

# Yeast Ty1 retrotransposon: the minus-strand primer binding site and a *cis*-acting domain of the Ty1 RNA are both important for packaging of primer tRNA inside virus-like particles

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## ABSTRACT

Reverse transcription of the yeast retrotransposon Ty1 is primed by the cytoplasmic initiator methionine tRNA (tRNA<sub>i</sub><sup>Met</sup>). The primer tRNA<sub>i</sub><sup>Met</sup> is packaged inside virus-like particles (VLPs) and binds to a 10 nucleotides minus-strand primer binding site, the (–)PBS, complementary to its 3' acceptor stem. We have found that three short sequences of the Ty1 RNA (box 1, box 2.1 and box 2.2) located 3' to the (–)PBS are complementary to other regions of the primer tRNA<sub>i</sub><sup>Met</sup> (TΨC and DHU stems and loops). Reconstitution of reverse transcription *in vitro* with T7 transcribed Ty1 RNA species and tRNA<sub>i</sub><sup>Met</sup> purified from yeast cells shows that the boxes do not affect the efficiency of reverse transcription. Thus the role of the boxes on packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs was investigated by analysing the level of tRNA<sub>i</sub><sup>Met</sup> packaged into mutant VLPs. Specific nucleotide changes in the (–)PBS or in the boxes that do not change the protein coding sequence but disrupt the complementarity with the primer tRNA<sub>i</sub><sup>Met</sup> diminish or abolish the packaging of the tRNA<sub>i</sub><sup>Met</sup> within the VLPs. We propose that base pairing between the primer tRNA<sub>i</sub><sup>Met</sup> and the Ty1 RNA is of major importance for tRNA<sub>i</sub><sup>Met</sup> packaging into the VLPs. Moreover the intactness of the boxes is essential for retrotransposition as shown by the transposition defect of a Ty1 element harboring an intact (–)PBS and mutated boxes.

## INTRODUCTION

Ty elements are a family of retrotransposons from the yeast *Saccharomyces cerevisiae* (reviewed in 1). The best characterized element Ty1 (2), is related to the  *copia*  class of plant and animal elements. It is 5.9 kilobases (kb) in length and consists of a central

region with two open reading frames TYA and TYB, flanked by two long terminal repeat (LTR) sequences of 334 base pairs (bp). The life cycle of Ty1 is very similar to that of several animal retroviruses which alternate their genetic material between RNA and DNA (3). Ty1 is transcribed from LTR to LTR forming an RNA that is packaged into intracytoplasmic virus-like particles (VLPs) containing the Ty1 encoded reverse transcriptase (RT), the core proteins, the Ty1 RNA and the replication primer tRNA<sub>i</sub><sup>Met</sup>. Initiation of cDNA synthesis by reverse transcriptase starts near the 5' end of the retrotransposon RNA just 3' to the upstream LTR using the base-paired cellular initiator tRNA<sub>i</sub><sup>Met</sup> as a primer (4, 5). The acceptor stem of the primer tRNA<sub>i</sub><sup>Met</sup> is unwound, 10 nucleotides at the 3' end of the primer form a base-paired duplex with the specific site in the Ty1 RNA termed the minus-strand primer binding site [(–)PBS] and cDNA synthesis proceeds from the 3'-hydroxyl end of the primer in the 5' to 3' direction. Until now the *cis*- or *trans*-acting components within the Ty1 VLPs involved in packaging or positioning of the primer tRNA<sub>i</sub><sup>Met</sup> have not been determined. Chapman *et al.* (4) have found that complementarity between the 3' terminus of the acceptor stem of the primer tRNA<sub>i</sub><sup>Met</sup> and the (–)PBS is not required for packaging of the tRNA<sub>i</sub><sup>Met</sup> within VLPs suggesting that the acceptor stem region is not a critical domain for the tRNA<sub>i</sub><sup>Met</sup> packaging signal. Here we demonstrate that a *cis*-acting domain of the Ty1 RNA is important for the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs. This domain is located 3' to the (–)PBS and comprises three short sequences (box 1, box 2.1 and box 2.2) complementary to the TΨC and DHU stems and loops of the tRNA<sub>i</sub><sup>Met</sup>. Specific nucleotide changes in the (–)PBS or in the boxes that do not change the TYA protein coding sequence but that disrupt the complementarity with the primer tRNA<sub>i</sub><sup>Met</sup> diminish or abolish the packaging of the tRNA<sub>i</sub><sup>Met</sup> within the VLPs. We propose that base pairing between the primer tRNA<sub>i</sub><sup>Met</sup> and the retrotransposon RNA is of major importance for tRNA<sub>i</sub><sup>Met</sup>

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packaging into the VLPs. A mutant Ty1 element with an intact (–)PBS but with mutations in the boxes was checked for its ability to transpose and showed a transposition defect. Thus the domain of Ty1 RNA comprising the boxes is not only important for packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs but is also essential for transposition.

## MATERIALS AND METHODS

### Strains, plasmids and media

The yeast strain FF18733 (*Mat a*, *leu 2-3,112*, *ura 3-52*, *trp1-289*, *his 7-2*, *lys 1-1*) was kindly provided by F.Fabre. Plasmids pX3 (6) and pJEF1105 (4), kindly provided by J.Boeke, are high copy number (2 $\mu$ m) plasmids marked with URA3 containing a Ty1-TRP1 or a Ty1-*neo* element fused to the GAL1 promoter. Culture media and growth conditions were as described (5,7).

### Ty1 VLPs and RNA isolation

Ty1 VLPs were isolated from strain FF18733 transformed with plasmid pX3 or its derivatives. Ty1 VLPs were purified using a method described by Eichinger and Boeke (8) with minor modifications and a further purification on Sephacryl S1000 (5,7). Extraction of nucleic acids from VLPs and electrophoresis of tRNA on monodimensional polyacrylamide gel (1D PAGE) were performed as described (5,7).

### Quantification of initiator tRNA<sub>i</sub><sup>Met</sup>

Ty1 RNA was extracted from VLPs isolated from 700ml cultures, fractionated on 1D PAGE, blotted on a Hybond N<sup>+</sup> membrane and hybridized with tRNA<sup>Ser</sup> GCU or initiator tRNA<sub>i</sub><sup>Met</sup> specific probes (5) Given that the recovery of VLPs varies from one preparation to another, the amount of material to be loaded on the gel was estimated from the reverse transcriptase activity of purified VLPs. tRNA<sup>Ser</sup> GCU, a tRNA species previously shown to be specifically packaged into VLPs (5) was used as an internal control to evaluate the actual amount of RNA loaded and to take into account the losses occurring during RNA preparation and transfer on the blot.

### Construction of mutants

Mutants P, B and PB were constructed using the method of Kunkel (9). A 0,57 kb *Xho*I – *Hpa*I fragment from plasmid pX3 was cloned into plasmid pSL1180 (Pharmacia 27-4384) digested with *Xho*I and *Hpa*I. The P and B mutations were done with the oligodeoxynucleotides P 7508, 5'CCAATTCTCATGGGT-CGGCGTGTGCTTCGG3', and B 7526, 5' CACAAATCAA-GACCCCTAGACGTTTCAGCAAGTAAAACAGAAGAAT-GTGAGAAGGCAAGTACCAAGGCTAACT3'. Mutation PB was made using the two mutagenic oligodeoxynucleotides P 7508 and B 7526. The mutated 0,57 kb *Xho*I – *Hpa*I fragments were subcloned into pX3 or pJEF1105.

### Transposition assay

The transposition assay was performed as described by Chapman *et al.* (4): Yeast strains harboring the wild type or mutant Ty1 elements on pJEF1105 were grown as patches on synthetic complete medium lacking uracil and containing 2% galactose (SC-Ura+Gal) at 22°C for 5 days. The cells were replica-plated to non-selective medium YPD to allow for plasmid loss. Following 1 or 2 days of growth at 30°C the patches were replica-

plated to SC medium containing 1 mg of 5-fluoroorotic acid (5FOA) per ml to select for cells that have lost the pJEF1105 plasmid containing the URA3 gene. After one day of growth at 30°C the plates were again replicated to SC+5FOA medium and grown at 30°C overnight. Cells were finally replica-plated to YPD containing 75  $\mu$ g of G418 per ml to identify colonies that had undergone transposition of the Ty1-*neo* element.

### Reverse transcription *in vitro*

*In vitro* reverse transcription was done with avian myeloblastosis virus (AMV) reverse transcriptase as follows: tRNA and template RNA were mixed, heated at 90°C for 2 min, cooled at 70°C for 10 min and slowly returned to room temperature. tRNA-primed DNA synthesis was done at 37°C in 50mM Tris–HCl, 50mM KCl, 10mM dithiothreitol, 12 mM MgCl<sub>2</sub>, 2,5 mM of each dNTP, 3,75  $\mu$ g tRNA<sub>i</sub><sup>Met</sup>, 0,16  $\mu$ g template RNA, 0.5U AMV reverse transcriptase, in a final volume of 25  $\mu$ l. Yeast tRNA<sub>i</sub><sup>Met</sup> purified according to published procedures (10) was 5' end labelled with T4 polynucleotide kinase in the presence of [ $\gamma$ -<sup>32</sup>P] ATP.

A 5' end labelled oligodeoxynucleotide primer complementary to 19 bases of the template was used as a control for the addition of equal amounts of template RNA to each reaction.

Reactions were continued for 5 to 60 min. The products were collected by ethanol precipitation and analysed on 8% polyacrylamide/8M urea denaturing gels.

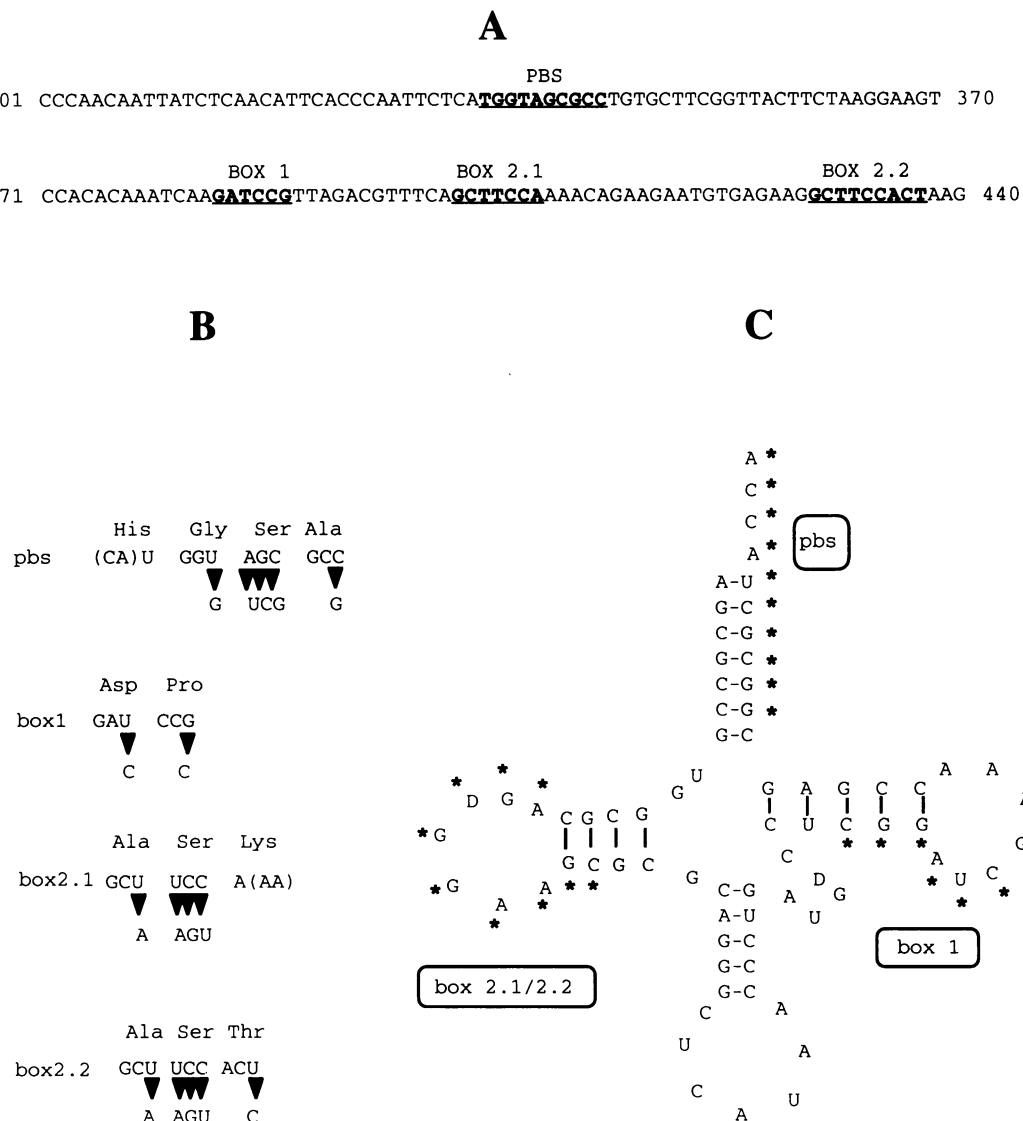
Gels were analysed with a Fuji Bio-Imager BAS2000 and quantified by direct computer analysis of the stored images.

### Production of synthetic templates

Two fragments (A and B) of Ty1 DNA were amplified by PCR and cloned into the *Eco*R5 and *Bgl*2 sites in the polylinker of the plasmid pSP72 (Promega P2191). Fragment A and B include nucleotide 245 to nucleotide 383 and nucleotide 245 to nucleotide 463 of the Ty1 genome respectively. Recombinants were named pSP1 (fragment A) and pSP1-4 (fragment B). Templates 1 and 3 were produced by run-off transcription with T7 polymerase of plasmid pSP1 digested with *Eco*R5 or *Xho*I respectively. Plasmid pSP1-4 was digested with *Cl*aI to produce template 2.

## RESULTS

The Ty1 sequence (2) extending from nucleotides 301 to 440 is shown in Fig.1A. This sequence includes the minus-strand PBS and the three short sequences (box 1, box 2.1 and box 2.2) complementary to the T $\Psi$ C and DHU stems and loops of the initiator tRNA<sub>i</sub><sup>Met</sup> primer. The regions of complementarity in the tRNA<sub>i</sub><sup>Met</sup> are indicated in figure 1C: box 1 is complementary to 6 bases in the T $\Psi$ C stem and loop of the tRNA. Boxes 2.1 and 2.2 comprise respectively 7 and 9 nucleotides complementary to the same region of the tRNA<sub>i</sub><sup>Met</sup> (two nucleotides in the DHU stem and 5 or 7 nucleotides in the DHU loop). Interactions between the boxes and the primer tRNA<sub>i</sub><sup>Met</sup> may be important for the retrotransposon replication and we hypothesized that they could play a role in the positioning of the primer tRNA<sub>i</sub><sup>Met</sup>, in the efficiency of initiation of reverse transcription and/or in the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs. Reconstitution of reverse transcription *in vitro* was used to test the role of the boxes in the initiation of reverse transcription. To investigate the role of the boxes in the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs, mutations that disrupt the complementarity with the



**Figure 1.** (A) Nucleotide sequence of the Ty1 RNA including the (-)PBS and the three boxes (1, 2.1 and 2.2) complementary to the TΨC and DHU stems and loops of the tRNA<sub>i</sub><sup>Met</sup>. The Ty1 RNA is 5918 bp in length. The nucleotide sequence shown here extends from nucleotide 301 to 440. (B) Construction of mutant Ty1 genes. Mutations in the (-)PBS and in the boxes that do not disrupt TYA protein coding but disrupt complementarity with the tRNA<sub>i</sub><sup>Met</sup> are indicated. (C) Cloverleaf structure of yeast tRNA<sub>i</sub><sup>Met</sup>. The regions of the tRNA<sub>i</sub><sup>Met</sup> complementary to the (-)PBS and to the boxes are indicated.

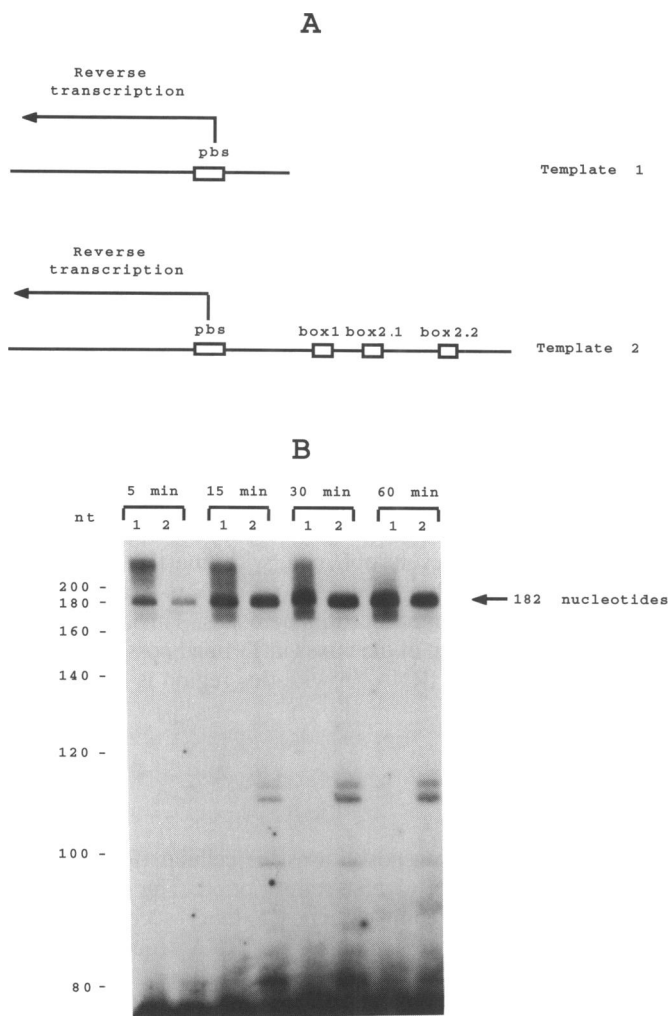
tRNA<sub>i</sub><sup>Met</sup> without changing the TYA protein coding sequence were introduced in the three boxes and/or in the (-)PBS (Fig. 1B). Yeast cells were transformed with the mutant Ty1 genes and the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> in the VLPs isolated from the transformed cells was examined.

**Reconstitution of reverse transcription *in vitro***

Reverse transcription experiments were done *in vitro* with T7 transcribed RNA templates and tRNA<sub>i</sub><sup>Met</sup> purified from yeast cells to test whether the interactions between the boxes and the primer tRNA<sub>i</sub><sup>Met</sup> play a role in the efficiency of reverse transcription. Three template RNAs were produced: template 1 is a 162 bases segment of Ty1 RNA which does not contain the boxes but contains the (-)PBS and 45 bases 3' of the (-)PBS; template 2 is a 238 bases segment containing the three boxes,

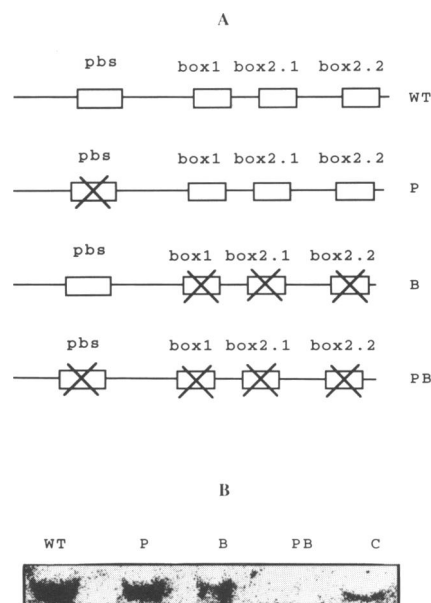
the (-)PBS, 107 bases 5' of the (-)PBS and 28 bases 3' of box 2.2; template 3 is a 236 bases segment in which the boxes have been replaced by sequences from the procaryotic plasmid pSP72.

Template Ty1 RNAs were annealed to the primer tRNA<sub>i</sub><sup>Met</sup>. Once annealed, the reconstituted Ty1 RNA-tRNA<sub>i</sub><sup>Met</sup> duplexes were analysed for their abilities to support cDNA synthesis catalysed by AMV reverse transcriptase. The primed run-off molecule is predicted to be 182 bases in length (107 nucleotides of cDNA covalently attached to 75 nucleotides of the primer tRNA). The reaction products were analysed on a 8% denaturing polyacrylamide gel followed by autoradiography. Results obtained with templates 1 and 2 are shown in Fig.2B. A quantification of the 182 nucleotide band shows that the amount of this specific run-off is identical for both templates. It seems therefore that the boxes are not required for efficient initiation of reverse



**Figure 2.** Reconstitution of reverse transcription *in vitro*. (A) Schematic representation of the Ty1 RNA templates used in the reverse transcription assay. Reverse transcription is from the (-)PBS using yeast tRNA<sub>i</sub><sup>Met</sup> as a primer. Template 1 is 162 nucleotides in length and ends one nucleotide 5' of box 1. Template 2 is 238 nucleotides in length and comprises the (-)PBS and the three boxes. (B) Autoradiography of a polyacrylamide gel showing products of the tRNA<sub>i</sub><sup>Met</sup> primed DNA synthesis obtained with template 1 and 2. Reverse transcription was performed for 5 min., 15 min., 30 min. and 60 min. as indicated in Materials and Methods. The arrow points to the run-off transcript of the appropriate size (107 nucleotides of DNA covalently attached to 75 nucleotides of the primer tRNA).

transcription. There is however a qualitative difference between the cDNA patterns obtained with template 1 and 2: for template 2 the 182 nucleotide fragment is the only run-off product whereas fragments of higher molecular weight running above the 182 bases band are obtained with template 1. This is particularly marked at short times (5 min. and 15 min.) of incubation of the Ty1 RNA-tRNA<sub>i</sub><sup>Met</sup> duplex with reverse transcriptase. Similar results were obtained with template 1 and 3 (data not shown) indicating that the presence of non-specific bands is not due to the smaller size of template 1. The presence of longer non-specific transcripts could be due either to non-specific initiation of reverse transcription or to strand transfer processes. Experiments are in progress to test these two possibilities. Another difference between template 1 and 2 is the presence of low molecular weight

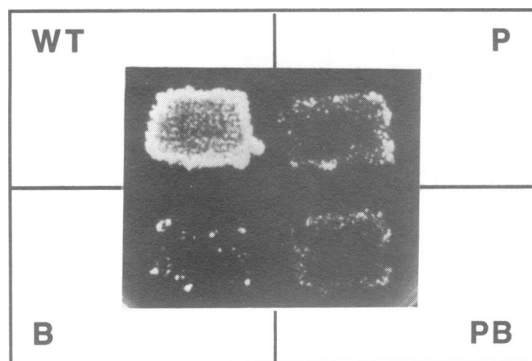


**Figure 3.** Packaging of tRNA<sub>i</sub><sup>Met</sup> in wild type (WT) and mutant (P, B and PB) Ty1 VLPs. (A) Schematic representation of the wild type (WT) and mutant (P, B and PB) Ty1 genes. Specific nucleotide changes that do not disrupt protein coding have been introduced in the (-)PBS (mutant P), in the boxes (mutant B) and both in the (-)PBS and in the boxes (mutant PB). Yeast cells were transformed with the Ty1 genes and the packaging of the tRNA<sub>i</sub><sup>Met</sup> in the VLPs isolated from the transformed cells was examined. (B) Northern blot analysis of tRNA extracted from wild type (WT) or mutant (P, B, PB) VLPs. RNA extracted from Ty1 VLPs was electrophoresed on a polyacrylamide gel and transferred to a nylon membrane. RNA blot analysis was carried out with a tRNA<sub>i</sub><sup>Met</sup> specific probe. Purified tRNA<sub>i</sub><sup>Met</sup> was loaded in lane C as a control. The blot was analysed with a Fuji Bio-Imager BAS2000 and quantified by direct computer analysis of the stored images.

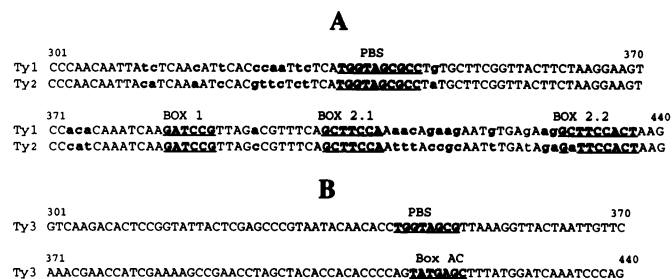
bands in the reverse transcripts of template 2. A possible explanation of this observation is that the interaction of the tRNA<sub>i</sub><sup>Met</sup> with the boxes forms a tight template-tRNA<sub>i</sub><sup>Met</sup> duplex which partially blocks the progression of reverse transcription. Structural studies to determine at the molecular level how the primer tRNA<sub>i</sub><sup>Met</sup> interacts with the Ty1 RNA will be necessary to test this hypothesis.

**Packaging of initiator methionine tRNA in Ty1 VLPs: role of the (-)PBS and of the three boxes**

To investigate the role of the (-)PBS sequences and of the three boxes in the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> in the VLPs we have introduced specific mutations in the (-)PBS and/or in the boxes that do not change the TYA protein coding sequence (Fig.1B) but that disrupt the complementarity with the primer tRNA<sub>i</sub><sup>Met</sup>. Three Ty1 mutants were constructed (Fig.3A): in mutant P the (-)PBS is mutated (the nucleotide changes are similar to those described by Chapman *et al.* (4)), in mutant B the three boxes are mutated and in mutant PB both the (-)PBS and the three boxes are mutated. Yeast cells were transformed with the plasmids bearing the mutant Ty1 genes and the presence of tRNA<sub>i</sub><sup>Met</sup> in the Ty1 VLPs was examined. The virus-like particles were purified as described in Materials and Methods and the nucleic acids were extracted. The RNA was fractionated on a polyacrylamide gel, transferred to a membrane and probed with a labeled oligonucleotide complementary to the tRNA<sub>i</sub><sup>Met</sup>



**Figure 4.** Transposition of wild type and mutant GAL1-Ty1 elements marked with *neo*. The transposition phenotype of wild type (WT) and three mutants (P, B and PB) elements is shown on YPD+G418 plates as described in Material and Methods.



**Figure 5.** Nucleotide sequence of Ty1, Ty2 and Ty3 around the (-)PBS. The (-)PBS and the sequences outside of the (-)PBS complementary to the tRNA<sub>i</sub><sup>Met</sup> are indicated in bold and are underlined. (A) The sequence divergence between Ty1 and Ty2 is about 20%. The nucleotides which differ in Ty1 and Ty2 are indicated in lowercase letters and are mostly located outside of the (-)PBS and of the boxes with the exception of one point mutation in box 2.2. (B) Ty3 has a (-)PBS of 8 nucleotides. A short sequence (designated Box AC) downstream of the (-)PBS is complementary to the anticodon loop of tRNA<sub>i</sub><sup>Met</sup>

(Fig.3B). As previously observed by Chapman *et al.*(4), tRNA<sub>i</sub><sup>Met</sup> is found in VLPs from cells transformed with mutant P. The tRNA<sub>i</sub><sup>Met</sup> is also found in VLPs from cells transformed with mutant B. In contrast the level of tRNA<sub>i</sub><sup>Met</sup> is very low in VLPs extracted from cells transformed with mutant PB. Quantification of tRNA<sub>i</sub><sup>Met</sup> indicates that, compared to wild type VLPs, 70% and 50% of tRNA<sub>i</sub><sup>Met</sup> is packaged in the P and B mutant VLPs respectively. In two independent PB mutant VLP preparations the level of primer tRNA<sub>i</sub><sup>Met</sup> packaged is less than 10% in one case (Fig.3B) and 16% in the second case (not shown). The difference observed between the two experiments could arise from a difference of contamination of the VLPs by cytoplasmic tRNA<sub>i</sub><sup>Met</sup>. The low level of tRNA<sub>i</sub><sup>Met</sup> in PB mutant VLPs is not due to a reduced amount of Ty1 RNA packaged into the VLPs since we have checked that PB mutant VLPs contain the same amount of Ty1 RNA as wild type VLPs. These results show that both the (-)PBS and the boxes contribute to the packaging of the tRNA<sub>i</sub><sup>Met</sup> in the VLPs. When the complementarity between the tRNA<sub>i</sub><sup>Met</sup> and both the (-)PBS and the boxes is disrupted, the packaging of primer tRNA<sub>i</sub><sup>Met</sup>

is greatly reduced, suggesting that base-pairing between the tRNA<sub>i</sub><sup>Met</sup> and the template RNA is a necessary condition for the packaging of primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs.

### Transposition of Ty1: role of the boxes

The mutations in the (-)PBS and in the boxes were introduced into a GAL1-Ty1 element marked with *neo* in order to look at the ability of these mutant elements to transpose. Growth on YPD/G418 indicates transposition of the Ty1-*neo* element in the host genome since the *neo* gene confers resistance to G418. As shown in Fig. 4, cells transformed with the wild type Ty1-*neo* element exhibit a high level of transposition. In contrast, transposition is greatly reduced in cells transformed with mutants P, B and PB. This is consistent with the observation that cDNA synthesis is impaired in P, B and PB VLPs (data not shown). The result obtained with mutants P and PB is not surprising since Chapman *et al.* (4) have previously shown that the nucleotide sequence of the (-)PBS region in Ty1 is essential for transposition. The result obtained with mutant B is more unexpected since in this mutant the (-)PBS is unchanged and mutations were only introduced in the boxes. This suggests that the nucleotide sequence of the boxes in Ty1 and possibly the base pairing of the primer tRNA<sub>i</sub><sup>Met</sup> with this region is essential for transposition.

## DISCUSSION

The yeast Ty1 retrotransposon uses the cellular tRNA<sub>i</sub><sup>Met</sup> as a primer for initiation of reverse transcription. The (-)PBS is a 10 nucleotides region complementary to the 3' acceptor stem of the primer. Closer examination of Ty1 RNA sequence reveals that three regions (box 1, box 2.1 and box 2.2) located downstream of the (-)PBS are complementary to the TΨC and DHU stems and loops of the primer tRNA<sub>i</sub><sup>Met</sup>. The ability to introduce specific mutations in the Ty1 elements provides an opportunity to examine the role of template sequences in the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> in the VLPs. We show here that the boxes and the (-)PBS both contribute to the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> into the VLPs, emphasizing the importance of the sequences outside of the (-)PBS in this process. Moreover a Ty1 element harboring an intact PBS and mutated boxes (mutant B) is defective for transposition and is impaired in cDNA synthesis although the primer tRNA<sub>i</sub><sup>Met</sup> is packaged into the mutant VLPs. This result must be compared to the results obtained *in vitro* with primer tRNA<sub>i</sub><sup>Met</sup> preannealed to RNA templates showing that interactions between the tRNA<sub>i</sub><sup>Met</sup> and the (-)PBS is sufficient for reverse transcription carried out by AMV reverse transcriptase. In contrast, *in vivo*, interaction of the primer tRNA<sub>i</sub><sup>Met</sup> with sequences outside of the (-)PBS seem to be necessary for the formation of a specific tertiary complex between tRNA<sub>i</sub><sup>Met</sup>, Ty1 RNA and Ty1 reverse transcriptase and for initiation of reverse transcription.

Interactions of the primer tRNA with sequences outside of the (-)PBS have also been found to be important in other systems. In ASLV, interactions between retroviral U5 RNA and the TΨC loop of the primer tRNA<sup>Trp</sup> are required for efficient initiation of reverse transcription (11). In Moloney leukemia virus, U5-IR stem mutations that disrupt the U5 RNA-tRNA<sup>Pro</sup> TΨC loop interaction result in a reverse transcription defect (12). Moreover, sequence and structure comparisons indicate that several

retroviruses have the potential to form primer/template interactions outside of the (-)PBS and suggest that these interactions may be important for the initiation of reverse transcription (11). On the other hand, Kohlstaedt and Steitz (13) show *in vitro* that recognition of viral sequences 3' to the primer binding site is important for the specificity of tRNA usage by HIV-RT. More recently it has been found that the interactions between HIV-1 RNA and its replication primer tRNA<sub>3</sub><sup>Lys</sup> is not limited to the base-pairing of the 18 3' terminal nucleotides of the primer with the (-)PBS but that a loop-loop interaction takes place between the anticodon loop of the tRNA<sub>3</sub><sup>Lys</sup> and a A-rich loop of the HIV1-RNA located upstream of the (-)PBS (14).

We have examined whether the other transposable elements from the yeast *Saccharomyces cerevisiae* can form primer/template interactions outside of the (-)PBS. Four functional families of transposable elements, Ty1 to Ty4, have been identified in *Saccharomyces cerevisiae* (2, 15-17). Ty1, Ty2 and Ty3 use tRNA<sub>i</sub><sup>Met</sup> as primer for minus strand cDNA synthesis whereas Ty4 uses tRNA<sup>Asn</sup>. The sequences around the (-)PBS of Ty1, Ty2 and Ty3 are shown in Fig.5. We find that in Ty2, which is closely related to Ty1, the three boxes 1, 2.1 and 2.2 have been conserved with the exception of one point mutation in box 2.2. In Ty3 a short sequence downstream of the (-)PBS has the potential to form an interaction with six bases of the anticodon loop of the primer tRNA<sub>i</sub><sup>Met</sup>. The retrotransposon Ty4 is quite different from the three others since it uses tRNA<sup>Asn</sup> as a primer and has a longer (-)PBS of 18 nucleotides. We have not found any region of complementarity longer than 4 bases between the Ty4 template and the primer tRNA<sup>Asn</sup> outside of the (-)PBS. It seems therefore that when the (-)PBS is short the boxes in Ty1 and Ty2 or the region complementary to the anticodon loop in Ty3 are part of a multipartite (-)PBS which ensures correct packaging and positioning of the primer tRNA in the VLPs. It is striking that nucleotides of the primer tRNA<sub>i</sub><sup>Met</sup> involved in the interactions with the sequences outside of the (-)PBS of Ty RNA are unpaired (i.d. seven nucleotides in the DHU loop for Ty1 and Ty2 or six nucleotides in the anticodon loop for Ty3). A first step of the recognition between the primer tRNA and the template could therefore take place without unwinding of the tRNA. Interactions with other factors (RT, nucleocapsid protein) would then promote the annealing of the primer 3'-hydroxyl end with the (-)PBS. Indeed a role for RT and/or nucleocapsid protein in the primer tRNA incorporation has been inferred from *in vitro* and *in vivo* experiments (18-23). Reverse transcriptase-negative avian sarcoma virions and mutant Moloney murine leukemia virions lacking reverse transcriptase include reduced amounts of primer tRNA (18, 19). *In vitro*, specific interactions between reverse transcriptase and their cognate primer tRNAs have been demonstrated for HIV-1 RT and AMV RT (20-23). Thus although base-pairing between the tRNA<sub>i</sub><sup>Met</sup> and the template RNA might be important for the packaging of the primer tRNA<sub>i</sub><sup>Met</sup> in the Ty1 VLPs, the mechanism of this process and of the cDNA priming is certainly more complex and may involve tRNA<sub>i</sub><sup>Met</sup> interactions with RT and nucleocapsid proteins. It will therefore be interesting to understand how reverse transcriptase interacts with the tRNA<sub>i</sub><sup>Met</sup> bound to the RNA template. Structural studies are now in progress to investigate the conformation of the Ty1 RNA and to determine at the molecular level how the primer tRNA<sub>i</sub><sup>Met</sup> interacts with the retrotransposon RNA and with reverse transcriptase.

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