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# A Novel Initiation Complex for Reverse Transcription of an Active LTR Retrotransposon in Sweetpotato

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**Sequence analysis of *Rtsp-1*, an active LTR retrotransposon in the sweetpotato genome, revealed a possible novel *Rtsp-1* RNA/tRNA$^{Met}$ complex for initiation of reverse transcription and the first DNA strand transfer. The *Rtsp-1* RNA has a primer binding site (PBS) that is partly complementary to the 3' end of tRNA$^{Met}$, and possesses an additional sequence complementary to the 5' end of tRNA$^{Met}$ downstream of the PBS. These additional base-pairings might stabilize the *Rtsp-1* RNA/primer complex. In the free form, the 5' LTR of *Rtsp-1* appears to form a stem-loop structure apparently preventing the initiation of reverse transcription. While the stem-forming site adjacent to the PBS is complementary to the tRNA$^{Met}$, the other stem-forming site on the LTR complements a region just upstream of the 3' LTR. Additionally, another region at the 3' end of the *Rtsp-1* RNA shows sequence complementarity to the tRNA$^{Met}$. As the 3' end of *Rtsp-1* approaches the tRNA$^{Met}$ bound to the PBS, the stem-forming strands dissociate and base-pair with their complementary regions in the tRNA$^{Met}$ and the 3' end of *Rtsp-1*, respectively. Consequently, the LTR loop opens, allowing reverse transcription to initiate. After the initial reverse transcription stops at the 5' end of the *Rtsp-1* RNA, the synthesized minus strand DNA needs to be transferred to the 3' end of the RNA to synthesize internal sequences. The *Rtsp-1* RNA/tRNA$^{Met}$ complex may have evolved to facilitate this DNA transfer. Similar RNA/tRNA initiation complexes have been reported from reverse transcription in retroviruses and yeast retrotransposons (Ty1 and Ty3).**

**Key words :** retrotransposon, reverse transcription, initiation complex, retrovirus, DNA strand transfer

## Introduction

### *Reverse transcription of LTR retrotransposons*

Retrotransposons are transposable genetic elements that require the action of reverse transcriptase on an RNA intermediate to move through the genome[1]. They are divided into two groups, depending on the presence of long terminal repeats (LTR). LTR retrotransposons are major components of a plant genome. However, only three families have been demonstrated to be transpositionally competent. These families are represented by *Tnt1*[2] and *Tto1*[3] in tobacco, and by *Tos17*[4] in rice. Recently, we have identified an active element of the LTR retrotransposon family in the sweetpotato genome (*Rtsp-1*), which had transposed during cell culture[5].

Transposition of LTR retrotransposons proceeds via a transcribed RNA that is reverse-transcribed into an extra-chromosomal double-stranded DNA prior to integration into host genomes[6]. It is initiated at the primer binding site (PBS), usually utilizing a host tRNA as a primer and a retrotransposon full-length transcript as a template (Fig. 1, step A)[7]. The reverse transcriptase (RTase) encoded by the LTR retrotransposon at first synthesizes a DNA complement of the R and U5 regions of the 5' LTR, since the full-length RNA is organized as R-U5-PBS-internal domain-PPT-U3-R (Fig.1, step B). Subsequently, RNaseH digests the RNA associated with the newly synthesized DNA, giving rise to a single stranded DNA copy (Fig.1, step C). This DNA fragment, termed the minus-strand strong stop DNA (minus-sssDNA), is subsequently transferred to the 3' end of the RNA (Fig. 1, step D) in the first of two strand exchanges during reverse transcription. The minus-sssDNA acts as a primer at the terminal R region and is elongated to form a copy of the internal sequences termed the minus-strand DNA (Fig. 1, step E). RNaseH degrades the RNA template except at the polypurine tract (PPT) region, leaving an RNA/DNA hybrid there (Fig. 1, step F). The undigested RNA serves as a primer for the plus-strand DNA synthesis

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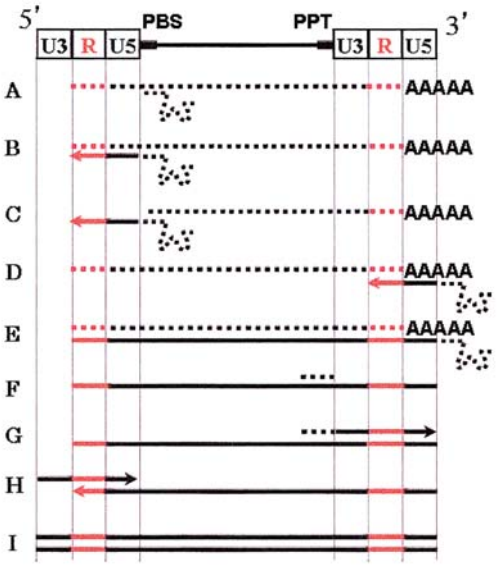


**Fig. 1 Reverse transcription mechanisms as described in the text.**
U3, unique at 3' region; R, repeated terminus of a transcript; U5, unique at 5' region; PBS, primer binding site; PPT, polypurine tract. Solid lines indicate DNA, whereas dotted lines indicate RNA. The DNA strands elongated by reverse transcriptase are shown as solid lines with arrowheads. The figures are adapted from Voytas and Boeke[7].

(Fig. 1, step G). When the plus-strand DNA is synthesized up to the end of the minus-strand DNA, it undergoes a second strand transfer to the 5' end of the minus-strand DNA (Fig. 1, step H). Elongation of both minus- and plus-strands complete a full-length linear LTR retrotransposon DNA (Fig. 1, step I).

***Proposed model for the reverse transcription initiation complex of Rtsp-1***

Sequence analysis of the *Rtsp-1* retrotransposon, registered as AB162659 in DDBJ/EMBL/GenBank, and primer $tRNA^{Met}$ revealed a possible novel retrotransposon RNA/tRNA complex (Fig. 2). While the PBS of the *Rtsp-1* retrotransposon is complementary to the 3' end of the $tRNA^{Met}$, the six nucleotides downstream of the PBS are complementary to the 5' end of the $tRNA^{Met}$ (Figs. 2a and 2b). The 3' end of the LTR also has a sequence complementary to the D-arm of the $tRNA^{Met}$. Additionally, the sequences near the carboxyl terminus of the RNaseH region, which is close to the 3' end of the transcribed *Rtsp-1* sequence, show significant complementarity to the T-*psi*-C-arm of the $tRNA^{Met}$. Furthermore, the downstream region of the RNaseH is a complement to the LTR U5 region, which is upstream of a possible binding site for $tRNA^{Met}$ D-arm (Fig. 2d). Finally, the 5' side of this LTR U5 region is complementary to the 3' end region of the LTR. Based on these sequence complementarities, we propose the following mode of formation of the *Rtsp-1* RNA/tRNA complex for reverse transcription initiation. In the free form, the *Rtsp-1* RNA LTR forms a stem-loop, leaving the PBS region as a single strand (Fig. 2a). The $tRNA^{Met}$ initially binds *Rtsp-1* RNA at the PBS and the adjacent downstream region (Fig. 2c). This binding involves 19 base pairs and ensures the stability and specificity of priming. However, the 3' end of LTR end and the two intervening nucleotides (5'-AU-3'), which serve as a template for the bound $tRNA^{Met}$ to initiate elongation, remain double-stranded and thus nonfunctional. As the RNaseH region of the *Rtsp-1* RNA approaches the T-*psi*-C-arm of the $tRNA^{Met}$, it loosens the LTR stem structure and opens the loop by base pairing of the T-*psi*-C-arm of the $tRNA^{Met}$ and the RNaseH downstream-untranslated region with the LTR stem-forming sites (Fig. 2d). This structural change in the *Rtsp-1* RNA/tRNA complex is a switch-on process from a closed stem structure with the template RNA inaccessible for the primer $tRNA^{Met}$, to an open structure in which the primer $tRNA^{Met}$ becomes accessible for elongation by reverse transcription. As RTase synthesis of the minus-sssDNA progresses, the D-arm of $tRNA^{Met}$ and the RNaseH downstream-untranslated region dissociate from the 3' end of the LTR. However, $tRNA^{Met}$ remains attached to both the PBS and the RNaseH region of the *Rtsp-1* RNA at least until the minus-sssDNA transfer.

***The initiation complexes for LTR retrotransposons and retroviruses***

The interactions between LTR retrotransposon RNA and the primer tRNA have been studied most comprehensively in *Saccharomyces cerevisiae* Ty1 and Ty3. The yeast LTR retrotransposon RNAs form a highly ordered initiation complex with the primer tRNA to stabilize the reverse transcription priming process. Besides binding of the PBS to the 3' end of $tRNA^{Met}$, the yeast Ty1 retrotransposon RNA has three short regions downstream of the PBS, which interact with the T-*psi*-C and D-arms of the $tRNA^{Met}$ (Fig. 3)[8]. Disruption of complementarity between these retrotransposon regions and $tRNA^{Met}$ dramatically decreases transposition efficiency, primarily affecting initiation of the synthesis of minus-sssDNA[9]. Additionally, the yeast Ty1 retrotransposon has two complementary 14 nucleotide sequences (CYC sequences), one downstream of the PBS and one in the U3 region of the LTR[10]. The U3 region is present only at the 3' end of a full-length RNA molecule, and the base pairing between the two CYC sequences results in close association of the 5' and 3' ends of the Ty1 RNA. Surprisingly, this association greatly enhances the efficiency of the initiation of reverse transcription.

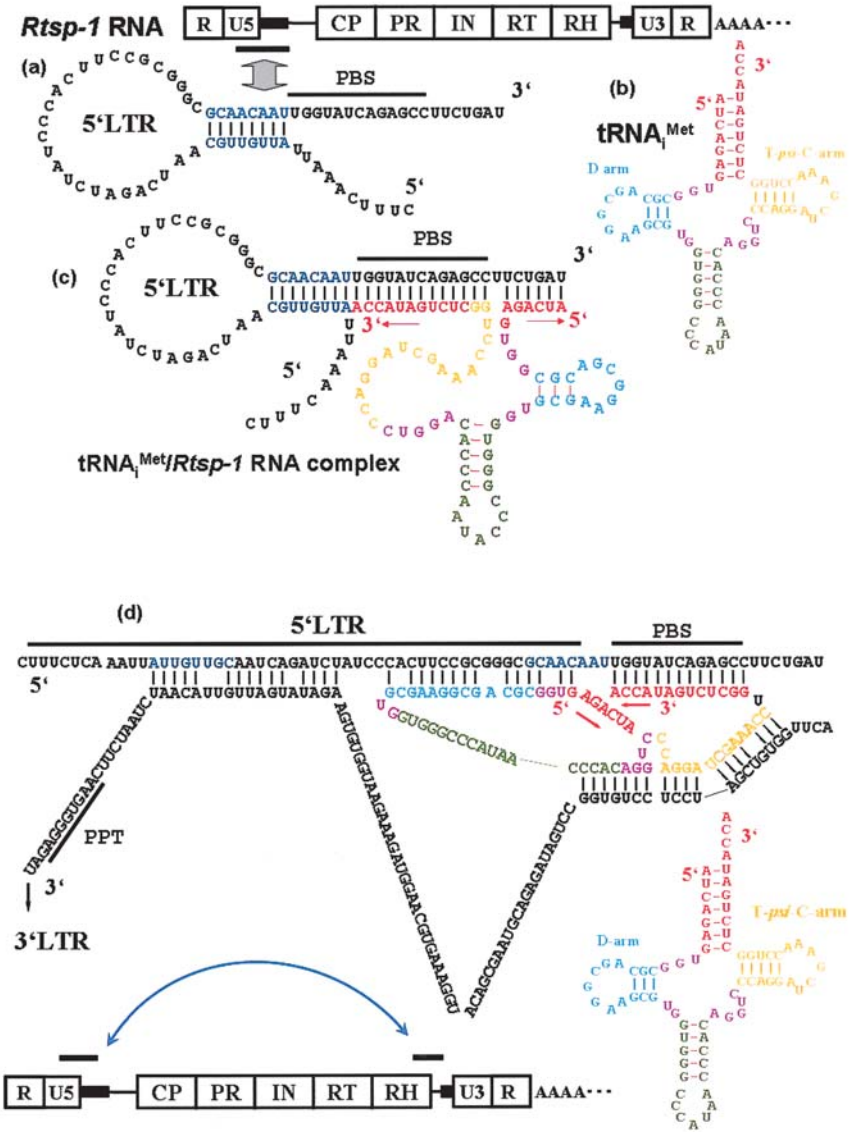


Fig. 2 A secondary structure model of the reverse transcription initiation complex consisting of *Rtsp-1* RNA and the initiator tRNA$^{Met}$.
(a) Free form of *Rtsp-1* RNA
(b) Initiator tRNA$^{Met}$
(c) Initial binary complex of *Rtsp-1* RNA and tRNA$^{Met}$
(d) Binary complex after base-pairing between the RNaseH terminus of *Rtsp-1* RNA and the T-*psi*-C-arm of tRNA$^{Met}$.

Cristofari *et al.*[10] have suggested that 5'-3' pairing induces a switch from a closed Ty1 RNA/tRNA$^{Met}$ structure (as shown by Friant *et al.*[8]) to an open structure that is competent for reverse transcription initiation. In this case, the synthesis of sssDNA is initiated only when the 3' end of the full-length RNA template is proximal to the initiation complex.

The 5'-3' pairing may have evolved to prevent aberrant reverse transcription of defective elements. In contrast to Ty1, the yeast Ty3 RNA has an additional tRNA binding site at the 3' LTR, which interacts with the T-*psi*-C and D-arms of the tRNA$^{Met}$ [11]. Initiation of cDNA synthesis requires the primer tRNA$^{Met}$ to anneal at both the PBS and 3' LTR sites. This double annealing causes circularization of Ty3 RNA via a tRNA bridge. Furthermore, the formation of a dimeric Ty3 RNA/tRNA$^{Met}$ structure appears to be essential for initiation of reverse transcription. The dimerization is likely to occur through interactions of acceptor-arms between two molecules of tRNA$^{Met}$, which contains a palindrome sequence of 12 nucleotides. In a proposed dimeric structure, the 5' end of the Ty3 RNA in one RNA/tRNA$^{Met}$ complex is placed in close proximity to the 3' end of the Ty3 RNA in the other complex[11]. This structure also facilitates the 5' to 3' transfer of the minus-sssDNA during minus-strand DNA synthesis.

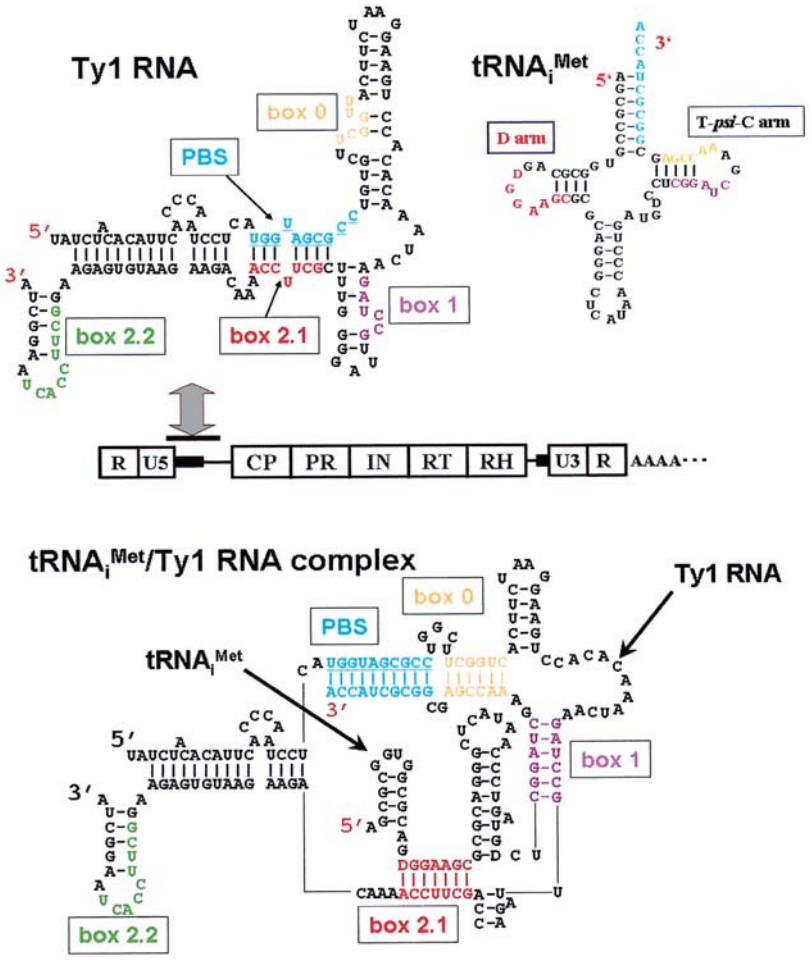


**Fig. 3 Structural models of Ty1 RNA, tRNA$^{Met}$, and the Ty1 RNA/tRNA$^{Met}$ complex.**
A sequence of 10 nucleotides in the PBS is complementary to the 3' end of the tRNA$^{Met}$. The sequences in boxes 0, 1, 2.1 and 2.2 are complementary to parts of the T and D stems and loops of tRNA$^{Met}$. Adapted from Friant *et al*.[9].

Retroviruses and LTR retrotransposons undergo a quite similar process of reverse transcription[12,13]. Lentiviruses, a subgroup of the retrovirus family that includes the human immunodeficiency virus (HIV), also possess a conserved sequence at the 3' end of their genomes, which is complementary to the anticodon stem of the primer tRNA$_3^{Lys}$. Interaction between the 3' end of the HIV genome and tRNA$_3^{Lys}$ increases the efficiency of the minus-sssDNA transfer[14].

## Conclusion

A highly ordered initiation complex is necessary for yeast LTR retrotransposons because only 10 or 8 nucleotides of the canonical PBS are complementary to the primer tRNA 3' end in Ty1 and Ty3, respectively. The *Rtsp-1* retrotransposon identified in this study has 13 nucleotides that are complementary to the tRNA, which is still short compared to 18 nucleotides in retroviruses. The hypothetical complex of the sweetpotato *Rtsp-1* retrotransposon RNA with tRNA$^{Met}$ may stabilize the tRNA$^{Met}$ priming by additional binding between the 5' end of the tRNA$^{Met}$ and the PBS downstream region. The complex appears to facilitate the minus-sssDNA transfer by making direct association of the *Rtsp-1* 3' end with the complex as required for the initiation of reverse transcription. During evolution of retrotransposons, it has been necessary to establish an effective control of the initiation of reverse transcription and the transfer of the minus-sssDNA using the limited materials available (the transcribed RNA and primer tRNA) within a confined space. Intriguingly, several distinct mechanisms were developed. The proposed structures of the retrotransposon RNA/tRNA complexes are strikingly different between the yeast Ty1, the yeast Ty3, and the sweetpotato *Rtsp-1*, even though the complexes have the same biological functions and the interacting domains of the primer tRNA are identical (*i.e.* the T-*psi*-C and D-arms, and the 3' end).

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# 転移能を有するサツマイモ・レトロトランスポゾン 塩基配列から推定される逆転写開始複合体の特徴

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カルスにおける転移が示されたサツマイモ LTR 型レトロトランスポゾン（*Rtsp-1*）の塩基配列を調べたところ，逆転写が開始される際，転写された *Rtsp-1* の RNA と最初の逆転写のプライマーに使われる $tRNA^{MET}$との間で，特徴的な逆転写開始複合体を形成し，この複合体が最初の逆転写とその後の過程で必要な逆転写産物（cDNA）の転移などを確実なものとしていることが示唆された．その内容は，1）転写された *Rtsp-1* の RNA 逆転写開始部位の塩基配列は自身の LTR 配列とステム構造をとること，2）$tRNA^{MET}$が結合する *Rtsp-1* の Primer Binding Site 部位には，プライマーの機能を果たす $tRNA^{MET}$の 3'末端の相補配列に加えて，その隣接部位に $tRNA^{MET}$の 5'末端部位と相補的な結合部位が存在するために，$tRNA^{MET}$の両末端が結合すること，3）*Rtsp-1* の 3'末端側に，$tRNA^{MET}$及びステム構造に関わる 5'LTR の部位との相補配列があり，この 3'末端側が転写開始複合体と結合することにより，ステム構造が崩れて逆転写が開始されると推定されること，4）逆転写が開始された後も，$tRNA^{MET}$の結合によって *Rtsp-1* の 5'末端と 3'末端側に近接した状態が保たれることである．*Rtsp-1* の 3'末端側の転写開始複合体への結合を転写開始の条件とすることにより，最初に合成される cDNA の 3'末端への転移が容易となることなどが示唆された．