pseudoknot core consists of two 7 bp stems (S1 and S2) with a 1-nucleotide interhelical connector, and long structured loops that bridge the stems. The essential packaging region between nucleotides 237 and 380 overlaps pseudoknot sequences in the S1 stem and the S2 stem and its structured loop [74]. Surprisingly though, mutations that destabilize the S1 stem of the pseudoknot do not diminish Ty1 RNA packaging [20]; ergo, pseudoknot formation may play a minor role in packaging of Ty1 gRNA.

Like the gRNA of retroviral particles, Ty1 gRNA is packaged in the VLP as a dimer [116]. The dimer interaction is non-covalent. *In vitro*, Ty1 RNA dimerizes in the presence of $tRNA_i^{Met}$ and the RNA binding domain of Gag; however, single nucleotide resolution structural analyses of Ty1 gRNA indicated that the $tRNA_i^{Met}$ does not participate in dimerization of Ty1 RNA [74,117]. Retroviral RNA dimerization and packaging are tightly coupled processes, both facilitated by the nucleocapsid activity of Gag [118,119]. Purzycka et al. [74] identified three palindromic sequences (PAL1-PAL3) in the 5′ terminus of Ty1 RNA that were less reactive in gRNA *in virio* than in gRNA *ex virio*. Based on analogy with retroviral dimerization sites, the authors proposed that PAL sequences are sites where the nucleic acid chaperone activity of Gag promotes a transition from intramolecular pairing to intermolecular pairing of Ty1 RNA [74,120].

Ty1AS RNA co-purifies with Ty1 VLPs, but there is no evidence that it interacts with Ty1 gRNA within the VLP [33,74]. Cellular mRNAs can be co-incorporated into VLPs and subsequently copied into cDNA using Ty1 cDNA as a primer. Most mRNAs gain access to the VLP on the basis of their abundance; however, a small number of mRNAs, including the transcript of the subtelomeric repeat, Y′, which encodes a helicase of unknown function, are highly enriched in VLPs [121-123].

B. Protease-dependent Gag and Gag-Pol processing

Ty1 protease (PR) is a 20 kDa monomeric aspartyl protease encoded by the C-terminus of the *GAG* ORF and N-terminus of the *POL* ORF. Processed p20-PR is very difficult to detect, even when pGTy1 is expressed [124]. Ty1 PR activity is required for all cleavages of Ty1 Gag and Gag-Pol proteins and for retrotransposition activity [124-126]. The p199-Gag-Pol precursor has three distinct cleavage sites at the Gag/PR (RAH/NVS), PR/IN (TIN/NVH) and IN/RT (LIA/AVK) junctions, which were defined by sequencing of various termini of mature Ty1 proteins and mutagenesis [125,127,128]. The 6-amino acid cleavage site, while necessary, is not sufficient to define the processing site. The Gag-Pol precursor must be cleaved at the Gag/PR site for subsequent cleavages of the Gag-Pol precursor or p49-Gag precursor to occur [125]. Pulse-chase experiments of pGTy1 expression and mutagenesis of PR cleavage sites support semi-ordered processing of the p49-Gag and p199-Gag-Pol precursor proteins [124,125]. Mutations in Ty1 that abolish PR activity result in VLPs with significant reverse transcriptase activity when an exogenous primer and template are supplied, but reverse transcription of the endogenous template is undetectable, perhaps because VLPs produced from a PR-defective element contain significantly less Ty1 gRNA, and Ty1 RNA dimerization is reduced [116,126].

C. cDNA synthesis

Cis*-acting sequences required for reverse transcription—Cis*-acting RNA motifs in the 5′ termini of Ty1 RNA participate in the initiation of reverse transcription. Critical sequences have been defined by introducing silent nucleotide substitutions into the 5′ end of a pGTy1 element, or by using a non-autonomous Ty1 as an RT template donor (Figure 4). Immediately adjacent to the 5′ LTR is the primer binding sequence (PBS; nucleotides 95 to 104), a 10-nucleotide sequence that is complementary to the 3′ end of $tRNA_i^{Met}$ (Figure 7). This tRNA species is selectively packaged into the VLP, where it functions as the primer for initiation of reverse transcription [129,130]. Two adjacent 7- and 6-nucleotide regions, known as Box 0 and Box 1, respectively, are complementary to sequences within the T or D hairpins of $tRNA_i^{Met}$ [131,132]. Analyses of mutations in both Ty1 RNA and $tRNA_i^{Met}$ have established a role for an extended interaction between $tRNA_i^{Met}$ and the PBS, Box 0 and Box 1 regions of Ty1 RNA in the initiation of reverse transcription [132,133]. Adjacent to Box 1 is a 14-nucleotide region known as CYC5, which is perfectly complementary to a sequence in the 3′ UTR known as CYC3. CYC5:CYC3 complementarity promotes efficient reverse transcription *in vitro* and retrotransposition *in vivo* [117,134]. In addition, intramolecular pairing of nucleotides 1-7 to nucleotides 264-270 promotes efficient reverse transcription [19,20]. The secondary structure model of the 5′ terminus of Ty1 gRNA *in virio* supports many aspects of earlier structural models that were based on mutational analyses [19,133], including pairing of the $tRNA_i^{Met}$ to the PBS, Box 0 and Box 1 regions and circularization of Ty1 RNA via the CYC5:CYC3 interaction. Moreover, the functionally defined pairing of nucleotides 1-7 to nucleotides 264-270 forms the S1 stem of the Ty1 RNA pseudoknot. All of the RNA motifs that are known to be required for reverse transcription are in the multi-branched loop that is formed by the S1 stem of the pseudoknot, suggesting that this subdomain may be functionally as well as structurally distinct (Figure 7).

Reverse Transcriptase—The Ty1 reverse transcriptase is a 63 kDa protein encoded at the carboxy terminal end of the *POL* ORF. Like retroviral RTs, Ty1 RT has an amino-terminal domain with an RNA- or DNA-dependent DNA polymerase activity and a carboxy terminal RNase H domain whose activity specifically degrades the RNA strand of an RNA:DNA duplex. These dual activities allow a full-length, double-stranded Ty1 cDNA to be synthesized from the single-stranded Ty1 RNA.

Ty1 RT, like all RTs, is dependent on a divalent cation, preferably Mg^{2+}, for catalytic activity. The Ty1 reverse transcription reaction also occurs in the presence of Mn^{2+}, although to a much lesser extent [135-137]. Ty1 RT contains a triad of aspartate residues in the polymerase active site that is conserved among RTs. A mutant bearing a substitution of asparagine for the aspartate at position 211 of Ty1 RT, the second aspartate in the canonical YXDD motif, retains some catalytic activity but has altered properties, including a preference for Mn^{2+} over Mg^{2+} and a marked defect in pyrophosphate binding and release, which affect the ability of polymerase to translocate on the template [136,138]. Interestingly, specific mutations in the RNase H domain increase the polymerase activity of Ty1 RT without affecting the RNase H activity [139].

Despite the fact that the RT and IN domains are arranged in an order opposite to that in the *POL* gene of retroviruses, Ty1 RT activity, like that of retroviruses, is dependent on an interaction between RT and IN. *In vitro*, the biochemical activity of recombinant Ty1 RT purified from *E. coli* requires the expression of the contiguous 115-amino acid C-terminal portion of IN fused to the N-terminus of the entire RT ORF [137]. Fusion of a small acidic tail to the RT protein restores polymerase and RNase H activity *in vitro*, supporting the hypothesis that a highly acidic domain of IN, located between amino acids 521 and 607, interacts with basic residues at the N-terminus of RT to allow RT to adopt an active conformation [140]. Consistent with this model, complementation assays based on the co-expression of two Ty1 elements show that IN acts exclusively in *cis* to activate RT *in vivo*. Furthermore, RT purified from Ty1 VLPs is not active unless it remains in close association with IN [141].

Reverse transcription reaction—Reverse transcription of the Ty1 RNA dimer within the VLP occurs by a series of ordered steps that are generally analogous to those of reverse transcription of retroviral RNA within virion cores [11,12,142] (Figure 9). Reverse transcription is initiated from the 3′ OH of the $tRNA_i^{Met}$ primer that anneals to the complementary PBS sequence immediately downstream of the 5′ LTR [129]. The initial DNA product, known as minus-strand strong-stop cDNA, contains the U5 and R regions of the LTR linked to the $tRNA_i^{Met}$ [143]. Once RT reaches the 5′ end of the Ty1 RNA template, the Ty1 RNA portion of the RNA:DNA hybrid is degraded by the RNase H activity of RT. Degradation of the RNA facilitates transfer of minus-strand strong stop cDNA from the 5′ end of the Ty1 RNA template to the 3′ end, where it anneals to the R region. Minus-strand strong stop cDNA is thought to anneal to the full-length Ty1 RNA and not the Ty1 RNA whose 5′ end has been degraded by RNase H [144]. Using minus-strand strong-stop cDNA as a primer, a minus-strand Ty1 cDNA that extends into the U5 and R regions present in the undegraded Ty1 RNA template is synthesized. The Ty1 RNA is then degraded by RNase H, except for two polypurine tracts, one directly upstream of U3 (PPT)

and one near the center of Ty1 (cPPT) [145,146]. PPT serves as the primer for synthesis of plus-strand strong stop cDNA to the 5′ end of the minus-strand cDNA [144]. This plus-strand cDNA fragment is transferred from the 5′ end to the 3′ end of the minus-strand cDNA, where it hybridizes to R and U5 sequences and serves as a primer for synthesis of the complete plus strand. Finally, the minus-strand cDNA is extended through the U3 region, using the plus-strand strong-stop cDNA as a template. These reactions synthesize linear double-stranded cDNA with complete 5′-U3-R-U5-3′ sequences at the 5′ and 3′ termini. Synthesis of plus-strand cDNA from cPPT leads to the formation of a flap in the plus strand of Ty1 cDNA, but is not essential for retrotransposition [145,147-149].

The yeast RNA lariat debranching enzyme, Dbr1, initially identified by characterization of a Ty1 retrotransposition-defective host mutant [150], is thought to be required for Ty1 cDNA synthesis [151-154]. Notably, the human homolog of Dbr1 is required for HIV-1 cDNA synthesis at a stage subsequent to minus-strand strong-stop cDNA synthesis [155]. A hypothesis that Dbr1 was required for debranching of a 2′, 5′-branched form of Ty1 RNA that serves as a template for transfer of the minus-strand strong stop cDNA provided an alluring model to explain the role of Dbr1 in Ty1 cDNA synthesis [154]; however, neither the existence of a lariat form of Ty1 RNA *in vivo* nor the proposition that Ty1 RT can read through a 2′, 5′ branched RNA were substantiated in further studies [156,157].

Nucleocapsid activities of Gag and PR—A nucleic acid chaperone activity that promotes annealing of the $tRNA_i^{Met}$ primer to the PBS of Ty1 RNA has been mapped to the C-terminus of p45-Gag. A peptide containing the C-terminal 103 amino acids of p45-Gag binds Ty1 RNA and promotes annealing of $tRNA_i^{Met}$ to the PBS and initiation of reverse transcription *in vitro*. Ty1 Gag lacks the canonical zinc finger motif that is found in the nucleocapsid domain of many retroviral and retrotransposon Gag proteins, and instead contains three stretches of basic amino acids in its nucleic acid chaperone domain [117]. Remarkably, the Ty1 PR also has nucleic acid chaperone activity that is essential for Ty1 retrotransposition. The N-terminal 35 amino acids of PR, encoded within the overlapping *GAG* ORF upstream of the frameshifting site, is required for reverse transcription but is dispensable for the proteolytic activity of PR. Expression of a pGTy1 element lacking the N-terminal PR domain in the absence of endogenous Ty1 element expression results in defective minus-strand strong-stop cDNA synthesis, despite the presence of Ty1 RNA and $tRNA_i^{Met}$ within VLPs [158].

Ty1 cDNA—When the pGTy1 element is expressed, full-length, double-stranded Ty1 cDNA can be purified from cell fractions enriched for VLPs [12,159]. Unintegrated Ty1 cDNA can also be detected in total DNA from a wild-type yeast strain grown at 20°C, the permissive temperature for retrotransposition. Unintegrated Ty1 cDNA is differentiated from genomic Ty1 elements on the basis of its lack of flanking DNA [160]. It is not known where the pool of unintegrated cDNA is in the cell, or what fraction can be utilized in retrotransposition. However, cDNA levels are correlated with rates of retrotransposition in a large number of mutants, suggesting that stable unintegrated Ty1 cDNA represents a functional retrotransposition intermediate [161-164]. Unintegrated Ty1 cDNA is present in less than one copy per haploid cell [160,165,166]. Treatment of cells with

phosphonoformate, an inhibitor of Ty1 RT, has been used to measure the rate of decay of Ty1 cDNA [167]. The half-life of Ty1 cDNA in wild-type cells is between 93 and 252 minutes in different studies, and is similar to the doubling time of the cell [167-169], which suggests that degradation of Ty1 cDNA does not play a major role in regulating transposition.

D. cDNA integration

Characteristics of the integration process—cDNA integration is performed by Ty1 IN, a 71 kDa protein of 635 amino acid residues produced by cleavage of the 140 kDa IN-RT processing intermediate [125]. Although Ty1 integration probably relies on a variety of cellular co-factors *in vivo*, it is possible to faithfully reproduce this process *in vitro* using DNA substrates and VLPs as a source of IN [128,159,170,171]. The requirements for efficient Ty1 IN activity *in vitro* have been reviewed in detail [140,172]. *In vitro*, the integration machinery recognizes as little as 4 bp at Ty1 cDNA termini [170,173]. The conserved inverted dinucleotides 5′-TG…CA-3′ at the ends of the cDNA duplex are absolutely required for integration *in vivo*, although very few sequences alterations have been explored [7]. During the integration process, Ty1 IN catalyzes a nucleophilic attack by the 3′ OH moieties at the blunt ends of the cDNA molecule on each strand of the target DNA at 5-bp staggered sites. These concerted transesterification reactions, called the strand transfer step (reviewed in [174]), result in a newly exposed 3′ OH and 5-nucleotide gap on each strand of the target DNA at the cDNA:target DNA junctions. Repair of the gaps generates a 5-bp target site duplication (TSD) flanking the newly integrated Ty1 cDNA, which is a hallmark of Ty1 integration. Ty1 IN does not repair the gaps, suggesting that either Ty1 RT or an unidentified cellular DNA repair protein performs this function. Ty1 IN has been shown to dimerize *in vitro* and by analogy with retroviral integrases, is thought to assemble as a tetramer on cDNA to form the pre-integration complex (PIC) [175].

In vivo, IN overexpression from the *GAL1* promoter allows the integration of blunt double-stranded DNA fragments, which have no similarity with Ty1 DNA, except for the conserved terminal dinucleotides (5′-TG-CA-3′) [176]. Most insertion events present hallmarks of IN-mediated events, such as 5-bp TSDs and Ty1 target site preferences. IN-mediated insertion of non-Ty1 DNA fragments has also been observed in cells lacking the Ku70 protein, suggesting that a Ku70-dependent mechanism restricts endogenous IN activity to Ty1 cDNA [177].

IN structure and function—Ty1 IN has the same three-domain organization as that of other LTR-retrotransposon and retroviral integrases (Figure 10A). The N-terminal domain contains a conserved $H(X_{3-7})H(X_{23-32})C(X_2)C$ zinc-binding motif (ZBD). Alanine substitution of each of the four amino acids ($H_{17}H_{22}C_{55}C_{58}$) of Ty1 IN ZBD motif results in IN instability and inefficient proteolytic processing, leading to substantially decreased cDNA levels and retrotransposition. Alanine substitution of additional hydrophobic residues between H_{22} and C_{55}, which are conserved between LTR-retrotransposons and retroviruses, alters IN-IN interaction, *in vitro* integration and *in vivo* retrotransposition [175].

The catalytic core domain in the central region of Ty1 IN is characterized by a DD(X_{35})E motif that is conserved among retroviral and retrotransposon integrases as well as some bacterial proteins [178] (Figure 10A). This motif binds divalent metal ions (Mg^{2+} or Mn^{2+}) and is critical for the transesterification reaction. An in-frame linker insertion of four amino acids at position 187 in this motif prevents Ty1 retrotransposition *in vivo* and *in vitro* [159].

The C-terminal domain is less conserved and larger than that of other retroviral integrases (Figure 10A). It begins approximately 35 amino acids downstream of the DDE motif and includes a GKGY motif unique to the *Pseudoviridae* family of LTR-retroelements [179]. This domain contains a bipartite nuclear localization signal (NLS) in the last 74 C-terminal residues consisting of two identical basic clusters (KKR) separated by 29 amino acids [180,181] (Figure 10A). The NLS is recognized by the classical importin-α receptor and is absolutely required for nuclear localization of Ty1 IN and retrotransposition but not for VLP production, protein processing, reverse transcription and *in vitro* IN activities [180-182]. IN also interacts closely with RT during reverse transcription and contributes to the proper folding and active conformation of RT *in vivo* [141].

IV. Regulation of Ty1 retrotransposition

A variety of mechanisms that limit the potentially deleterious effects of retrotransposition on the host genome have been described. These fall into two major categories: limiting the frequency of retrotransposition and confining integration events to specific regions of the genome that are gene-poor. A common theme that emerges from analysis of several types of host-mediated control of the frequency of Ty1 retrotransposition is the regulation of reverse transcriptase activity.

A. Control of retromobility by Ty1 copy number

Transcriptional co-suppression—All examined *S. cerevisiae* strains contain no more than 40 full-length Ty1 elements [23,183]. This copy number limit is not likely to be the consequence of a growth disadvantage conferred on cells harboring more Ty1 copies, since a two to ten-fold increase in the number of Ty1 elements, obtained experimentally, does not alter the growth rate, except when strains are challenged with DNA damaging agents [184]. Two mechanisms that limit retrotransposition in strains with a high Ty1 copy number have been described. One type, called transcriptional co-suppression, was detected using Ty1(*GAG:URA3*) reporter constructs and 5-fluorotic acid counterselection, and is characterized by transient and rapid switches between Ura^+ and Ura^- states, in which all Ty1 elements are transcribed or silenced within a cell population, respectively [185]. Co-suppression requires Ty1 elements in high copy number and actively transcribed from the native promoter. In contrast to this system that depends on counterselection to identify cells in which Ty1 RNA expression is suppressed, analysis of the expression of a Ty1(*GAG:GFP*) reporter construct in single cells by flow cytometry failed to detect a population of cells lacking expression of Gag-GFP [168]. Thus, Ty1 transcriptional co-suppression may be strain or marker-specific.

Transcriptional co-suppression of Ty1 elements may be mediated by Ty1AS RNA [68]. In different studies, stabilizing Ty1AS RNA by deleting *XRN1* decreased the level of Ty1 RNA

to about 20% to 60% of that in a congenic wild-type strain[68,70,71]. Notably, an internally initiated Ty1 sense-strand transcript identical in size to a 4.9 kb transcript previously reported in the *spt3*Δ mutant was detected in the *xrn1*Δ mutant [68,186]. Expression of Ty1AS RNA from the *GAL1* promoter silenced the expression of a Ty1(*GAG::URA3*) reporter in *trans*, which is consistent with a potential role for Ty1AS RNA in co-suppression. Interestingly, truncated Ty1AS RNA species lacking either U3-R, U5 or *GAG* sequences did not repress Ty1 transcription in *trans*, revealing that the integrity of Ty1AS RNA is critical for transcriptional repression. Although *S. cerevisiae* lack RNAi, introduction of Dicer and Argonaute proteins from *Saccharomyces castellii* results in strong repression of Ty1 sense-strand RNA expression and retrotransposition, suggesting that Ty1 RNA and Ty1AS RNA may form double-stranded RNAs that trigger RNAi-mediated repression [187].

Post-transcriptional copy number control—Using a strain of the related species, *Saccharomyces paradoxus* that lacks full-length Ty1 elements, Garfinkel *et al.* [32] discovered a robust post-transcriptional form of Ty1 copy number control (CNC). This regulatory system results in repression of retrotransposition of a chromosomal RIG-marked Ty1 element when unmarked Ty1 elements are present in the same cell, with the extent of repression being roughly proportional to the copy number of Ty1 elements. The level of total Ty1 RNA increases with Ty1 copy number, demonstrating that repression occurs at a post-transcriptional step. A minimal segment of Ty1-H3 spanning nucleotides 238 to 1702 is sufficient to confer post-transcriptional CNC, although inclusion of the 3′ LTR enhances CNC activity of this fragment. Deletion of nucleotides 238 to 281 or nucleotides 1600 to 1702 abolishes the repressing activity of this minimal construct [32,33]. A high copy number of Ty2 but not Ty3 or Ty5 confers CNC on Ty1, which is consistent with a homology-dependent mechanism. Ty1AS transcripts map to the minimal region required for CNC, and the strength of CNC is correlated with the abundance of Ty1AS RNA [33]. Nevertheless, ectopic expression of two different Ty1AS RNA species from the *GAL1* promoter did not recapitulate the level of repression observed when Ty1AS RNA is expressed from its own undefined promoter. The failure of Ty1AS RNA to act in trans is not consistent with a causative role in CNC.

The mechanism of CNC-mediated repression of retrotransposition was investigated by constructing strains with an integrated *GAL1*-driven Ty1 element, which promotes the formation of VLPs, and either a high copy number of Ty1 elements whose sense-strand transcript was abolished by the *spt3*Δ mutation, and thus high levels of Ty1AS RNA (CNC^+) or only one endogenous Ty1 element and low levels of Ty1AS RNA (CNC^-). VLP fractions from CNC^+ strains were enriched for Ty1AS RNA and had reduced levels of Gag and RT and barely detectable levels of IN [33]. CNC^+ VLPs had reverse transcriptase activity on an exogenous primer-template added to VLPs, but no cDNA was synthesized from the endogenous primer and template within CNC^+ VLPs. Moreover, $tRNA_i^{Met}$ binding to Ty1 RNA in CNC^+ VLPs was robust, but no minus-strand strong-stop cDNA was detected.

SHAPE analysis of the overall structure of the minimal region required for CNC in Ty1 RNA extracted from VLPs (referred to as "*in virio*") revealed similar secondary structures in the RNA from CNC^+ and CNC^- samples, and provided no evidence for extensive annealing

between Ty1 RNA and Ty1AS RNA *in virio* in CNC^+ strains [74]. The major difference between CNC^+ and CNC^- RNA samples was that the nucleotide sequence 5′-AUGAUGA-3′, present at nucleotide positions 321-327, 694-700 and 1406-1412, was more accessible to 2′-hydroxyl acylation in CNC^+ RNA. The same regions also became more accessible in CNC^- RNA when the VLPs were gently stripped of protein before the 2′-hydroxyl acylation reactions were performed (*ex virio*). These findings suggest that Gag binds these regions in CNC^- RNA and that disruption of Gag binding in CNC^+ RNA blocks steps in the initiation of cDNA synthesis, perhaps by interfering with requisite chaperone activities of Gag [74]. The mechanism of CNC-mediated repression of Ty1 cDNA synthesis, while correlated with high levels of Ty1AS RNA and specific alterations in the structure of Ty1 RNA *in virio*, remains to be elucidated.

CNC and transpositional dormancy—CNC is likely to be the major factor responsible for transpositional dormancy of Ty1 elements in laboratory strains. This view is consistent with the observation that a single RIG-marked Ty1 element mobilizes at a much higher rate in the absence of other elements than it does in the presence of multiple unmarked elements, even though the level of RIG-marked Ty1 RNA remains constant [32]. Moreover, both CNC and transpositional dormancy are overridden at a posttranscriptional level by expression of a pGTy1 element [32,72]. Expression of a pGTy1 substantially increases the levels of processed IN and RT and cDNA, and retrotransposition is elevated as much as 100-fold per Ty1 transcript, which is consistent with the idea that CNC is negated [72]. Deletion of many host repressors of Ty1 retrotransposition has been demonstrated to stimulate a post-translational step in retrotransposition, and therefore it is possible that the absence of these factors counteracts CNC as well. For example, deletion of *RTT101*, which causes activation of the DNA damage checkpoint, results in increased levels of p63-RT and p71-IN and cDNA, precisely the intermediates that are suppressed by CNC [162]. Deletion of *FUS3*, which triggers the activation of the invasive-growth signaling pathway, causes similar if not identical alterations in Ty1 replication [166]. This hypothesis implies that the activation state of the DNA damage checkpoint pathway or invasive growth pathway directly impacts the potency of CNC. Finally, a common mechanism of release from CNC-mediated repression in host mutants and in cells expressing pGTy1 would explain why deletion of many host repressors including Rtt101 and Fus3 only stimulates the retrotransposition of LTR-driven endogenous Ty1 elements and not that of pGTy1.

B. Host-retrotransposon interaction

S. cerevisiae is an unparalleled system for characterizing the role of the eukaryotic host in the mobility of LTR-retrotransposons and retroviruses (reviewed in [188]). The major reasons are the ease of connecting genotype and phenotype, the availability of simple genetic assays for retromobility, and the extensive variety of genomic tools available in yeast. A plethora of host factors required to maintain transpositional dormancy (known as restrictors of Ty1 transposition and encoded by *RTT* genes) and host factors that promote efficient Ty1 retromobility (known as retromobility host factors and encoded by *RHF* genes) have been identified. *RTT* genes were initially identified by screening for mutants with increased levels of retromobility of a chromosomal Ty1*his3AI* element [160,164,166] (Figure 3). Because retromobility of a RIG-marked chromosomal Ty1 in a wild-type strain

is detected in only about 1 in 10^7 cells, this strategy only identified mutants with elevated retromobility. Subsequent studies used various approaches to increase retrotransposition of genetically marked Ty1 elements so that hypotransposition mutants could be found. For example, Griffith et al. [151] introduced a plasmid-borne pGTy1*HIS3* element into each of the ~5000 homozygous diploid non-essential ORF deletion strains, and screened for those with decreased retromobility of Ty1*HIS3* into the genome. 99 non-essential *RHF* genes were identified. Another screen employed an integrating plasmid-based Ty1*his3AI* element introduced into the haploid ORF deletion strain collection. While a single copy of this plasmid-based element is presumably integrated into the genome, retromobility of the integrating plasmid-based Ty1*his3AI* element is significantly higher than that of a chromosomal Ty1*his3AI* element for reasons that are not understood. This screen identified 168 non-essential *RHF* genes and 91 *RTT* genes [78,161]. Most recently, iterative synthetic genetic array screens in which two different *rtt* mutations were used to increase the mobility of a chromosomal Ty1*his3AI* element were performed. A chromosomal Ty1*his3AI* element and one of two *rtt* mutations was introduced into the haploid ORF deletion collection. Screens for ORF deletions that suppressed the hypermobility phenotype of either *rtt* mutation resulted in the identification of 275 non-essential *RHF* genes [163]. The large number of host factors that influence retrotransposition is consistent with the fact that LTR-retrotransposons and retroviruses have a limited coding capacity and complex replication cycle (Figure 2), and therefore substantial host participation in replication is necessary.

More than 200 *RTT* and *RHF* genes have been verified by at least one of the following criteria: isolation in two independent screens; isolation in one screen and confirmation in a secondary screen for changes in the level of a retrotransposition intermediate, such as Gag or cDNA, that mirror the changes in retrotransposition; or characterization of effects on retromobility frequency and retrotransposition intermediates, irrespective of isolation in a genetic screen (Table 1). A few of these verified genes have been identified as *RTT* genes in one screen and *RHF* genes in another. The reason for this is not understood, but the observation underscores the variety of methodologies employed in individual screens and suggests that screen design influences the subset of host factors that are identified.

C. Regulation by genome integrity

Activation of retrotransposition by DNA lesions—Ty1 is an exquisitely sensitive sensor of the integrity of the genome. The majority of host restriction factors encoded by *RTT* genes are involved in different aspects of host genome maintenance [160-162,164,168,169,188-191]. These genome caretakers do not act directly to repress retrotransposition; rather, their absence results in DNA lesions that activate DNA damage signaling pathways that in turn induce Ty1 retrotransposition.

An example of this class of host co-factors is telomerase, an enzyme complex required for the lengthening of simple repeats at ends of chromosomes known as telomeres. The telomerase holoenzyme consists of a reverse transcriptase, Est2, associated with an RNA template, *TLC1* RNA. Telomerase adds telomeric DNA repeats to chromosome ends using *TLC1* RNA as template. In the absence of either telomerase component, telomeres become shorter with every generation because DNA polymerase cannot synthesize to the 5′ end of

the DNA template. Telomere shortening has no major effect on cell growth or Ty1 retrotransposition until about 60 to 80 generations after either subunit of telomerase is rendered dysfunctional. When the telomere reaches a critically short length, it is recognized as a double-strand break, triggering damage signals that in turn result in progressive loss of growth, or senescence [192,193]. In the senescence phase, the levels of Ty1 cDNA and retrotransposition increase in parallel with the shortening of telomeres and gradual loss of cell viability [168]. A fraction of yeast cells temporarily recover from senescence by invoking an alternative method of telomere lengthening. As survivors with elongated telomeres restore the viability of the culture, retrotransposition and cDNA are concomitantly reduced. The inverse correlation between retrotransposition levels and the length of telomeres and viability of the culture demonstrates that DNA lesions created by the absence of telomerase, rather than the absence of telomerase per se, activates Ty1 retrotransposition.

Spontaneous DNA lesions occurring in S-phase in the absence of genome caretakers stimulate Ty1 retrotransposition completely or partially through two S-phase specific checkpoint pathways [162]. The components of these checkpoint pathways are not needed for the basal level of retromobility in wild-type strains, but are required to induce retromobility in genome caretaker mutants. In the absence of recombinational repair genes (e.g. *RAD50*, *MRE11*, *XRS2*, *RAD51*, *RAD52* and *RAD55*), or the *RTT101* gene, which encodes a cullin involved in replication fork progression, activation of retrotransposition is Rad9-dependent and thus, occurs primarily through the DNA damage checkpoint pathway. The absence of other genome caretakers, notably Elg1, a component of a replication factor C complex, results in Rad9-independent, Rad53-dependent hypermobility, indicating that the replication stress pathway induces hypermobility in these mutants. In a third class of mutants that includes *rad27*Δ, *rmm3*Δ and *rtt109*Δ, mobility is activated partially through one of these S-phase checkpoint pathways and partially by independent mechanisms, as discussed below.

Hypermobility of Ty1 in the absence of genome caretakers is usually accompanied by an increase in the level of Ty1 cDNA but not Ty1 RNA or Gag protein. The target of the S-phase checkpoint pathways has not been identified, but it is likely to be encoded by a domain of Ty1 that is divergent in Ty2, since Ty2 is not mobilized by these pathways. When the DNA damage checkpoint pathway was stimulated by deletion of *RTT101*, VLPs resulting from pGTy1 expression had elevated levels of p63-RT and p71-IN, and 20-fold higher levels of reverse transcriptase activity on an exogenous template relative to an *RTT101* strain [162]. Notably, these increases were not observed when a pGTy1 lacking a domain encoding 30-amino acids of the N-terminus of p20-PR was expressed in the presence of the *rtt101*Δ mutation [162]. This domain is divergent between Ty1 and Ty2 and has nucleocapsid function required for the initial steps in reverse transcription [158,194]. As such, increased pRT-63 and p71-IN and RT activity may be downstream effects of stimulating the nucleocapsid function of p20-PR, or another unknown function of this region within the Gag-Pol precursor protein.

Exposure of yeast cells to various DNA-damaging agents, such as UV light, γ-rays, 4-nitroquinoline-1-oxide (4-NQO) and methylmethane sulfonate (MMS), increases the level of Ty1 RNA and retrotransposition [38,40,41,195-197]. Treatment with γ-rays or 4-NQO does

not increase the level of Gag protein [38,41], suggesting that DNA lesions that result from treatment with mutagens stimulate a posttranslational step in retrotransposition, like those generated in the absence of genome caretakers.

Repression of multimeric cDNA formation—Sgs1, Rrm3 and Rad27 are genome caretakers that suppress Ty1 retromobility by inhibiting the introduction of multiple Ty1 cDNAs as tandem arrays, or concatenates, into the host genome. Rad27, also known as flap-endonuclease 1 (FEN1), is a structure-specific nuclease that degrades a 5′ single-stranded flap that is generated during DNA replication of the lagging strand, as well as in base excision repair [198]. Rad27/FEN1 plays critical roles in genome stability. Functional defects in Rad27 result in large increases in Ty1 retromobility that are only partially mediated via S-phase checkpoint pathways [162]. In *rad27*Δ mutants, concatenates of cDNA are introduced into the genome at a high frequency [169]. These concatenates presumably form by recombination between the 3′ LTR of one cDNA molecule and the 5′ LTR of another cDNA molecule, resulting in joints with a single LTR. Homologous recombination between cDNA and genomic Ty1 sequences is also substantially increased in the *rad27*Δ mutant [169], indicating that cDNA concatenates may enter the genome by integration or recombination. The phenotype of mutants lacking Sgs1, a nucleolar RecQ-family DNA helicase, or Rrm3, a Pif1-family helicase is distinct. In these mutants, increased incorporation of Ty1 cDNA into the genome is strongly dependent on the homologous recombination factor Rad52, but recombination between cDNA and chromosomal Ty1 elements remains at its normally low level [5,190,191]. Instead, recombination generates concatenates of cDNAs that are inserted into the genome at typical Ty1 integration sites. Thus, the involvement of multiple cDNAs in each integration event explains the increased retromobility of Ty1 cDNA in *sgs1*Δ and *rrm3*Δ mutants. Multimeric Ty1 integration events are associated with aberrantly sized chromosomes in *rrm3*Δ mutants, suggesting that cDNA multimers are large enough to change the migration of chromosomes in pulse-field gels, or that their formation is associated with chromosomal rearrangements. Remarkably the formation of multimeric insertions in an *rrm3*Δ strain, but not a wild-type or *sgs1*Δ strain, is suppressed by overexpression of RNase H. This finding supports a model in which newly inserted Ty1 cDNA is broken within an RNA:DNA hybrid region, and the resulting break in Ty1 is repaired by homology-driven incorporation of multiple cDNAs [191]. RNA:DNA hybrids may be present in the newly integrated Ty1 cDNA because of incomplete plus strand cDNA synthesis [143,199].

Cell cycle restriction of retromobility—There is some data to suggest that Ty1 retrotransposition is restricted to certain stages of the cell cycle. Treatment of cells with 25 to 100 mM hydroxyurea, which results in nucleotide depletion, replication fork pausing and delayed progression of S phase, dramatically increases Ty1 cDNA synthesis and retrotransposition [162]. In contrast, transient cell-cycle arrest at G2/M by nocodazole has no effect on retrotransposition, and arrest of the cell cycle in G1 by mating pheromone strongly reduces retrotransposition of a pGTy1 element [200]. Treatment of cells with mating pheromone results in a post-translational block in Ty1 retrotransposition. VLPs prepared from mating pheromone treated cells have very low levels of reverse transcriptase activity both *in vivo* and *in vitro*. Together, these data are consistent with the model that

retrotransposition is potentially restricted to S phase, or to the S and G2 phases of the cell cycle.

Three observations are consistent with the idea that a feature of the DNA replication fork may be recognized as a target for integration of Ty1 cDNA. First, Ty1 cDNA autointegration events are preferentially targeted to the region near the central PPT, where a DNA flap structure resembling a replication fork forms [148]. Second, genes transcribed by RNA Polymerase III (Pol III) are not only hotspots for integration of Ty1 cDNA but also barriers to replication fork progression [201-203]. Third, the increase in multimeric Ty1 integration events in the absence of the Rrm3 helicase, which travels with the replication fork, suggests that integration might occur just prior to passage of the replication fork [191]. It is tempting to speculate that replication pausing at Pol III-transcribed genes influences the efficiency of Ty1 targeting, although this hypothesis remains to be tested.

D. Integration Targeting

A wealth of evidence supports the idea that the host cell strictly controls not only Ty1 mobility but also target site selectivity with the consequence of limiting deleterious mutations and promoting repair of double-strand breaks. In the yeast genome, most Ty1 and Ty2 elements and solo LTRs reside upstream of genes transcribed by Pol III, in a window that generally begins ~80 bp upstream and extends over several hundred base pairs [24,204,205]. Regions harboring tRNA genes, which comprise the major class of Pol III-transcribed genes, are generally devoid of protein coding genes; consequently, Ty1 integration into these regions is thought to minimize negative impacts on host cell growth and survival [206,207]. In addition, regions harboring tRNA genes are sites of replication pausing, chromosome fragility, and Ty1-mediated chromosomal rearrangement [201-203,208,209]. The presence of Ty1 sequences at these fragile sites likely facilitates repair of deleterious double-strand breaks by recombination with non-allelic Ty1 elements and therefore is likely to promote host cell survival in conditions of replication stress [209].

Characterization of *de novo* integration events on chromosome III provided the first indication that the chromosomal position of Ty1 is due to a non-random integration pattern and not to selection [210]. Ty1 integrates preferentially within a 700 bp window upstream of transcriptionally active RNA Pol III-transcribed genes [210,211]. An extensive analysis of several hundreds of *de novo* insertions events at $tRNA^{Gly}$ and $tRNA^{Thr}$ gene families uncovered a periodic insertion pattern upstream of tRNA genes, with approximately 80 bp between insertion peaks. Interestingly, the frequency of integration upstream of individual tRNA genes within the same family was highly variable, suggesting that local features near the tRNA genes also influence targeting [212]. Two recent studies that employed deep-sequencing approaches to identify Ty1 integration sites showed that the upstream region of virtually all RNA Pol III-transcribed genes, including tRNA genes, *SNR6, RPR1, SNR52, SCR1* and the repeated *RDN5* loci, were targets for Ty1 integration [5,213]. Two loci, *RNA170* and *ZOD1*, which recruit incomplete Pol III transcription complexes [214,215], were not targeted. These studies confirmed that there is significant variation in the frequency of insertion at RNA Pol-III transcribed genes, although genomic features specifically associated with hot or cold targets were not identified. Insertions into ORFs were

significantly under-represented [5,213]. Importantly, the same insertion profiles were observed in diploid and haploid cells [5], demonstrating that avoidance of ORFs is not due to selection.

Deep sequencing further revealed that Ty1 integrates into nucleosomal DNA, with the first three nucleosomes upstream of Pol III genes being the hottest targets. Two integration hotspots per nucleosomal DNA separated by about 70 bp and located near the H2A/H2B interface were identified (Figure 10B). The unexpected asymmetry in the Ty1 insertion sites relative to the nucleosome dyad axis suggests that a dynamic process of nucleosome remodeling exposes a specific H2A/H2B surface permissive to Ty1 integration. Mutation of the ATP-dependent chromatin remodeling factor Isw2 alters nucleosome positioning and Ty1 integration periodicity upstream of tRNA genes, suggesting that periodic integration is at least partially dependent on a specific chromatin structure imposed by the Isw2 complex [216]. The Bdp1 subunit of the Pol III transcription factor TFIIIB is important to target Isw2 to tRNA genes and consequently influences the periodicity of Ty1 integration [217].

The mechanism underlying targeting of Ty1 integration to Pol III genes has been the subject of intense interest. Two histone deacetylases of the Set3C complex, Hos2 and Set3, enhance the efficiency of Ty1 integration upstream of Pol III-transcribed genes, but neither is sufficient to designate a Pol III-transcribed gene as a hotspot of integration [153]. Ty1 integration into ORFs is increased in *rrm3*Δ, *rtt109*Δ and *rad6*Δ mutants [5,161,191,218], but there is no change in the pattern of integration upstream of Pol III-transcribed genes [5]. A physical interaction between Ty1 integrase and a subunit of Pol III was discovered recently, and several lines of evidence indicate that this interaction is essential for the targeting of Ty1 to the upstream region of Pol III-transcribed genes (A. Bridier-Nahmias, A. Tchalikian-Cosson and P. Lesage, unpublished data). It remains to be determined how the interaction between Ty1 IN and Pol III facilitates integration into nucleosomal regions upstream of Pol III-transcribed genes.

V. Impact of ty1 on the yeast genome

A. Regulation of adjacent gene transcription by stress

The yeast Ty1 element is arguably the best model to understand the activation of LTR-retrotransposons by environmental stress and the impact of this activation on adjacent gene expression [67,219]. In diploid cells, nitrogen starvation activates the Ty1 promoter through the invasive/filamentous signaling pathway by a mechanism involving the transcription factors Ste12 and Tec1 that bind Ty1 promoter sequences [34]. In haploid cells, Ty1 transcription increases in adenine-deprived *bas1*Δ cells defective in *de novo*-AMP biosynthesis (a condition known as severe adenine starvation [35]). The low levels of ATP and ADP in these cells suggest that a decrease in adenylic nucleotides could be a signal for activation of Ty1 transcription [58]. Although the mechanism of promoter activation has not yet been solved, it involves Snf2-dependent chromatin remodeling in the Ty1 promoter region and primarily affects weakly transcribed Ty1 elements [35]. Transcriptional activation by severe adenine starvation also requires the 5′ LTR and a motif in the *GAG* ORF that is recognized by the Tye7 transcription factor [46,58]. Notably, severe adenine

starvation represses transcription of the major Ty1AS RNA species in a Tye7-dependent manner [58].

Elevated Ty1 cDNA levels and a 15-fold increase in retrotransposition of Ty1 elements that are normally weakly expressed accompany the increase in Ty1 RNA in severe adenine starvation [35]; however, the level of Gag protein is not altered (A.L.Todeschini and P. Lesage, unpublished data), suggesting that induction of Ty1 expression may not be the primary mechanism of stimulating retrotransposition. Alternatively, Ty1 retrotransposition may be stimulated by derepression of CNC or Ty1AS RNA-mediated control, as may also be the case with activation of retrotransposition via other environmental stress pathways [36,162]. Thus, the major significance of Ty1 promoter activation in severe adenine starvation could be its resulting effect on the transcription of adjacent genes. Although Ty1 insertions have modest effects, if any, on transcription of adjacent Pol III-transcribed genes [220], they can have substantial effects on expression of adjacent Pol II transcribed-genes, as reviewed in detail previously [35,219]. Briefly, integration of Ty1 upstream of an ORF and in the opposing orientation situates the Ty1 promoter near the 5′ end of the adjacent ORF and places expression of that ORF under the control of environmental signaling pathways that regulate Ty1 transcription. Indeed, transcription of ORFs from cryptic sites within the 5′ LTR of an adjacent Ty1 is stimulated in response to severe adenine deprivation [46]. The transcriptional regulator, Tye7 is required for Ty1-dependent activation of adjacent target genes [58]. Given that Tye7 also controls Ty1AS RNA transcription, it is possible that Ty1AS RNA directly influences the regulation of genes adjacent to Ty1 insertions.

Ty1 can exert its regulatory effect at a relatively long distance. For example, severe adenine starvation activates the *ESF1* gene, which is located 320 bp away from a full length Ty1 element in the yeast genome [58]. A consequence of activating Ty1 transcription by stress could be the reprogramming of the yeast transcriptome by modulating the expression of coding or non-coding sequences that are adjacent to endogenous Ty1 sequences, as proposed by Servant et al. [46].

B. Contribution to genome plasticity

Ty1-mediated repair of double-strand breaks (DSBs)—Ty1 elements in yeast, like repetitive elements in many organisms including humans, mediate many types of simple and complex chromosomal rearrangements, including deletions, segmental duplications, inversions and reciprocal and non-reciprocal translocations [221-231]. Remarkably, the introduction of a single Ty1 element near the non-essential terminus of the left arm of chromosome V in haploid cells results in a 380-fold increase in gross chromosomal rearrangements (GCRs) with breakpoints near the introduced Ty1 element [221]. The formation of GCRs at Ty1 elements is primarily a result of DSB repair within or near the Ty1 element, mainly by *RAD52*-mediated homologous recombination between non-allelic Ty1 elements [221,232]. In haploid cells, Ty1-mediated repair results in a remarkably high rate of cells that survive DSB formation [221,232,233]. In diploid cells, where homologous recombination (HR) is highly favored over repair through non-homologous end-joining (NHEJ), nearly all repair of DSBs leading to chromosomal aberrations results from recombination among non-allelic Ty1 sequences [230,232]. Notably, multiple Ty1 loci can

be involved in rearrangement-associated recombination events, suggesting that both ends of the DSB may be able to find homology with different Ty1 loci [221]. Alternatively, Ty1 cDNA or tandem cDNA arrays may be incorporated at sites of Ty1-mediated DSB repair [230]. By activating DNA damage checkpoint pathways, DSBs and other DNA lesions can trigger the synthesis of Ty1 cDNA, which in turn can be captured at the sites of DNA damage [122,162,168,191,234]. This capture can be initiated by retrotransposition or repair of DSBs in Ty1 elements or resected back to nearby Ty1 elements [122,191,234].

When homologous recombination is blocked, a small fraction of DSB repair events (1%) involves the capture of Ty cDNA fragments by NHEJ [235-238]. In some cases, the portion of Ty1 cDNA found at the breakpoint junction corresponds to minus-strand strong stop cDNA and few bases that are part of the tRNA primer, suggesting the possibility of formation of a RNA:DNA hybrid during the integration process. Capture of longer Ty1 fragments has also been described and requires RT but not IN [236]. Ty1 cDNA capture may have occurred several times during *S. cerevisiae* evolution [235].

Ty1 as an initiator of DSBs—In addition to being sites of ectopic homologous recombination, Ty1 elements can themselves be fragile sites where chromosomes tend to acquire DSBs. At least two types of fragility are associated with Ty1 sequences. First, two adjacent inverted Ty1 elements can induce DSB formation, probably because of their capacity to form a long hairpin structure in the lagging strand [239]. Fragility at inverted Ty1 elements is greatly enhanced in mutants with a low level of DNA polymerase, which retards replication fork progression. Fragile sites containing inverted Ty1 elements are naturally occurring in the yeast genome, and these sites are frequently at the breakpoints of GCRs [239,240]. A second mechanism is through the formation of R-loops, which are regions of the genome in which one strand of the DNA duplex is hybridized to RNA. Formation of RNA:DNA hybrids is associated with genome instability and replication stress across many species, likely because its presence results in DNA replication fork stalling [241,242]. Genome-wide profiling has shown that retrotransposon Ty1 and Ty2 sequences are greatly enriched in R loop regions [199]. The same study found a strong correlation between regions of RNA:DNA hybrid formation and antisense transcription, suggesting that transcription of Ty1AS RNA contributes to R-loop formation in Ty1 sequences and may play a role in the formation of DSBs and spontaneous chromosomal aberrations at Ty1 elements.

Despite the well-established role of Ty1 in DSB repair by homologous recombination, Ty1 elements function as repressors of recombination at meiotic hotpots. This repression is a consequence of the compact chromatin structure of Ty1 and requires the first 4 kb of Ty1 sequence [243].

Consequences of Ty1 activity for genome plasticity, adaptation and longevity —Both in the wild and in laboratory evolution experiments, yeasts adapt readily to a variety of environmental stresses that limit their growth, including nutrient deprivation and exposure to toxic compounds [178,222,227,234,244-247]. Rapidly evolving adaptive phenotypes are often the result of duplicated chromosomal fragments that arise at high frequency and alter expression of multiple genes [222,227,234,244,246-248]. The formation

of segmental duplications by ectopic recombination between Ty1 elements is a common mechanism of creating mutations that answer selective pressures [222,227,234,244,249]. Adaptive chromosomal duplications can have negative consequences for cells when they are grown in the absence of selective pressure, probably because of the expression changes in large numbers of genes [250,251]. Therefore, chromosomal duplications that can be rapidly reversed when the stress is relieved or when a more finely tuned mutation arises may be optimal for adaptation to rapidly changing environments [222,244]. Ty1-mediated chromosomal duplications are flanked on both sides by a Ty1 element or array, or bounded on one side by Ty1 elements and on the other by the telomere. They are highly dynamic and reversible by ectopic recombination between the Ty1 elements that flank the duplication [244]. Thus, the presence of Ty1 elements within intergenic regions that are also fragile sites provides a dynamic mechanism by which cells can rapidly generate repeatable and reversible adaptive chromosomal duplications without significant insult to protein coding genes.

Not only the presence of Ty1 in the genome, but also the mobility of Ty1 cDNA is likely to influence genome plasticity and adaptation. Retrotransposition events are commonly seen in strains that emerge from short-term evolution experiments. Ty1 insertion mutations, although often neutral or deleterious, can result in positive fitness effects by contributing to GCR formation or by modifying the expression of adjacent genes [183,206,234]. The impact of Ty1 retrotransposition on genome plasticity has been observed during chronological aging in yeast. Ty1 retrotransposition is substantially increased in very old cells, and the resulting Ty1 insertions are frequently found on aberrant sized chromosomes. Moreover, aging-associated loss of heterozygosity and chromosome loss are consistently diminished by treatments or mutations that reduce Ty1 retrotransposition [252]. These findings support the argument that Ty1 retrotransposition is a significant cause of the increased chromosomal instability that is characteristic of aging. Perhaps retrotransposition in aging cells is a response to stresses associated with chronological aging. In fact, the presence of multiple Ty1 elements in the genome can promote longevity of yeast [253]. Under certain growth conditions, strains of *Saccharomyces paradoxus* containing approximately 20 *S. cerevisiae* Ty1 elements have a longer chronological life span than a congenic strain lacking Ty1 elements. Ty1 copy number-mediated longevity is correlated with reduced levels of reactive oxygen species, and accordingly, is phenocopied by treatment of the strain lacking Ty1 with antioxidants, which reduces reactive oxygen species. These findings suggest that the presence or expression of Ty1 retrotransposons can alter the level of reactive oxygen species. The relationship between Ty1 and reactive oxygen species is likely to be complex, as chemicals that increase reactive oxygen species are potent inducers of Ty1 retrotransposition [254]. The longevity-promoting role of Ty1 elements, albeit context-dependent, is a unique example of a beneficial function associated with the presence of retrotransposons in a eukaryotic genome.

Retrosequence formation—Occasionally, cellular mRNAs are used as templates to synthesize cDNAs that are introduced into the host genome, a process known as retrosequence or processed pseudogene formation. Retrosequence formation has been detected in yeast by expressing the *his3AI* RIG from the strong *GAL1* promoter or by

embedding *his3AI* into the 3′ UTR of a cellular mRNA [121,122,255,256]. Reverse transcription-dependent duplication of the ATCase domain of the *URA2* gene has been detected by positive selection for an expressed *URA2* retrosequence [257,258]. Retrosequence formation is initiated by reverse transcription of the cellular mRNA within Ty1 VLPs, using Ty1 cDNA as a primer to initiate synthesis at the 3′ poly(A) tail of the mRNA [121-123,258]. Most often the poly(A) tail sequence is joined to the end of a complete LTR, suggesting that RT can use the 3′ OH end of a complete Ty1 cDNA as a primer, even when there is no homology with the mRNA template [121,122]. Overexpressing Ty1 RNA stimulates retrosequence formation, as does stimulating Ty1 RT activity posttranslationally by deleting either subunit of telomerase [121,122,203,258]. In telomerase-negative cells, cDNA of highly expressed genes joined to Ty1 LTR sequences, detected by PCR, is dramatically increased as telomeres erode [121,122]. The cDNA of cellular mRNAs can be introduced into the genome by three major mechanisms. First, gene conversion of mRNA-encoding genes by their homologous cDNAs replaces part of the original gene with an intronless copy [122,123,255,256]. These gene conversion events, which do not retain the poly(A) stretch or flanking Ty1 sequences, occur only in strains expressing Rad52 [122,123,255]. Gene conversion of protein-coding genes by homologous cDNA has been proposed to explain the dearth of introns in *S. cerevisiae* [259]. Second, chimeric retrosequence-Ty1 cDNA can undergo Rad52-dependent homologous recombination with genomic Ty1 elements at sites of Ty1-mediated DSB repair [188,258]. This mechanism results in the introduction of retrosequences that are flanked on both sides by Ty1 sequences. Ty1 cDNA sequences upstream of cellular retrosequences are usually at junctions with microhomology, suggesting that the upstream Ty1-retrosequence junction is created by template switching during plus-strand cDNA synthesis. Chimeric retrosequences, probably formed by RT switching between one cellular mRNA template and another, are frequently observed. Also commonly observed are large regions of Ty1 sequence, possibly Ty1 cDNA arrays, flanking the retrosequence on one or both sides [188]. When Ty1 promoter sequences are present upstream of the retrosequence, they can contribute to its expression [258]. Ty1-flanked retrosequences are introduced at the breakpoints of chromosomal rearrangements, suggesting that the retrosequences and flanking Ty1 cDNA are incorporated as “molecular band-aids” to facilitate DSB repair [122]. Finally, retrosequences can enter the genome via rare *RAD52*-independent events such as non-homologous end joining or Ty1 IN-mediated integration [122,255].

The mRNA of the subtelomeric repeat element, Y′, is selectively packaged within Ty1 VLPs by an unknown mechanism. Thus, even though Y′ RNA is not particularly abundant, chimeric Y′-Ty1 cDNA can be detected by PCR and, like other retrosequence cDNAs, increases substantially as telomeres erode in telomerase-negative cells. Y′-Ty1 cDNA reaches levels as high as 10^{-2}-10^{-4} copies per genome in the absence of telomerase [121], which are high enough for this cDNA to play a major role in extending telomeres. Y′ retrosequences are typically inserted at chromosome ends by homologous recombination between a subtelomeric Y′ element and Y′-Ty1 cDNA. This mechanism places Ty1 cDNA sequences at the unprotected chromosome end, and therefore the ends frequently undergo ectopic recombination with internal Ty1 elements throughout the genome, resulting in a very high incidence of chromosomal rearrangements [121,123].

VI. Perspectives

From the time of their discovery as retrotransposons in 1985, Ty elements have been at the forefront of research that has elucidated fundamental mechanisms of retrotransposition and the impact of retrotransposons on their eukaryotic hosts. Since the publication of Mobile DNA II nearly 15 years ago, the trajectory of discoveries in Ty1 and retrotransposon biology has been shaped by the major advances of the post-genomic era, particularly DNA microarray analysis, high throughput sequencing and functional genomics tools, especially in yeast [249]. Studies have illuminated the role that Ty1 elements and the expression of Ty1 elements play in the rapid and reversible formation of chromosomal rearrangements in response to selective pressures. This type of abrupt large-scale change is similar to chromosomal aberrations that are often associated with human cancers [260,261]. As our understanding of Ty1-mediated mutations expands, we increasingly uncover examples of recurring mutations that are tailored to the stress by which they are evoked, such as the induction of Ty1 RT-mediated synthesis of chimeric cDNA that elongates telomeres when telomerase is inactivated [121]. These findings underscore the importance of retrotransposons in shaping the cell's response to stress. Looking ahead, we can expect the increasing use of Ty1 as a model to understand whether retrotransposons are drivers or passengers in the retrotransposon-related genomic alterations that accompany aging, neurodegenerative disorders and the formation of tumors.

Other advances in the post-genomic era include the discovery of Ty1AS RNA and its correlation with copy number-mediated silencing of Ty1 elements [33,68]. This system has many parallels to RNA interference, which does not occur in *S. cerevisiae* [187]. Future studies are likely to define the mechanism by which Ty1AS RNA mediates changes in the levels and functions of VLP-associated proteins. Addressing these questions will provide clues to the evolution of RNA interference and the roles that non-coding RNAs play in modulating gene expression. Recent studies have also begun to reveal the secondary structure of Ty1 RNA and its functional significance [20,74]. In the future, structure-function analysis will address a fundamental question in retrotransposon and retrovirus biology: how does the RNA function as both an mRNA and a gRNA?

A striking advance in retrotransposon biology over the last 15 years is the identification of hundreds of host factors that positively or negatively influence the rate of Ty1 retrotransposition. Evolutionarily conserved genes with functions in DNA replication and repair, histone modification, chromatin remodeling, transcription and protein modifications were initially identified as RTT factors, illustrating the axiomatic contributions of Ty1 research to the discovery of fundamental host processes. Most retrotransposition host factors are conserved in eukaryotes and many, including mRNA silencing and decay proteins, have parallels in regulating the replication of mammalian retrotransposons and retroviruses. The sheer number of Ty1 host factors and the variety of cell processes in which they are involved illuminate the complex symbiosis between Ty1 and its host. Characterization of these host factors continues to reveal that the host carries out fundamental steps in Ty1 retrotransposition from transcription to protein trafficking and integration targeting. For example, recent work has shown that a universally conserved co-translational chaperone is critical for the synthesis of Gag and the nucleation of VLP assembly sites [76]. Future work

will determine where VLP assembly takes place and how the transition of Ty1 RNA from translation to packaging is regulated. Since the events that initiate assembly of retroviral nucleocapsids are only beginning to be described, studies on Ty1 are likely to provide an essential paradigm for understanding fundamental aspects of retroviral replication. Future studies will likely characterize additional essential genes that are required for Ty1 retrotransposition. The identification of essential genes holds the promise of revealing mechanisms by which the cell cycle controls retrotransposon and retroviral replication, which in turn will provide clues to the connection between retrotransposons and the development of cancer.

In summary, the golden age of Ty1 retrotransposons as a model is in its adolescence, with burgeoning opportunities for studies on Ty1 to advance our understanding of the tangled tête-à-tête between retroelements and their hosts. These studies hold promise for improving the treatment of a variety of human diseases by suggesting novel approaches to controlling retrovirus and retrotransposon-mediated genome rearrangements and cell pathologies.

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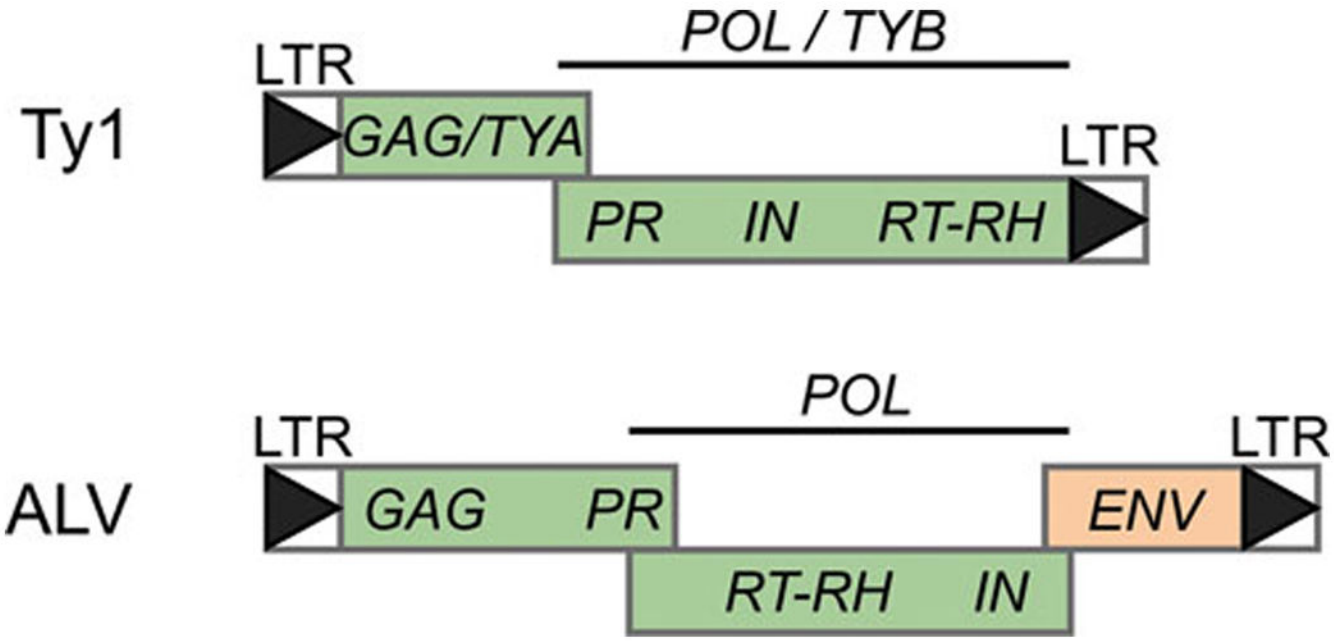

Figure 1. Structure of the Ty1 element relative to the simple retrovirus, avian leukemia virus (ALV)

Ty1 consists of long terminal repeats (LTRs; boxed arrowheads) flanking a central coding region that contains two overlapping ORFs, *GAG* (*TYA1*) and *POL* (*TYB1*), which are analogous to retroviral *GAG* and *POL* genes, respectively. Separate functional domains of *POL* that are conserved in LTR-retrotransposons and retroviruses and are synthesized as part of the Gag-Pol polyprotein and cleaved into separate proteins posttranslationally include PR (protease), RT-RH (reverse transcriptase-RNase H) and IN (integrase). The retroviral envelope gene (*ENV*) is not present in Ty1.

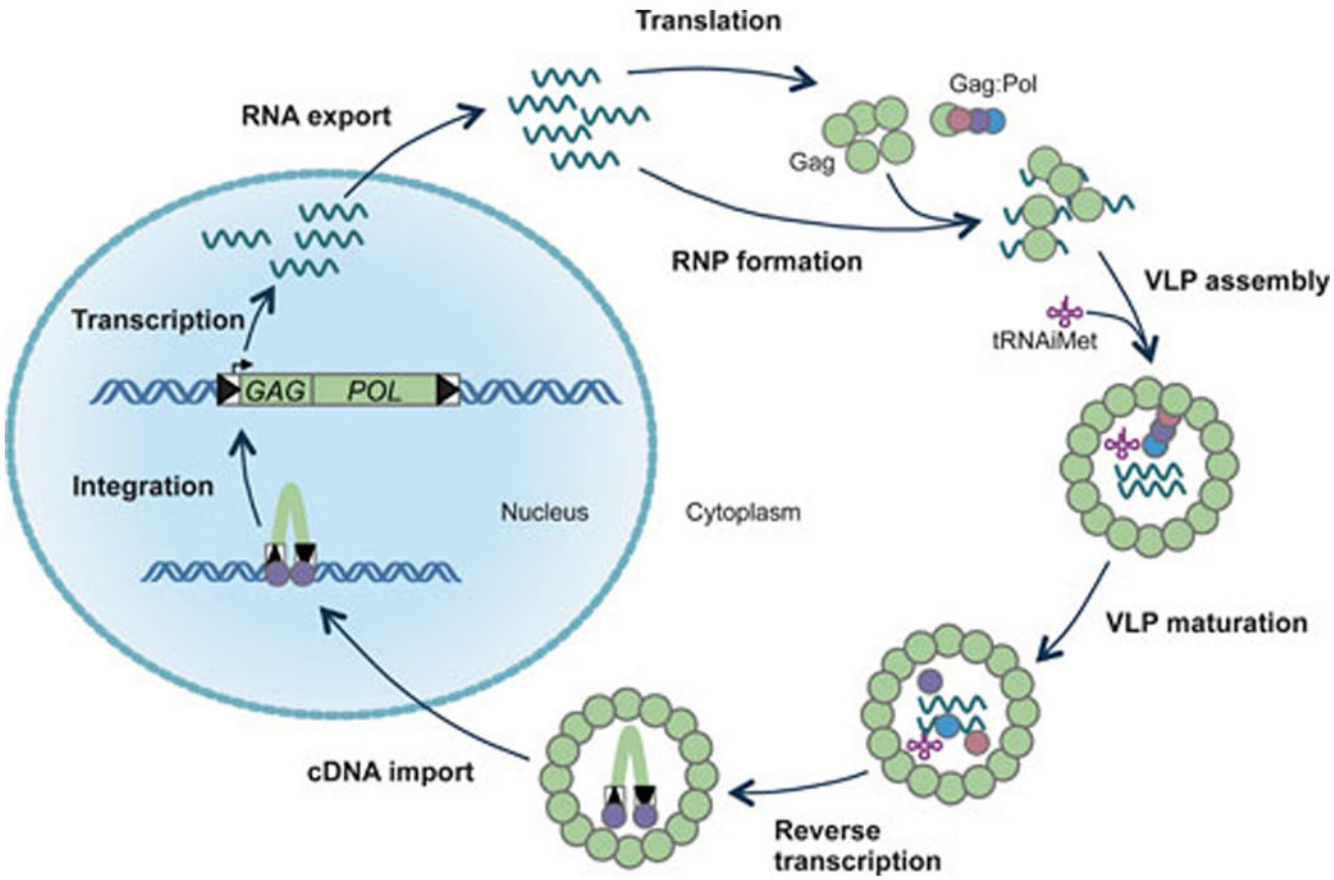

Figure 2. Ty1 replication cycle

The major steps in replication of Ty1, which results in the introduction of a new copy of Ty1 into the host genome, are illustrated. A Ty1 element in the host genome (blue double helix) is transcribed and the Ty1 RNA (wavy teal lines) is exported to the cytoplasm. The RNA is translated into Gag and Gag-Pol proteins, and associates with these proteins to form Ty1 RNPs, also known as retrosomes. Ty1 RNPs give rise to VLPs that encapsidate a dimer of Ty1 RNA and $tRNA_i^{Met}$. Within the VLP, Gag and Pol proteins are cleaved by PR (maroon ball) to form mature Gag, PR, IN and RT proteins. Following VLP maturation, Ty1 RNA is reverse transcribed into cDNA by RT (blue ball) using $tRNA_i^{Met}$ as a primer. The cDNA is bound by IN (purple ball) to form the pre-integation complex, which is imported into the nucleus. IN integrates Ty1 cDNA into the yeast genome.

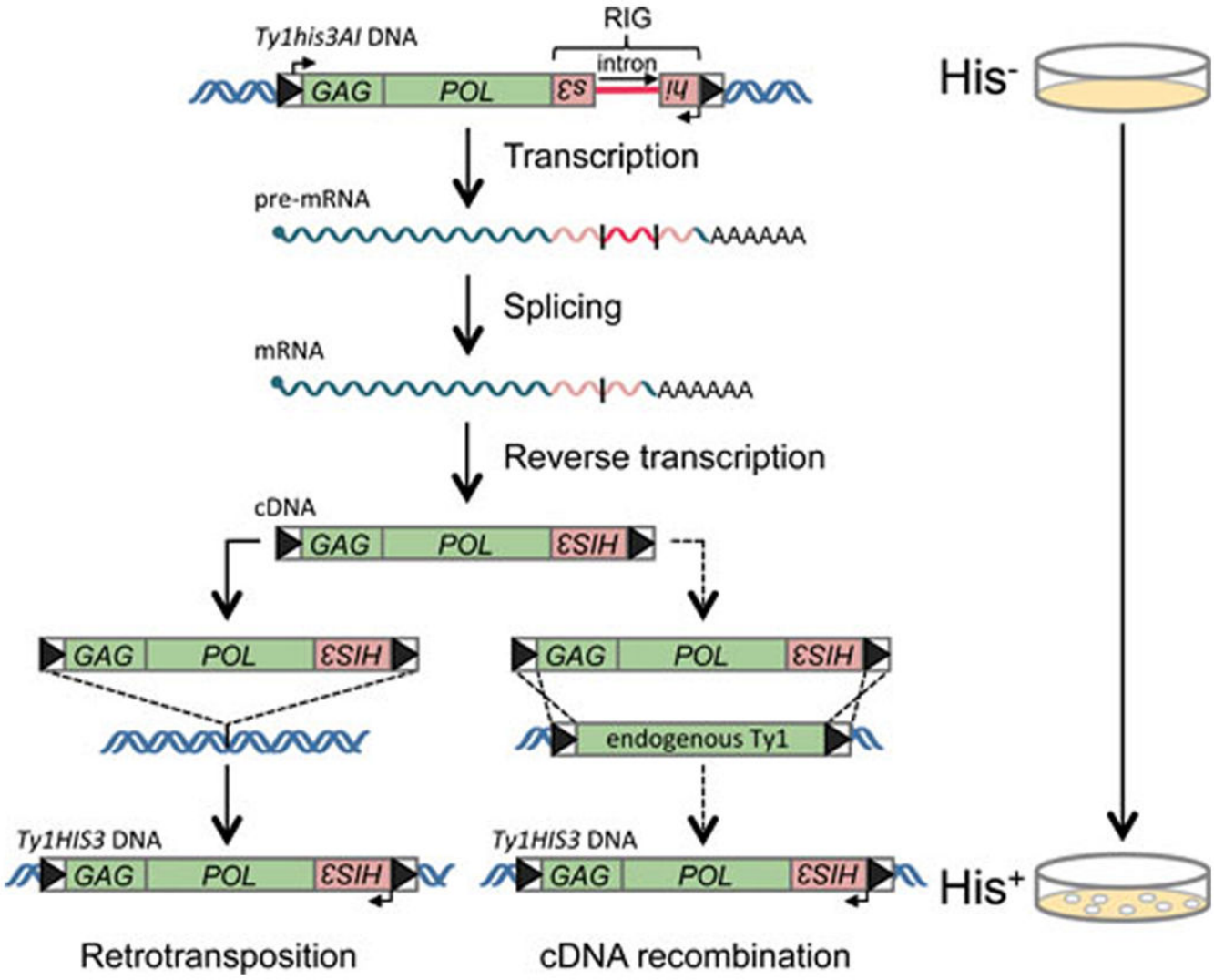

Figure 3. Assay for Ty1 retromobility using a retrotranscript indicator gene (RIG)

A chromosomal Ty1 element tagged with the *his3AI* RIG gives rise to Ty1 cDNA bearing a functional *HIS3* gene. The cDNA can enter the host genome (represented by a blue double helix) by two retromobility pathways. Retrotransposition occurs when Ty1 IN mediates the integration of cDNA into the genome, while cDNA recombination occurs when the cDNA recombines homologously with an endogenous Ty1 element. The dashed line represents the low frequency of Ty1 cDNA recombination in wild-type cells. Cells that sustain a Ty1*HIS3* retromobility event give rise to His^+ colonies. Other RIGs used in yeast include *kanMXAI*, which is selectable with G418 and *ade2AI*, selectable by adenine prototrophy [268,269].

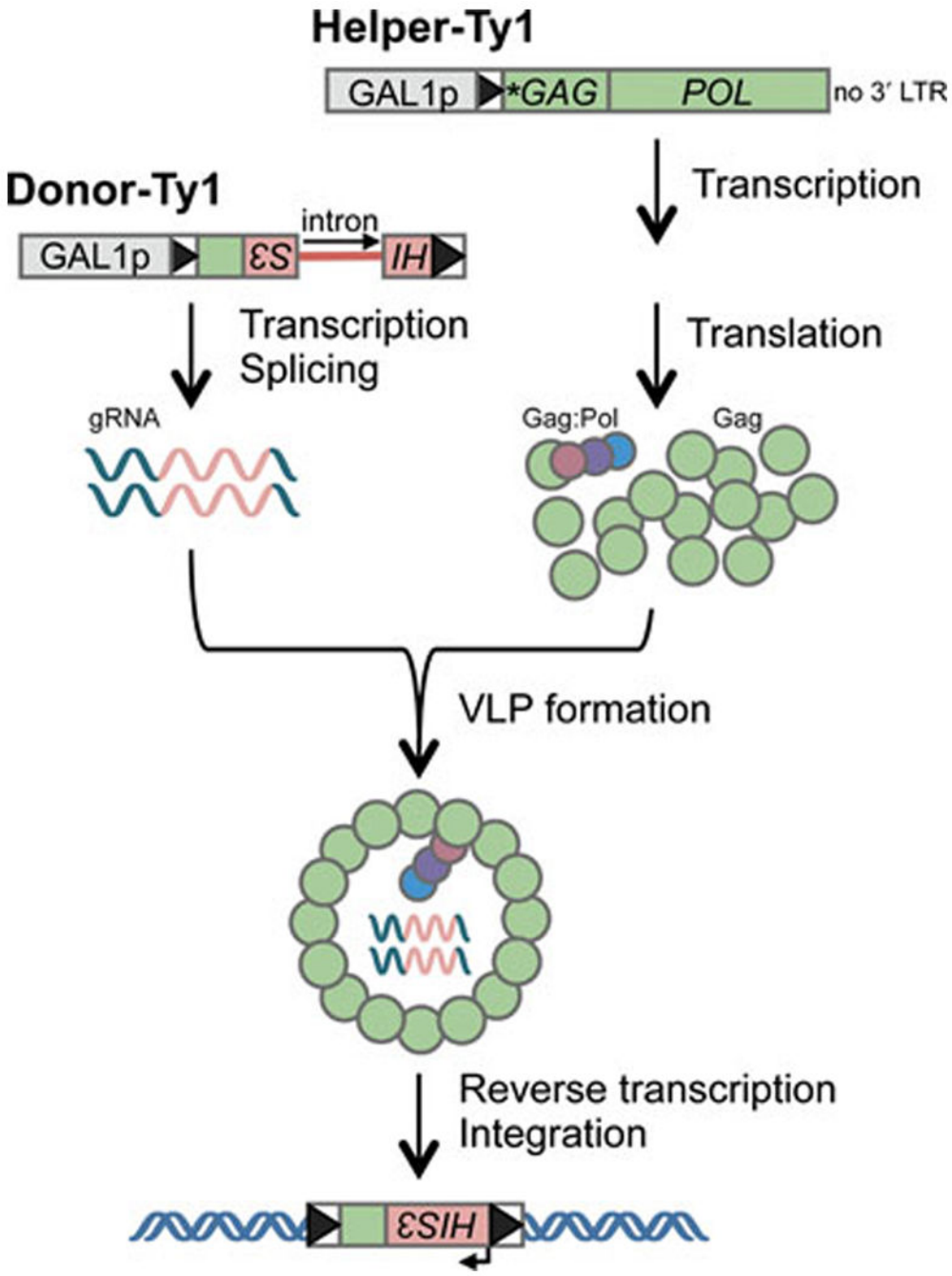

Figure 4. Helper-donor assay for separation of mRNA and gRNA functions

In this assay, expression of both Ty1 elements is driven from the *GAL1* promoter in an *spt3* strain, which lacks endogenous Ty1 expression. The helper-Ty1 encodes a functional mRNA with *GAG* and *POL* ORFs and silent mutations at the 5′ end of *GAG* (indicated by an asterisk) that disrupt *cis*-acting signals required for reverse transcription. The absence of a 3′ LTR also precludes the use of the helper-Ty1 RNA as a template for reverse transcription. The donor-Ty1 RNA encodes a functional gRNA that lacks ORFs but contains *cis*-acting signals for dimerization, packaging and reverse transcription. The *his3AI* RIG is also

contained in this element to detect retromobility of mini-Ty1*HIS3* cDNA. The minimal donor element capable of retromobility is depicted.

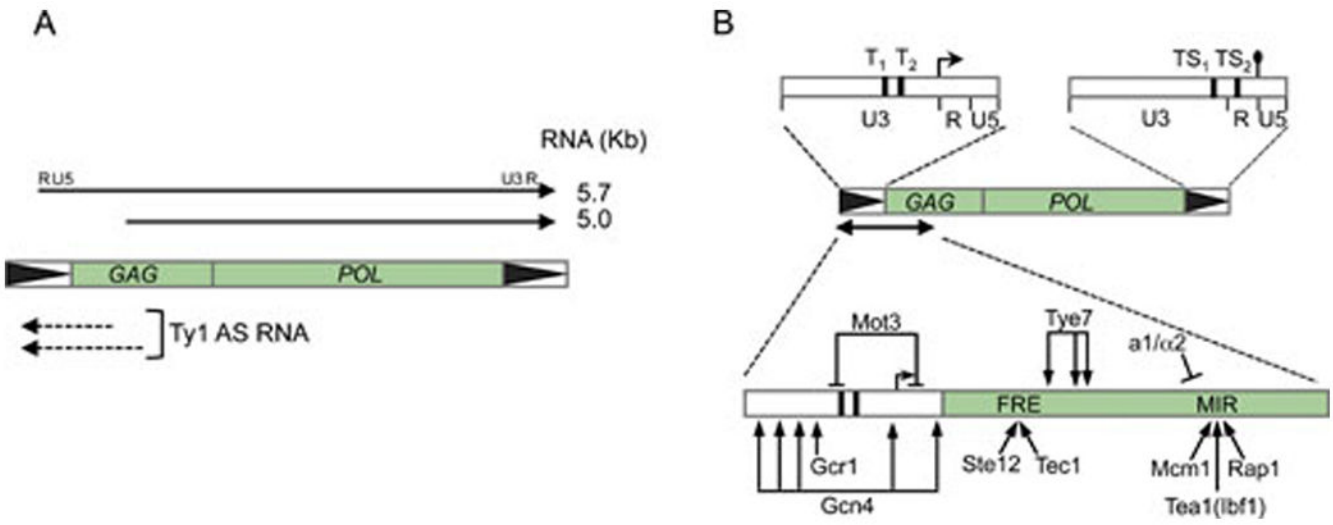

Figure 5. Ty1 transcription

A. Sense and antisense RNAs transcribed from Ty1. The Ty1 sense-strand transcript starts at the U3/R junction of the 5′ LTR and ends at the R/U5 junction of the 3′ LTR. The 5 kb Ty1 RNA species is detected in *spt3* mutants. Ty1AS RNA 5′ and 3′ extremities have been mapped by RACE to positions 661 [68] and 760 [33] of Ty1-H3 and to positions 136 and 178 of Ty1-H3 [33], respectively. **B**. Organization of the Ty1 promoter. Ty1 contains two TATA boxes, T_1 and T_2 (at positions 159-165 and 167-173, respectively) and two termination sequences TS_1 (5776-5781) and TS_2 (5837-5842). The arrow and lollipop indicate sites of transcription initiation and termination, respectively. The Ty1 promoter extends over 1 kb including the 5′ LTR and part of the *GAG* ORF. The positions of the Ty1 activator binding sites are: Gcn4 (five binding sites; 12-17, 79-84, 98-103, 155-160 and 318-323), Gcr1 (115-119), Ste12 (395-401), Tec1 (418-422), Tye7 (3 binding sites; 463-468, 661-666 and 727-732), Mcm1 (833-848), Tea1/Ibf1 (884-899), Rap1 (911-923). The filamentous response element (FRE) comprises Ste12 and Tec1 binding sites, while MIR comprises Mcm1, Tea1/Ibf1 and Rap1 binding sites. The positions of the Ty1 repressor binding sites are: Mot3 (several binding sites in the 5′ LTR; higher affinity site *in vitro* at positions 147-150) and a1/α2 (832-863). Positions are given relative to Ty1-H3 sequence [10].

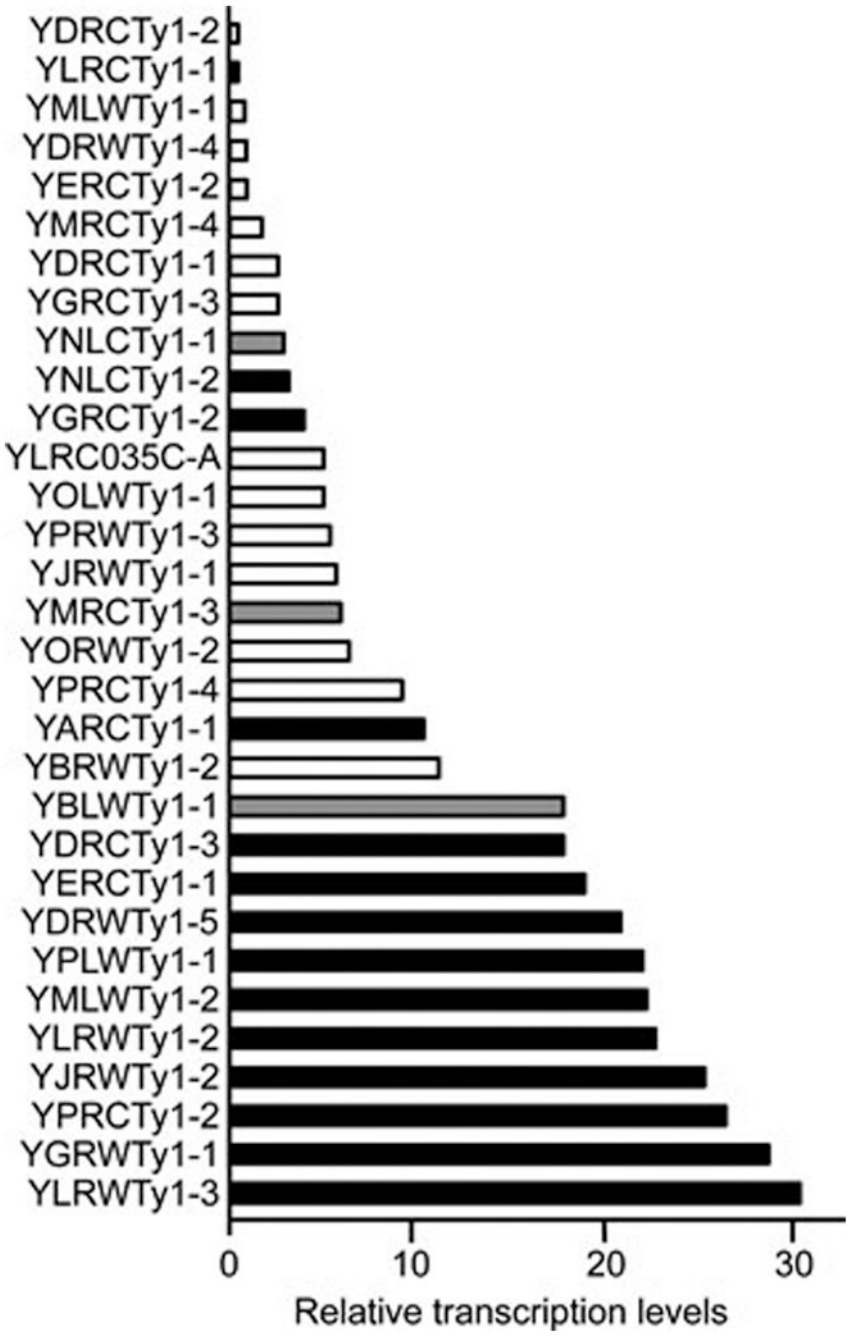

Figure 6. Expression of individual endogenous Ty1 elements

Relative transcriptional activities of 31 Ty1 elements present in the *S. cerevisiae* S288C strain (adapted from Morillon et al. [56]). Ty1/Ty2 hybrid elements are indicated by black filled bars and Ty′ elements are indicated by grey filled bars.

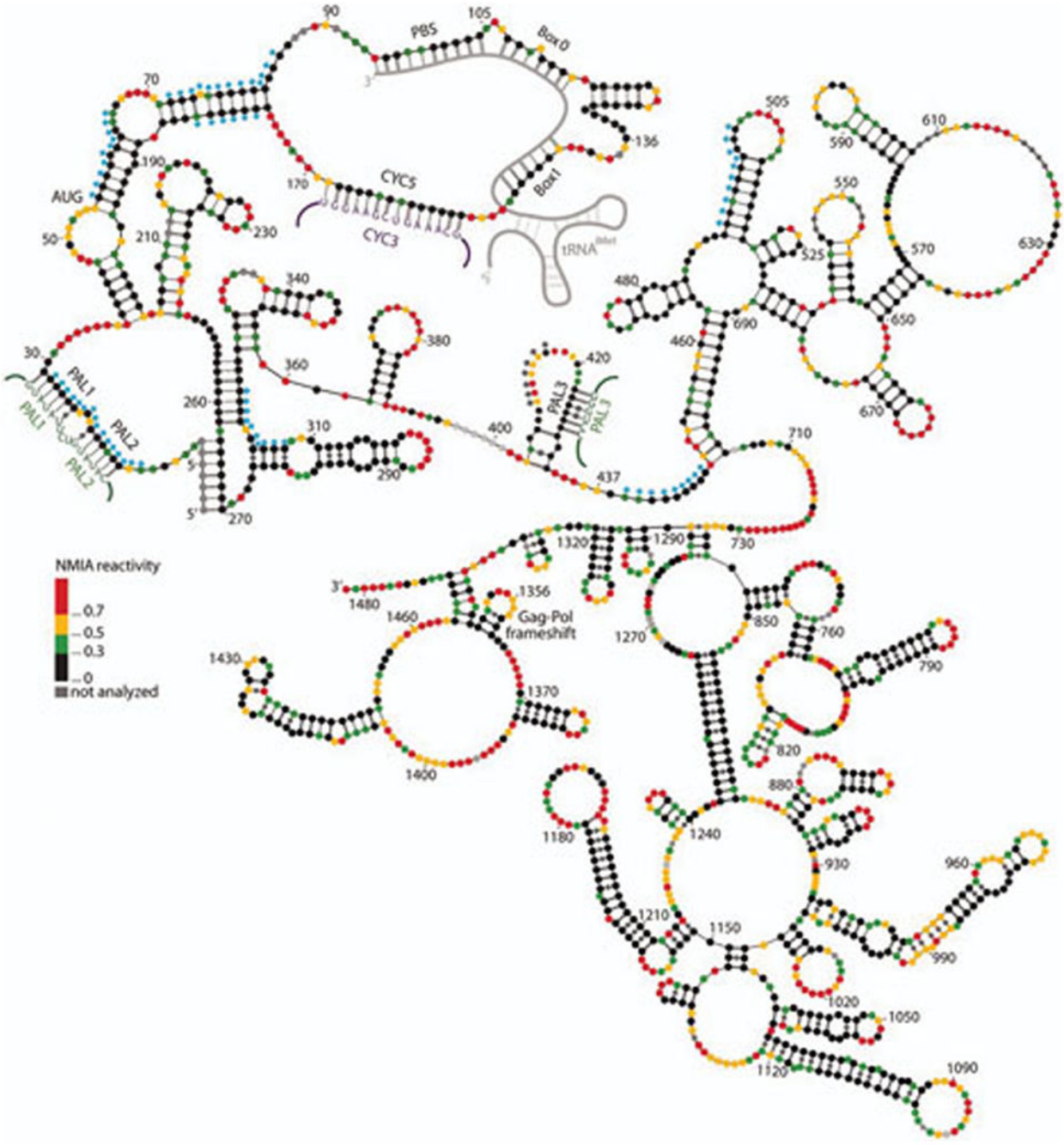

Figure 7. Secondary structure model of the 5′ end of Ty1 RNA

(Reproduced from Purzycka et al. [74].) Full-length Ty1 RNA within VLPs (*in virio*) was analyzed to determine the SHAPE reactivities of nucleotides 1-1482, which are individually represented by a color-coded ball; the color is indicative of the reactivity of that nucleotide to the SHAPE reagent, NMIA. The secondary structure model in which regions predicted to be based-paired (illustrated as bars linking balls) and regions predicted to be single stranded are shown was obtained from the SHAPE reactivities using RNAstructure 4.6 software [270]. Nucleotide positions at which SHAPE reactivities changed when the proteins were

gently stripped away from VLP-associated Ty1 RNA (*ex virio*) are marked with blue diamonds (increased reactivity) or grey diamonds (decreased reactivity). Annotated regions include the following: PAL1, PAL2 and PAL3 motifs, at which the differences in nucleotide reactivity *in virio* versus *ex virio* suggest that the Ty1 RNA pairs with a second molecule of Ty1 RNA to form a dimer; the AUG codon of *GAG* (nucleotides 54-57); CYC5, a region that hybridizes to the CYC3 domain near the 3′ end of Ty1 RNA; PBS, Box 0 and Box 1, where the $tRNA_i^{Met}$ primer (shown in grey) hybridizes to Ty1 RNA to initiate reverse transcription; and the Gag-Pol frameshift site at nucleotides 1356-1362.

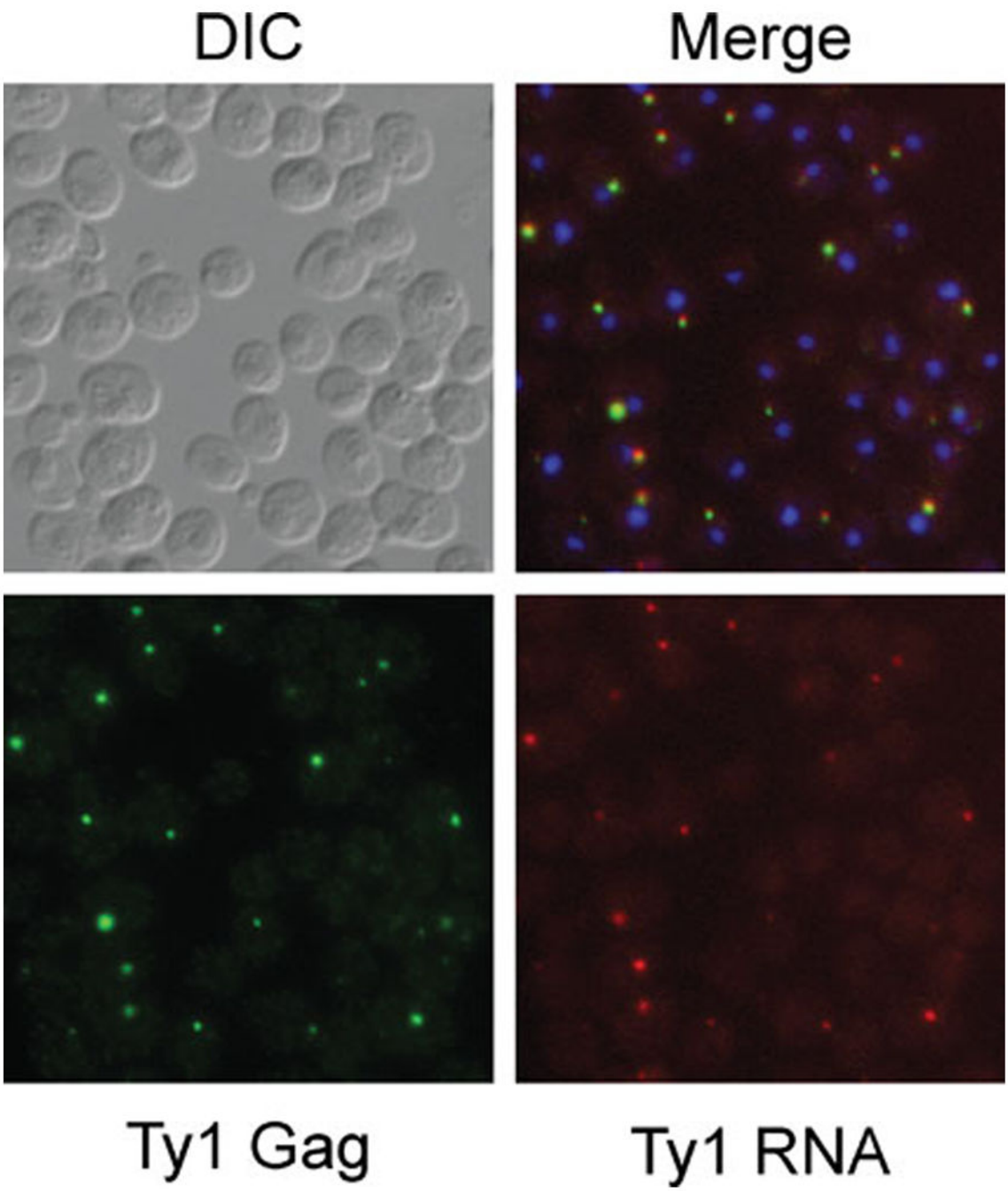

Figure 8. The Ty1 retrosome

Retrosomes are cytoplasmic foci in which Gag and retrotransposon RNA co-localize, as visualized by fluorescence microscopy. Ty1 retrosomes are detected here in fixed cells after fluorescence *in situ* hybridization and immunofluorescence. Ty1 RNA is detected using a DNA oligomer end-labeled with Cy3, and Gag is detected using anti-VLP antibodies that are bound by a secondary antibody coupled to Alexa Fluor® 488.

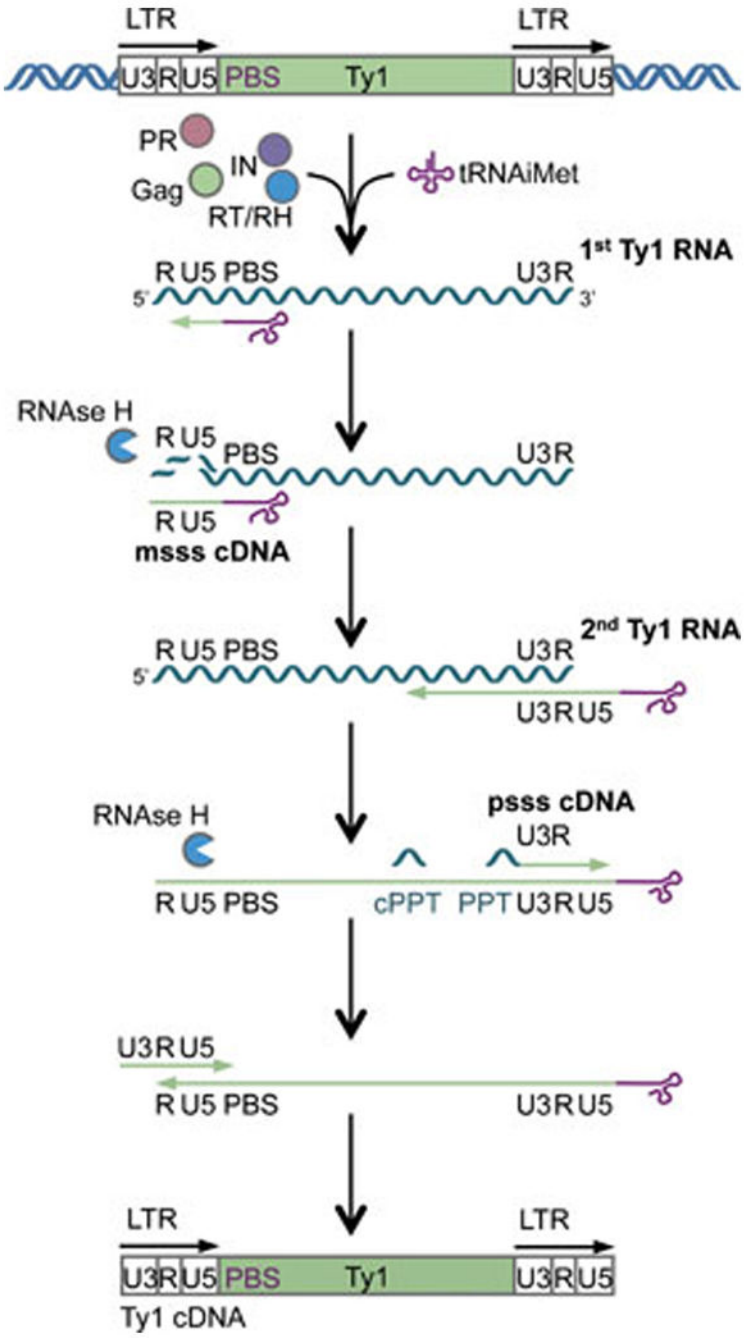

Figure 9. Steps in reverse transcription of Ty1 cDNA

Details of each step are provided in the text. Gag and PR have nucleic acid chaperone activities required for reverse transcription, and IN facilitates the reverse transcription reaction carried out by RT/RH. In this schematic, msss cDNA is shown being transferred to the second Ty1 RNA in the VLP for minus strand cDNA synthesis; however, it is formally possible that msss cDNA transfers to the 3′ end of the first Ty1 RNA, as long as this RNA, following RNAse H-mediated degradation, contains a remnant of R-U5 sequence to template minus-strand cDNA synthesis so that minus-strand cDNA can hybridize to psss

cDNA in the final step of the RT reaction. Single strands of cDNA are represented by thin green lines; the presence of an arrowhead indicates strands being extended by reverse transcription. Blue wavy line: Ty1 RNA; short blue squiggles: polypurine tracts of Ty1 RNA, cPPT and PPT, remaining after RNase H endonucleolytic activity; blue pacman shape: RNase H activity of RT/RH; psss: plus-strand strong-stop; msss: minus-strand strong-stop.

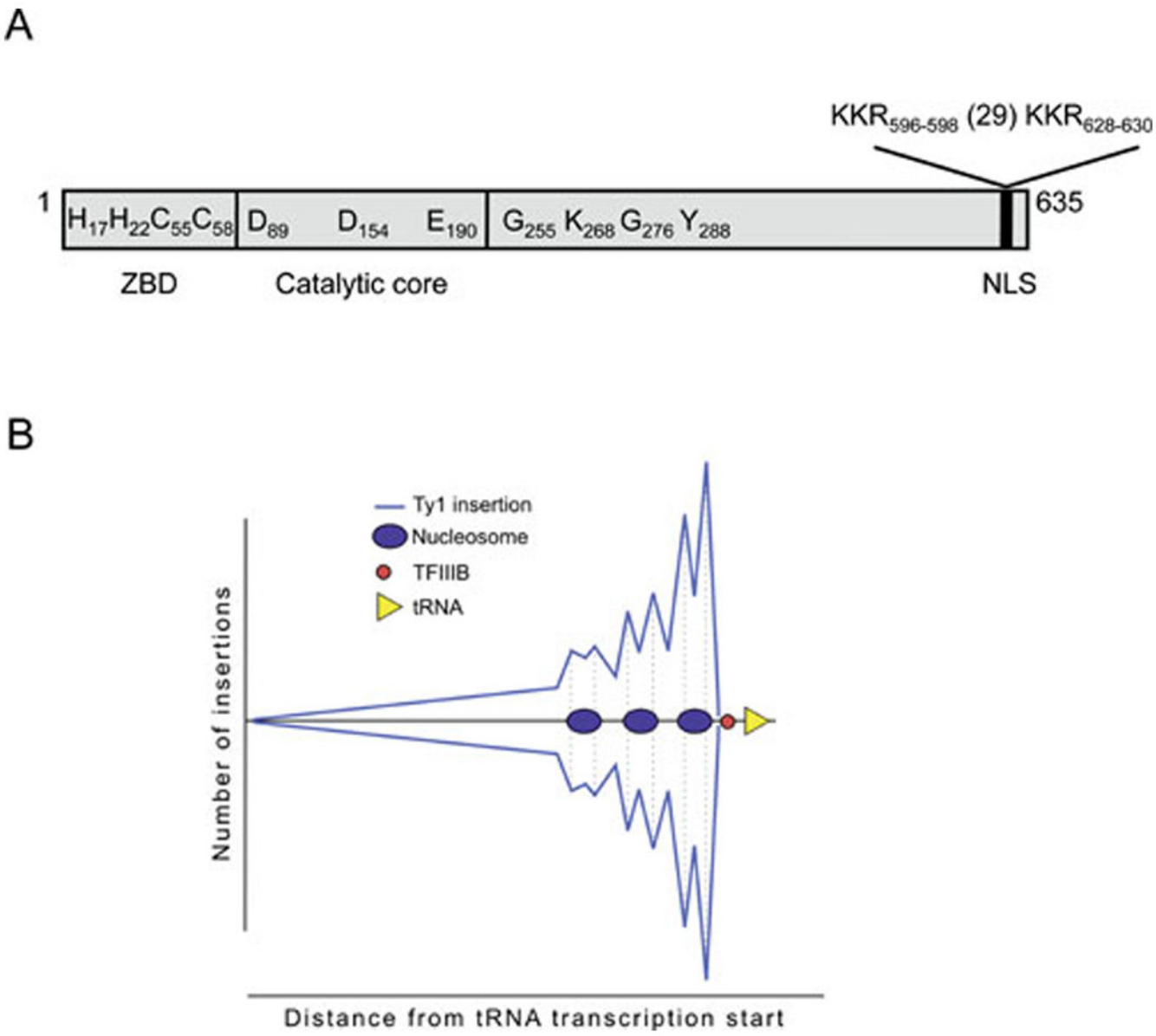

Figure 10. IN and the integration target region

A. Schematic representation of Ty1 integrase domains. Amino acid residues in the zinc binding motif (ZBD) and the catalytic core domain that are conserved in retroviral integrases, or residues in the C-terminal domain that are conserved in the *Pseudoviridiae* family of LTR-retrotransposons and retroviruses are indicated. Identical clusters of basic residues that define the bipartite nuclear localization signal (NLS) are also indicated. B. Plot of Ty1 insertions upstream of tRNA genes. (Reproduced from Bridier-Nahmias and Lesage [271].) The blue curve above the midline represents Ty1 insertions in tandem with the tRNA genes, and that below the midline represents elements inserted in inverted orientation.

Table 1
List of verified host factors that regulate Ty1 retromobility

CELLULAR FUNCTION	HOST ACTIVATORS (*RHF* genes)	HOST RESTRICTORS (*RTT* genes)
Chromatin/Transcription	*ARP5* [78], *CTK1* [151], *NPL6* [78], *RPC53*[a] [151], *SPT2* [65,151,186]	*ASF1* [65,161], *ELF1* [65,161], *HHT1* [161], *RTT103* [65,164], *RTT106* [65,161,164],
Pol II TFIIH subunits		*RAD3*[a] [167], *SSL2* [a] [160]
Transcription factors	*MCM1* [50,262], *MIG3* [78,163], *RAP1* [262], *STB5* [151], *STE12* [34,48,49,56,166], *TEC1* [54,65], *TEA1* [52]	
Mediator complex	*SIN4* [65,78,151], *SRB8* [78,151], *SSN2* [78,151]	*CSE2* [161], *MED1* [65,162,164], *NUT2* [a] [164], *SOH1* [65,161], *SRB5* [65,161]
Paf1 complex		*CDC73* [161], *LEO1* [65,161], *PAF1* [161], *RTF1*[b] [65,151,161]
SAGA complex	*SPT3* [65,78,163,186], *SPT7* [186], *SPT8* [78,163,186]	
SPT4/5 complex	*SPT4* [65,78,151], *SPT5*[a,b] [78,161,163]	
SWI/SNF complex	*SNF2* [48,56,60], *SNF5* [48,60,163], *SNF6* [60,163], *SWI3* [48,60,78,163]	
THO/TREX complex	*THP2* [151,163], *MFT1* [151]	
Histone acetylation/deacetylation	*HDA3* [78,163], *NAT4* [163]	*RTT109* [65,161,162,164]
Rpd3S/L complex	*SAP30* [151]	*EAF3* [161], *RPD3* [161], *SIN3* [161]
SetC3 complex	*HOS2* [153], *SET3* [153], *SNT1* [163]	
Elongator complex	*ELP2* [163], *ELP4* [78,151]	
Histone ubiquitylation		*BRE1* [161], *RAD6* [218,263,264]
Histone transcription	*SPT10* [65,78,151,163]	*SPT21*[b] [65،151،161], *HIR1*[c] [265], *HIR2*[c] [65،161،265], *HIR3*[c] [161,265]
Translation	*EFT2* [a] [266], *SPE1* [94], *SPE2* [94]	*ASC1* [161], *BUD27* [161]
Ribosome biogenesis factors	*BUD21* [76,163], *BUD22* [78], *DBP7* [163], *HCR1* [163], *MRT4* [163], *RKM4* [163], *RPA49* [151], *HMO1* [78,163], *NOP12* [78,151]	
Ribosomal subunits	*RPL16B* [151,163], *RPL19A* [163], *RPL19B* [151], *RPL20B* [151], *RPL27A* [78,151], *RPL31A* [163], *RPL43A* [163], *RPL6A* [151], *RPL7A* [76,163], *RPP0* [a] [266], *RPP1A* [151,163], *RPS0B* [78], *RPS10A* [163], *RPS19B* [78,163], *RPS25A* [78,163], *RPS30A* [163]	
RNA metabolism	*DBR1* [65,150-153], *LSM1* [68,70,71,78,151,163], *REF2* [78,163], *SKI8* [78,163], *TGS1* [78,163]	
Cap binding	*CBC2* [151], *STO1* [78]	
RNA binding	*CTH1* [163], *LOC1* [78,163,252], *PUF6* [151,163], *SCP160* [78,151]	
RNA degradation	*CCR4* [68,71,163], *DCP1* [a] [68،71], *DCP2* [a] [68,71], *DHH1* [70,71,78,163,252], *NAM7* [68,71,78,163], *NMD2* [71,78], *PAT1* [68,70,71,78,151], *POP2* [78,151], *PUB1* [76,78], *UPF3* [68,71,78,163], *XRN1* [68,70,71,78]	
tRNA biogenesis	*HSX1* [88,91,252], *LOS1* [163], *NCL1* [163], *RIT1* [78,151]	*RTT10* [161]
Protein Metabolism	*DFG10* [76,163,252], *DOA4* [78,151], *MCK1* [78,151]	*CKB2* [161]
Chaperones	*JJJ1* [a] [78،151], *KAR2* [a] [76], *SRP68* [a] [76], *SRP101* [a] [76], *SRP102* [a] [76], *SSE1* [78,163], *SSZ1* [78], *UMP1* [78,163], *YKE2* [78]	

CELLULAR FUNCTION	HOST ACTIVATORS (*RHF* genes)	HOST RESTRICTORS (*RTT* genes)
N-terminal acetyltransferase	*ARD1* [151], *NAT1* [151], *NAT3* [151]	
Peptidyl-prolyl *cis-trans* isomerase	*CPR7* [78,151,163], *ESS1* [a] [78]	*RRD2* [161,162,164]
Protein transport/sorting	*CLC1* [78], *LST7* [163], *SEC22* [151], *SIT4* [78], *SLA2* [78], *STP22* [78], *VPS9* [151], *VPS16* [78]	
Nuclear/cytoplasmic transport	*APQ12* [78,163], *GSP1*[a] [182], *NTF2* [a] [182], *NUP133* [151,163], *NUP170* [78,163], *RSL1*[a] [182], *SRP1* [a] [182]	*KAP122* [164]
Cell polarity division	*AFR1* [163], *BEM1* [151], *BUD25* [78], *CDC50* [78,163], *DBF20* [163], *HOF1* [151], *NUM1* [151]	*BEM4* [161], *BUD27* [161]
Dynactin Complex	*JNM1* [151], *NIP100* [78,163]	
Cell cycle progression		*CDC40* [161], *CLN2* [161], *SIC1* [161]
Cellular energetics/ion balance	*ATP17* [163], *PDE2* [78,163], *PHO88* [78], *PMR1* [135], *QDR2* [78], *SPF1* [163], *TRK1* [78,151,163], *VPH1* [78,163]	*AGP3* [161], *RNR1* [164]
Inositol metabolism	*KCS1* [151]	*ARG82* [161], *IPK1* [161], *KCS1* [161]
Cell signaling	*ASI1* [78], *KSS1* [166]	
HOG pathway		*HOG1* [36,161], *PBS2* [161], *SSK2* [161], *SSK22* [161]
Mating response pathway	*RAM1* [78], *STE4* [78,166], *STE5* [78,166], *STE7* [49,166,267] *STE50* [78]	*STE11* [34,36], *FUS3* [36,65,162,166]
Genome Stability		
DNA Repair and Replication	*APN1* [151]	*CDC9* [189], *CTF4* [162,163], *ELG1* [65,161,162,164], *MMS1* [65,161,162,164], *MMS22* [161-163], *MRC1* [162], *RAD18* [161,162], *RAD27* [161,162,169], *RRM3* [65,161,162,164,191], *RTT101* [65,161,162,164], *RTT107* [65,161,162,164], *SAE2* [161,162,164], *SGS1* [13,161,164], *WSS1* [162]
Telomere maintenance		*EST2* [164,168], *TEL1* [162,164]
Recombinational repair		*RAD51* [65,161,162,189], *RAD52* [65,161,162,189], *RAD54* [161,189], *RAD55* [161,162], *RAD57* [161,162,164,189]
Mre11/MRX complex		*MRE11* [161,162,164], *RAD50* [161,162,164,189], *XRS2* [162,164]
Miscellaneous	*DGR2* [163], *GLO2* [78,163], *HIT1* [163], *HSP31* [78], *KGD1* [163], *OCA4* [78,163], *TPS2* [151], *YD124W* [163], *YIL102C* [78], *YOR292C* [151]	*RTT105* [164], *YOL159C* [151]

[a] essential or nearly essential genes

[b] gene was identified as an *RHF* and as an *RTT* in different genetic screens

[c] Phenotype of deleted gene requires a specific genetic background containing a *cac3* mutation.