

Influence of RNA structural elements on Ty1 retrotransposition

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The long-terminal repeat (LTR)-retrotransposon Ty1 is a mobile genetic element that replicates through an RNA intermediate. Retroelement genomic transcripts contain internal structures fundamental to gene expression and propagation. In addition, long non-coding antisense RNAs overlap the 5'-terminal region of the genomic RNA and confer post-translational copy number control. Although LTR-retrotransposons are functionally related to retroviruses, little is known about the structural determinants required for genomic RNA packaging or reverse transcription. This commentary summarizes two recent papers that provide the first snapshot of genomic RNA structures from the retrotransposon Ty1 involved in transposition. We combined structural approaches with functional and genetic assays to determine if antisense RNAs anneal with the genomic RNA. Analysis of various steps in the Ty1 life cycle showed that a novel RNA pseudoknot contributes to retrotransposon function. Comparing different RNA states provides additional information about regions potentially involved in Ty1 RNA dimerization or packaging.

The long-terminal repeat (LTR) retrotransposon Ty1 of the budding yeast *Saccharomyces cerevisiae* replicates via an RNA intermediate and shares several important structural and functional characteristics with retroviruses.¹ For example, Ty1 RNA serves as mRNA for synthesis of Gag and Gag-Pol polypeptide precursors, as well as the genomic (g) RNA

for reverse transcription. Ty1 gRNA is packaged as a dimer into virus-like particles (VLPs)² comprised of a Gag-like structural protein and the *POL*-encoded enzymes protease, integrase and reverse transcriptase. Gag is important for Ty1 RNA stability, nuclear export and localization.³ Initiator (i) tRNA-Met is also specifically packaged into VLPs where it acts as the primer for the first step in reverse transcription, (-) strand-stop DNA synthesis.⁴ Functional aspects of Ty1 reverse transcription have been analyzed extensively, taking full advantage of the genetic methods available for *S. cerevisiae*. In particular, a mini-Ty1 element has been developed that allows evaluation of *cis*-acting functions present on a minimal segment of Ty1 gRNA.^{5,6} These studies showed that the RNA sequences required for retrotransposition are located near the 5' terminus of the element. The terminal ~560 nucleotides are sufficient to support retrotransposition when co-expressed with a helper element.^{5,6} Among other roles, this region sequesters the cellular tRNA-Met, a component of the reverse transcription initiation complex. The primer binding site (PBS) region of Ty1 contains 10 contiguous nucleotides complementary to the 3' acceptor stem of tRNA-Met.^{7,8} A long-range interaction between a 5' region (CYC5) adjacent to the extended PBS with sequences near the 3' end of genomic RNA (CYC3) is also critical for efficient reverse transcription and transposition.⁹ Recent studies by Garfinkel and coworkers have provided evidence that long non-coding antisense (AS) RNAs that overlap the 5'-terminal regulatory region confer

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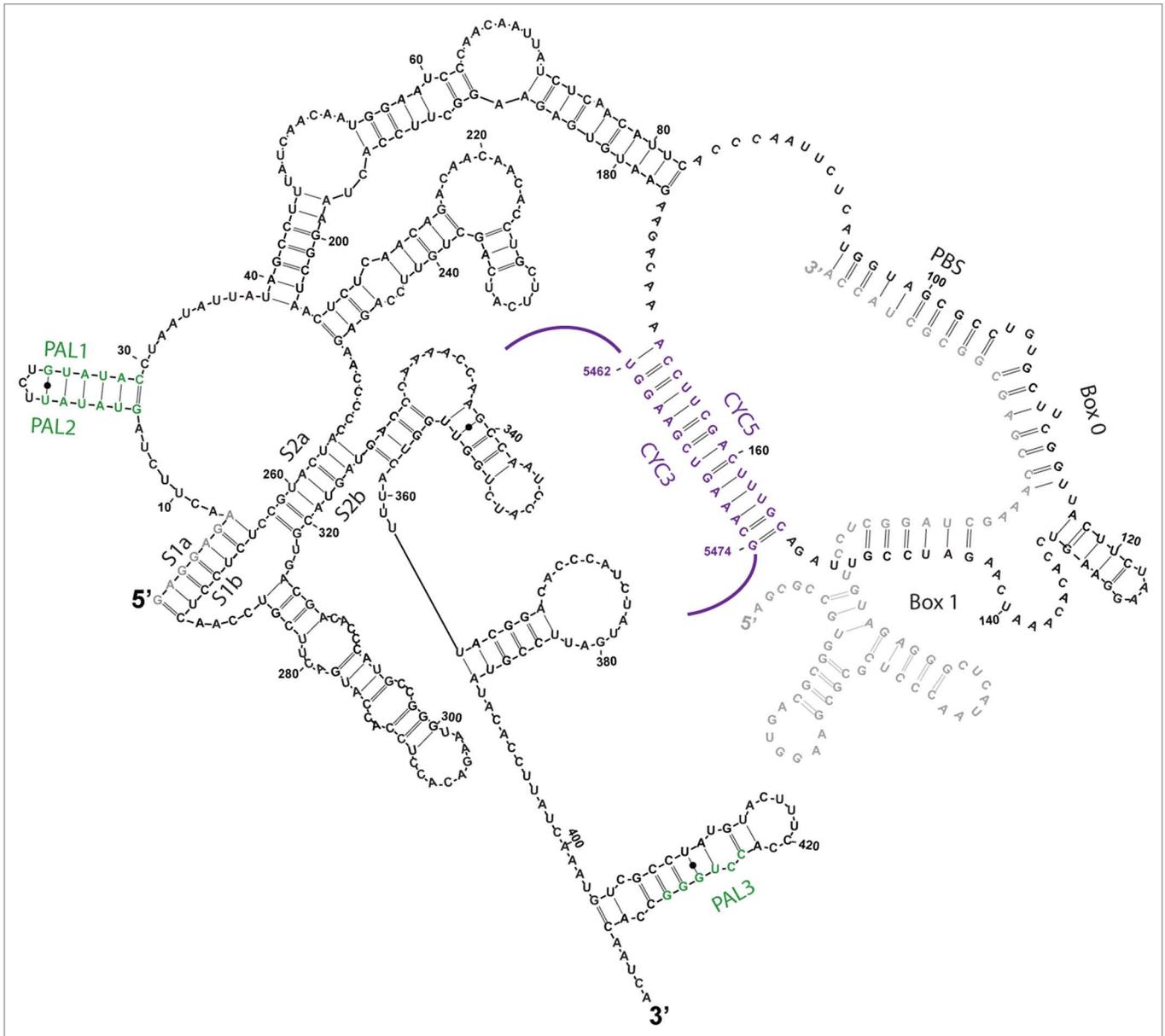


Figure 1. RNA secondary structure of nt 1–437 of Ty1 gRNA in the monomer state. tRNA-Met_i is shown in gray, cyclization motif in violet, PAL motifs in green.

copy number control (CNC) posttranslationally and are enriched in VLPs.¹⁰

Until recently, little was known about the structure of retrotransposon gRNA, or the complex it forms with tRNA-Met_i within VLPs. This commentary summarizes two recent papers,^{11,12} which provide the first gRNA structures from the retrotransposon Ty1 involved in transposition. The first of these¹¹ analyzed the structure of Ty1 gRNA in the presence (CNC⁺) or absence (CNC⁻) of long non-coding AS transcripts. Since the AS RNAs overlap the PBS region, we determined

whether they blocked reverse transcription by annealing with gRNA. We combined chemoenzymatic RNA probing of Ty1 RNA/tRNA complexes assembled *in vitro* with RNA structure probing within virus-like particles (*in virio*) and following gentle extraction of Ty1 proteins (*ex virio*), and proposed the first comprehensive model of the *in virio* Ty1 RNA region important for reverse transcription (Fig. 1). Surprisingly, comparative structural analysis of CNC⁻ and CNC⁺ VLPs showed that the structure of the PBS region was unaffected in the presence

of the Ty1 AS RNAs even though CNC⁺ VLPs were defective for reverse transcription. The replication block observed in CNC⁺ VLPs, as monitored by the production of full-length cDNA and endogenous reverse transcriptase activity,^{10,11} likely results from the low level of mature integrase.¹⁰ We reported, however, that several regions proximal to the 5'-end of Ty1 RNA are destabilized in VLPs associated with high levels of Ty1 AS RNAs. Interestingly, destabilization of the RNA 5' terminal domains in CNC⁺ VLPs was comparable to that achieved by total removal of Ty1

proteins from CNC- VLPs (ex virio), from which we proposed the notion that AS RNAs target a protein function, perhaps mediated by Gag, that destabilizes the regulatory region. The 5' terminus of Ty1 gRNA specifies, in addition to the PBS, essential structural determinants required for RNA packaging and/or dimerization. Interestingly, a ~1.5-fold defect in RNA packaging, monitored by nuclease protection, was observed for Ty1 gRNA in CNC⁺ cells. Our detailed structural studies demonstrated that 5'-terminal region contains a novel pseudoknot, and that this element was one of the motifs destabilized in the absence of Ty1 proteins. An AUGAUGA sequence in stem S2 of the pseudoknot (Fig. 1) may be involved in protein binding. Pseudoknots are known to play regulatory roles and intermolecular pseudoknots (kissing-loops) promote dimerization and packaging of retroviral gRNA.¹³ We hypothesized that the Ty1 gRNA pseudoknot may make an important contribution to proper VLP assembly.

In a subsequent study,¹² we combined chemoenzymatic probing with phylogenetic conservation analysis and molecular and genetic assays of various steps in the Ty1 life cycle to determine whether the predicted structure of the gRNA contributes to retrotransposon function. Analysis of pseudoknot and compensatory mutants demonstrates that this structure is critical for Ty1 retrotransposition. Mutations in stem S1 of the pseudoknot cause more severe transposition defects. To observe similar defects in transposition frequency for stem S2 mutants, double or triple nucleotide substitutions interfering with canonical base pairing were required. However, in vitro structure mapping (in the absence of protein factors) of pseudoknot mutants indicates that the single nucleotide substitution U260C sufficed to disrupt stem S2 (Fig. 1). Therefore, in agreement with previous findings,¹¹ the nucleic acid chaperone activity associated with Ty1 Gag may facilitate pseudoknot folding and maintenance of the correctly folded structure in vivo. Pseudoknot mutants display normal levels of Ty1 gRNA and Ty1 Gag. Intriguingly, reverse transcription, as opposed to gRNA packaging, was affected when pseudoknot architecture was compromised, raising the

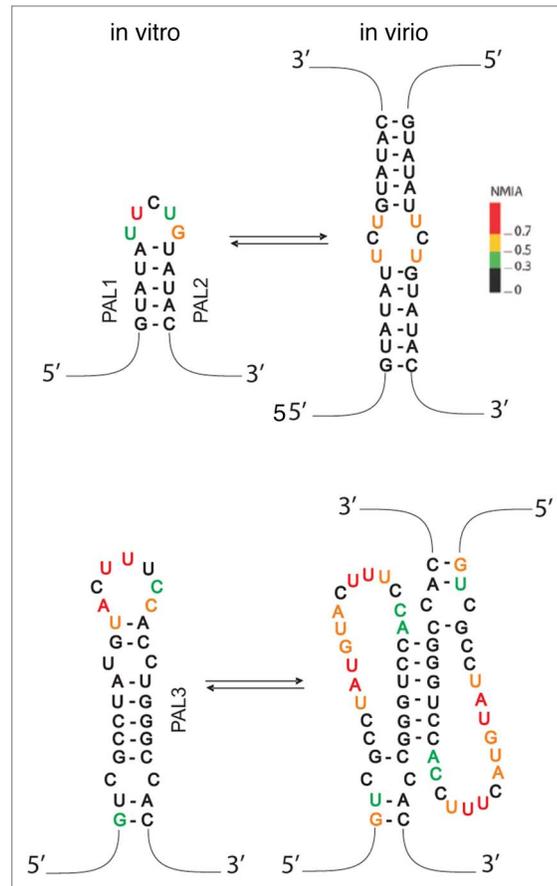


Figure 2. Reciprocal interstrand interactions of the PAL motifs. This model is based on SHAPE constraints derived from the in vitro and in vivo states of Ty1 RNA.

exciting possibility that interplay between structural elements in Ty1 RNA involved in packaging and reverse transcription mediates retrotransposon function. Maintaining a stable structure of the regulatory region, as shown by comparing either CNC⁺/CNC⁻ VLPs or Ty1 pseudoknot mutants, is clearly critical for transposition. These observations also suggest that the pseudoknot structure is essential for the overall architecture of the Ty1 gRNA regulatory region.

Little is known about structural determinants required for gRNA packaging of Ty1 or other LTR retrotransposons. We propose structural elements within regulatory region of Ty1 gRNA that may contribute to dimerization/packaging.¹¹ Previous reports suggest that like Ty3, Ty1 RNA might be maintained in a dimeric form as a consequence of interactions between two tRNA-Met_i molecules bound to the element encoded PBS.¹⁴ A recent study by the Sandmeyer

group¹⁵ demonstrated that sequences in the Ty3 3' UTR that bind tRNA-Met_i are not required for packaging. Our structural studies¹¹ do not exclude the possibility that two tRNA-Met_i molecules provide a dimerization interface but we found regions in Ty1 gRNA that are likely involved in this process. Three regions that may be involved in protein binding contain palindromic (PAL) sequences (Fig. 1). Even weak intermolecular interactions are readily detected by SHAPE¹⁶ and reactivity differences in vitro, in vivo and ex virio support intermolecular structures for the PAL regions (Fig. 2). Self-complementary sequences are involved in dimerization of retroviral genomes¹³ and dimerization is a prerequisite for packaging. For Murine leukemia virus, dimer formation exposes tandem UCUG sequences essential for protein binding and packaging.¹⁷ Since Ty1 PAL1 and PAL2 sequences are comprised of such tandem repeats, we

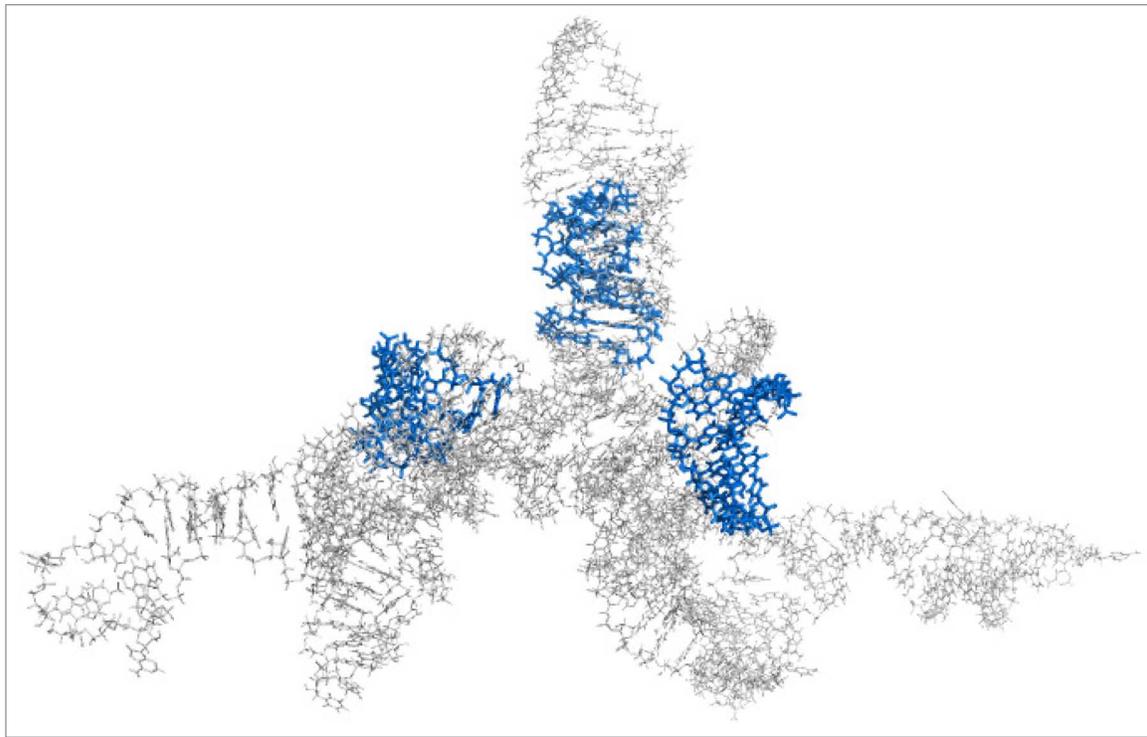


Figure 3. 3D structure model of Ty1 RNA. Proposed protein binding sites¹¹ are depicted in blue.

hypothesize that formation of the pseudoknot exposes the Ty1 PAL motifs for protein-mediated dimerization and packaging. Correct folding may be facilitated by the nucleic acid chaperone activity of Gag, since the pseudoknot structure fails to fold *in vitro* in the context of full-length Ty1 RNA.¹¹ The AUGAUGA sequence involved in protein binding to the pseudoknot occurs three times in the CNC region. Interestingly, all three regions displayed altered chemical reactivity *ex vivo*, strengthening the notion of their recognition by Gag. Detailed examination of the Ty1 RNA sequence reveals two copies of the AUGAUGA sequence at the 3'-end of the genome, bracketing the cyclization sequence CYC3. The fact that the cyclization motif is not folded *in vitro*¹¹ supports the idea that this sequence could be specifically recognized by Ty1 Gag and is important for the structural organization of Ty1 gRNA. To this end, the tertiary structure of Ty1 RNA (362 nt) was predicted using RNAComposer¹⁸ and the resulting model shows that the proposed protein binding sites cluster within the regulatory region and close

in space (Fig. 3). Therefore, these motifs may serve as a 'seed' for Ty1 RNA packaging. Further structural and functional analyses of Ty1 gRNA will improve our understanding of retrotransposon replication and the relationship between these elements and retroviruses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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