Molecular determinants of Ty1 reverse transcription initiation in yeast.

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ABSTRACT

The *S. cerevisiae* genome contains several Ty retroelements, segments of DNA which produce virus-like particles (VLPs) containing mRNA that can be reverse-transcribed into cDNA and integrated into the genome. The Ty element has two products analogous to retroviral proteins: Gag and a Gag-Pol. Pol is cleaved into three separate proteins: protease, reverse transcriptase (RT), and integrase, which along with the mRNA transcript and a tRNA, are packaged into a VLP made of Gag. In the first step of reverse transcription, $tRNA_i^{Met}$ binds to the Ty1 mRNA primer-binding site (PBS), which has complementarity to the $tRNA_i^{Met}$ acceptor stem, providing a primer for reverse transcriptase. This DNA strand is subsequently used as a template for the complementary strand, generating a double-stranded cDNA that is integrated into the genome. Previous work has shown that Ty1 transposition is substantially reduced by mutations outside of the $tRNA_i^{Met}$ acceptor stem, suggesting that the tertiary structure of $tRNA_i^{Met}$ has a significant role in the first priming step. Potential interaction sites can be explored by characterizing the interaction of Ty1 mutants that rescue transposition with mutant tRNA. We screened a library of mutations directed to distinct Gag and Pol regions of Ty1 for variations that rescue transposition using a mutant $tRNA_i^{Met}$. Ty1 transposition in yeast is assayed using a galactose-inducible plasmid-borne Ty1 element. The Ty1 element also contains a marker gene (*his3AI*) to select for transposition on media lacking histidine. Cells are patched to selective media to hold the plasmid, replica-plated to galactose-containing media to induce Ty1 transcription, and replica-plated to media lacking histidine to select for transposition. The first step in this project is to rescue selected Ty1 mutants and characterize their compatibility with wild type and mutant $tRNA_i^{Met}$ primer at permissive and non-permissive temperatures. Transposition assays have identified Ty1 mutants with compromised transposition at an elevated temperature and mutants that do not transpose with wild type $tRNA_i^{Met}$ at any temperature. Selected Ty1 mutants will be sequenced and also be tested for protein stability at the temperatures used in transposition assays.

BACKGROUND

- Retrotransposons in *S. cerevisiae* genome produce virus-like particles (VLPs).
- This project is investigating the Ty1 retroelement, which consists of a protein-coding region surrounded by long terminal repeats (LTRs).[3]
- Ty1 encodes
  - Gag protein
  - Gag-Pol fusion protein (Gag, protease (PR), integrase (IN), & reverse transcriptase (RT)).
- Gag encapsulates PR, IN, RT, mRNA template, & $tRNA_i^{Met}$.
- $tRNA_i$ serves as primer for reverse transcription.
- RT copies mRNA into cDNA.
- IN integrates cDNA into genome.[2]

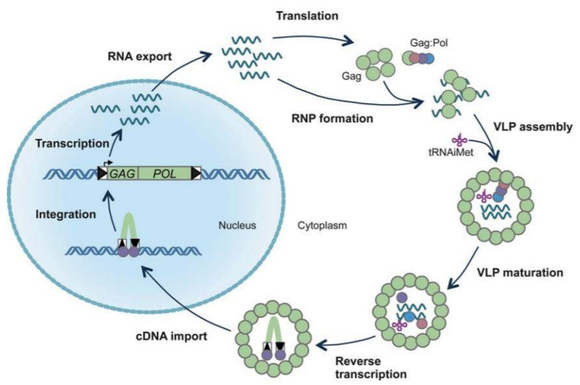

Fig. 1. Ty1 life cycle. Figure taken from Curcio, et al.

- Ty1 reverse transcription initiation
  - $tRNA_i^{Met}$ acceptor stem binds Ty1 primer binding site (PBS) (Fig. 2).

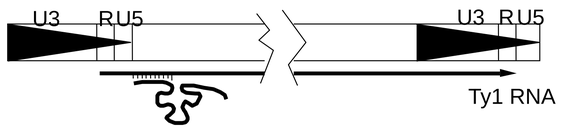

Fig. 2. tRNA priming of Ty1 reverse transcription. tRNA acceptor stem shown bound to Ty1 PBS. Dashed arrow represents cDNA extending from primer. Figure taken from Keeney, et al.

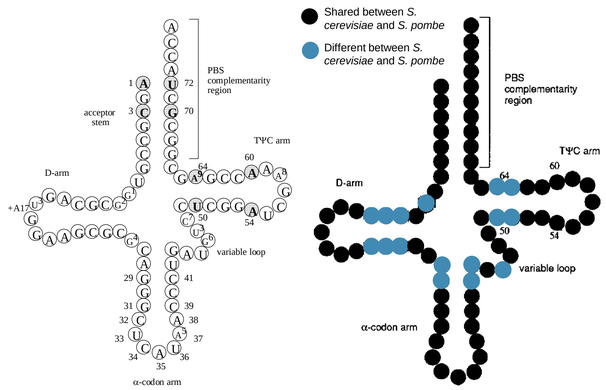

Fig. 3. A) *S. cerevisiae* $tRNA_i^{Met}$ sequence. Acceptor stem region is complementary to the Ty1 PBS. The site of pVIT83 mutation, position 50:64, is also highlighted. B) *S. pombe* $tRNA_i^{Met}$ with *S. cerevisiae* acceptor stem highlighting differences. Figure adapted from Keeney, et al.

- Ty1 transposition can be reduced by mutations outside the acceptor stem.
  - pVIT83 $tRNA_i^{Met}$ mutation (U50:A64 → G50:C64), see Fig. 3A.
    - Supports transposition at 20% of frequency from WT $tRNA_i^{Met}$.[3]
  - *S. pombe* $tRNA_i^{Met}$ with *S. cerevisiae* acceptor stem, see Fig. 3B.
    - Transposition not supported.[3]
- Some Ty1 mutants rescue transposition with mutant $tRNA_i^{Met}$.[3]

RESEARCH QUESTION

What factors outside of the PBS contribute to initiation of reverse transcription in Ty1?

- To learn how interactions outside the PBS influence reverse transcription initiation, we will look at Ty1 mutants that rescue transposition with mutant $tRNA_i^{Met}$.
- Ty1 mutants created by gap repair were screened for compatibility with *S. pombe* $tRNA_i^{Met}$ with *S. cerevisiae* acceptor stem.

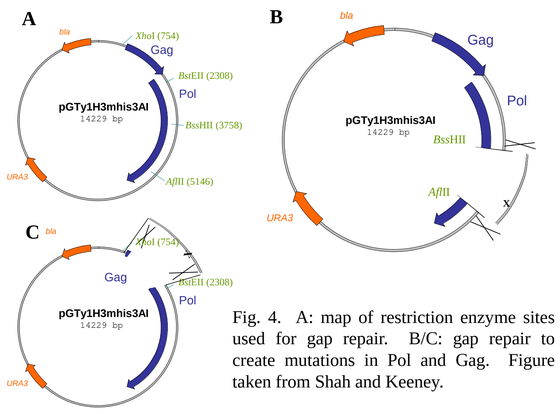

Fig. 4. A: map of restriction enzyme sites used for gap repair. B/C: gap repair to create mutations in Pol and Gag. Figure taken from Shah and Keeney.

- Mutants used in this project have previously been screened and selected to rescue transposition, but their level of transposition has not been characterized with WT $tRNA_i^{Met}$.

Assay System-galactose induction of Ty1

- Plasmid pGTy1H3*his3*AI (*URA3*/2-micron) containing galactose inducible Ty1 with *his3AI* marker.
- Growth on galactose containing media initiates transcription.
  - Galactose induction was done at 22°C and 33°C to maximize assay sensitivity.
- During reverse transcription, cells become histidine prototrophs through splicing-out of the artificial intron in the *his3* gene.
- Growth on media lacking histidine evidences Ty1 mobility.[1]

RESULTS

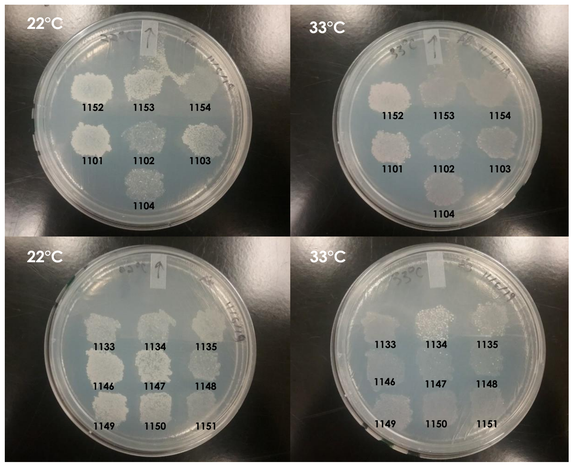

Fig. 5. A-B.) 1152-1154: Ty1 mutants with WT, pVIT83, and *imt4-9* $tRNA_i^{Met}$. Imt4-9 has acceptor stem mutations that prevent reverse transcription priming but allow translation. 1101-1104: WT Ty1 with WT, imt4-9, pVIT83, and *S. pombe* $tRNA_i^{Met}$. C-D.) 1133-1135: Ty1 mutants with *S. pombe* $tRNA_i^{Met}$. 1146-1148 & 1149-1151: Ty1 mutants with WT, pVIT83, and *imt4-9* $tRNA_i^{Met}$. Cells were patched thickly on glucose media lacking uracil, printed to galactose media after 1 day of incubation at either 22°C or 33°C, printed to media lacking histidine after 36 hrs, and imaged after 3 days.

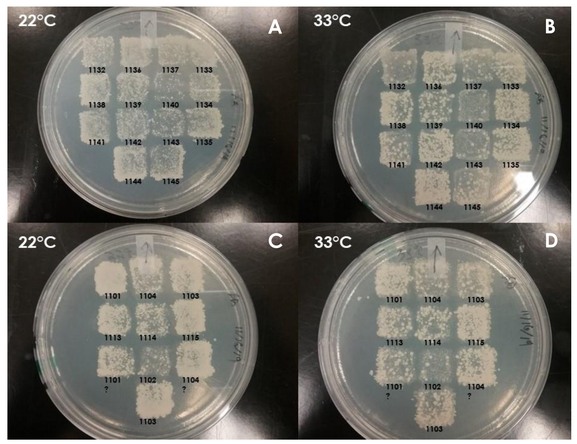

Fig. 6. A-B.) 1132-1143: Ty1 mutants with *S. pombe* $tRNA_i^{Met}$. 1144: WT Ty1 with WT $tRNA_i^{Met}$. 1145: WT Ty1 with *S. pombe* $tRNA_i^{Met}$. C-D.) 1101-1104: WT Ty1 with WT, *imt4-9*, pVIT83, and *S. pombe* $tRNA_i^{Met}$. 1113-1115: Ty1 mutants with *imt4-9*, *S. pombe*, and WT $tRNA_i^{Met}$. Cells were patched thickly on glucose media lacking uracil, printed to galactose media after 1 day of incubation at either 22°C or 33°C, printed to media lacking histidine after 36 hrs, and imaged after 3 days.

DISCUSSION

- Ty1 mutants were screened for rescue of transposition at 33°C (Fig. 5).
  - Differences in transposition between WT $tRNA_i^{Met}$/WT Ty1 and WT $tRNA_i^{Met}$/mutant TY1 or mutant $tRNA_i^{Met}$/WT Ty1 strains indicates that crucial interactions have been disrupted in priming of reverse transcription in the mismatched strain.
  - Rescue of transposition in mutant $tRNA_i^{Met}$/mutant Ty1 strains indicates that priming interactions have been restored.
  - 22°C is permissive of mobility, so growth may be too thick to see differences between samples.
  - Expect that molecular interactions will be sensitive to higher temperatures (33°C), allowing for a more sensitive assay.
- Transposition was compromised at 33°C in strains with mutants Ty1 and WT.
- Strains with mutant Ty1 and pVIT83 $tRNA_i^{Met}$ did not show transposition comparable to WT levels at 33°C.
  - Indicates that reverse transcription priming interactions have not been restored.
- Two out of three strains carrying mutant Ty1 and *S. pombe* with an *S. cerevisiae* acceptor stem rescued transposition.
- More Ty1 mutants were screened for rescue of transposition with *S. pombe* $tRNA_i^{Met}$ (Fig. 6).
- At 33°C, transposition was rescued to near-WT levels in mutants 1134, 1135, 1136, 1142, and possibly 1138.
  - Indicates restoration of reverse transcription priming interactions.

NEXT STEPS

- Retransform mutant Ty1 plasmids into strain carrying *S. pombe* $tRNA_i^{Met}$ to verify that the mutant plasmid rescues transposition.
- Rescue mutant Ty1 plasmids that appear to restore transposition at 33°C and transform into strain with WT $tRNA_i^{Met}$ to serve as comparison.
  - Reduced transposition with WT $tRNA_i^{Met}$ would indicate that a mutant did actually rescue transposition with *S. pombe* $tRNA_i^{Met}$.
  - WT level of transposition with WT $tRNA_i^{Met}$ would indicate that apparent rescue of transposition with *S. pombe* $tRNA_i^{Met}$ is due to stronger transposition overall with Ty1 mutant.
  - Plasmid rescue has been carried out; transformation of mutants into strain carrying WT $tRNA_i^{Met}$ is in progress.
- Sequence Ty1 mutants to map mutation locations and amino acid changes that may restore crucial interactions with *S. pombe* $tRNA_i^{Met}$ in initiation of reverse transcription.

REFERENCES

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