Two large-scale analyses of Ty1 LTR-retrotransposon de novo insertion events indicate that Ty1 targets nucleosomal DNA near the H2A/H2B interface

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Abstract

Background: Over the years, a number of reports have revealed that Ty1 integration occurs in a 1-kb window upstream of Pol III-transcribed genes with an approximate 80-bp periodicity between each integration hotspot and that this targeting requires active Pol III transcription at the site of integration. However, the molecular bases of Ty1 targeting are still not understood.

Findings: The publications by Baller et al. and Mularoni et al. in the April issue of Genome Res. report the first high-throughput sequencing analysis of Ty1 de novo insertion events. Their observations converge to the same conclusion, that Ty1 targets a specific surface of the nucleosome at the H2A/H2B interface.

Conclusion: This discovery is important, and should help identifying factor(s) involved in Ty1 targeting. Recent data on transposable elements and retroviruses integration site choice obtained by large-scale analyses indicate that transcription and chromatin structure play an important role in this process. The studies reported in this commentary add a new evidence of the importance of chromatin in integration selectivity that should be of interest for everyone interested in transposable elements integration.

Keywords: LTR retrotransposon, Ty1, Selective integration, Large-scale analysis

Findings

Retrotransposons are major components of eukaryotic genomes. They represent, for example, half of the human genome, up to 80% of some plant’s genomes and 3% of the compact genome of Yeast S. cerevisiae. They have a central role in shaping genomes, and have been shown to be a powerful force of evolution and to play a positive role in long-term adaptation. However, they can also be deleterious in the short-term, since their integration into the host genome can inactivate or deregulate gene expression or even induce large chromosomal rearrangements by homologous recombination of distant copies. LTR-retrotransposons are structurally and functionally related to retroviruses but their life cycle is exclusively intracellular since they do not encode an envelope glycoprotein. They replicate by reverse transcribing their RNA into cDNA, which is ultimately integrated into the host genome by the element-encoded integrase (IN). The non-random distribution of LTR-retrotransposons and retroviruses into genomes suggests that these elements actively select their integration sites (for review, [1]).

For the past 20 years, studies on Ty3 and Ty5 LTR-retrotransposons of S. cerevisiae have led to better understand the molecular bases of their targeted integration (Figure 1). It has been established that an interaction between Ty3 IN and the Brf1 subunit of TFIIB is sufficient to target Ty3 integration to Pol III transcription initiation sites in vitro [2]. Likewise, in vivo, Ty5 preferential integration into silent telomeric heterochromatin depends on the interaction between Ty5 IN and the Sir4 protein, a...
structural component of silent chromatin [3]. In the distant
Yeast S. pombe, interaction between Tf1 IN and the Atf1
transcription factor plays a direct and specific role in tar-
getting Tf1 integration in the fbp1 gene promoter [4]. These
studies have converged on a common targeting mecha-
nism, based on tethering of integration complexes to the
cell genome through interaction between IN and cellular
proteins bound at favored insertion sites. It is noteworthy
that this model may also account for the selectivity of the
integration of retroviruses, since HIV-1 integration in ac-
tive transcription units relies on the interaction of its IN
with the LEDGF/p75 transcription factor (reviewed in [5]).

Discovered in 1979 [6], Ty1 is the most abundant and ac-
tive LTR-retrotransponson in S. cerevisiae. It was first
noticed in 1982 that Ty1 insertions are located adjacent to
several tRNA genes [7]. Over the years, a number of
reports have found that Ty1 integration occurs in a 1-kb
window upstream of Pol III-transcribed genes with an
approximate 80-bp periodicity between each integration
hotspot and that this targeting requires active Pol III
transcription at the site of integration (Figure 1) [8-10].
However, despite 30 years of active research, the molecular
bases of Ty1 targeting are still not understood. Thus, the
recent articles of Baller et al. and Mularoni et al. are an
important advance for understanding the selection of Ty1
integration sites, by showing that Ty1 targets a specific sur-
fase of the nucleosome [11,12].

Both studies used a deep-sequencing approach to get
insights into Ty1 integration selectivity. To discriminate
de novo insertion events from resident Ty1 elements,
both used a short tag sequence introduced in one LTR
of a galactose-inducible donor Ty1 element, such that
after a complete retrotransposition cycle the tag would
be recovered in both LTRs (Figure 2), and this short tag
was used to specifically sequence newly transposed
sequences. In the Mularoni et al.’s study, sequences were
generated by an Illumina GAII apparatus. Of a total of
7,990,112 reads, 1,154,281 were characterized as non-
redundant Ty1 insertions. Baller et al. used a donor Ty1
element, which contained the his3AI reporter construct
conferring histidine prototrophy to the cells after Ty1
retrotransposition, and recovered only His+ insertions.
A single 454 run produced from 13,000 to 111,000 reads.
While Mularoni et al. analyzed 10- to 100-fold more
integration events than Baller et al., the latter analyzed
integration profiles in diploid and haploid wild-type cells
and in a panel of mutants affecting DNA-related pro-
cesses known to increase integration frequency (rrm3Δ,
rtt109Δ, hos2Δ) and integration in coding sequences and
transcription units (rad6Δ, RTT109 and HOS2 encode
histone-modifying enzymes, while RRM3 encodes a heli-
case and RAD6 an ubiquitin-conjugating enzyme.

Both reports describe the same general insertion profile
pattern in wild-type cells. Independently of cell ploidy, a
vast majority (~90%) of insertions were observed as predicted in the 5’ region of class III genes. However, Ty1 did not target class III genes equally. All but two tRNA genes, tEE(UUC)C and tIA(AAU)L1, received many insertions. Other class III genes, such as SNR6, RPR1, SNR52, SCR1 and the repeated locus RDN5 received insertions as well. However, two class III loci of unknown function, RNA170 and ZOD1, were not targeted, probably because the Pol III transcription machinery was not efficiently recruited at these loci. Insertions in Pol II-transcribed genes were rare, representing about 5% of total events. Most of them occurred near a Pol III gene, with a strong preference for the region closest to the class III gene. Considering ORFs which are more distant to tRNA genes (~5 kb), the few recovered insertions occurred at the gene 5’ end. Although insertions into mitochondrial sequences (mtDNA) were reported by Mularoni et al., they were not detected by Baller et al., probably because of a smaller dataset size or because those insertions did not confer histidine prototrophy and were, consequently, not selected for sequencing. Mularoni et al. suggest that these insertions might come from shattered mitochondria and could occur in the nucleus or even in the cytoplasm.

The novel and most striking observation of both studies is that Ty1 integration is positively correlated with nucleosome occupancy. An important role for chromatin in the selection process of insertion sites was already suspected after the discovery of an intriguing periodicity of ~80 bp between each integration hotspots that relied on the ATP-dependent chromatin remodeling factor Isw2 [10]. By comparing their deep-sequencing results with genome-wide nucleosome positioning data sets, they have discovered two hotspots per nucleosome, separated by about 70 bp. These observations were made for the first three nucleosomes directly upstream of a class III gene and the integration events were aligned with the nucleosome H2A/H2B interface (Figure 3).

In hos2Δ and rtt109Δ mutant strains analyzed by Baller et al., the pattern of Ty1 insertion events was not significantly different from that in wild-type cells. In contrast, integration events in verified ORFs increased significantly in rrm3Δ and rad6Δ mutant strains (by two- and three-fold, respectively), although the integration pattern upstream of tRNA genes was unmodified, leading to the conclusion that the determining factors for specific nucleosomal targeting upstream of class III genes were not affected in these mutants.

High-throughput sequencing of insertion events has provided a saturated profile of target activity for several retrotransposons and contributed to better understand their integration preferences. For example, integration of the LTR retrotransposon of S. pombe Tfl has been shown to be strongly biased for Pol II promoters with a clear preference for stress-induced promoters [13], and another report, in which 10,000 events have been analyzed, confirms all previous in vitro evidences on Ty3 integration at Pol III sites [14]. A high-throughput sequencing strategy has also been largely used to map the insertion profiles of different retroviruses (for review [15]). The reports of Mularoni et al. and Baller et al. reveal that Ty1 integration upstream of class III genes is strongly correlated with the chromatin structure at these loci and preferentially targets a specific nucleosomal DNA segment. Interestingly, the preference for nucleosome-rich regions is not a conserved feature of retroelements since it has been shown that elements such as Ty5 and Hermes (when expressed in yeast) prefer nucleosome-free regions [16,17].

Although these two studies clearly contribute to better understanding of Ty1 targeting, they do not characterize

![Figure 3](https://example.com/figure3.png)
whether a specific nucleosomal DNA conformation, a specific histone modification, or a nucleosome-bound factor enriched at sites of Pol III transcription, determine Ty1 preferred target sites, nor do they elucidate the role of RNA polymerase III and its co-factors in Ty1 targeting. Thus, further work is required to completely decipher the molecular bases of Ty1 targeting.

Abbreviations
H2A: Histone protein H2A; H2B: Histone protein H2B; His+: Histidine photopropy; HIV-1: Human Immunodeficiency Virus 1; IN: Integrate; LTR: Long Terminal Repeat; ORF: Open Reading Frame; Pol III: RNA Polymerase III; Ty: Transposon in yeast.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AB-N, wrote the manuscript PL, supervised the final manuscript. All authors read and approved the final manuscript.

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