RNA polymerase II induced transcription of tRNA genes and processing of the mRNAs in yeast*

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Abstract Only 5′-halves were produced when the terminator sequence for RNA polymerase (pol) III transcription was inserted into the intron of yeast $tRNA^{Tyr}$ gene. If a promoter and a terminator for pol II transcription flanked it, the tRNA gene could be transcribed by pol II, but the transcripts could not be processed into mature tRNAs. In contrast, tRNA gene could also be transcribed by pol III and the transcripts could be processed into mature tRNAs even if a promoter and a terminator for pol II transcription flanked it. Pol II transcripts, modified with a self-cleaved hammerhead structure at 3′-end, were processed into mature tRNAs in the medium containing 100 mmol/L Mg^{2+}, indicating that the 3′-long trailer sequence blocks the maturation of tRNA gene transcripts by pol II.

Keywords: RNA polymerase, tRNA, transcription, processing, yeast.

Eukaryotic tRNA genes are transcribed by RNA polymerase III (pol III) and the promoter for pol III is located in the coding sequence. Therefore, it is difficult to remove the pol III promoter and force tRNA genes to be controlled by other promoters. All the control of tRNA gene expressions are not based on altering the pol III promoter, such as governing the dosage of tRNA genes[1], inserting a bacterial operon sequence around the start site of pol III transcription so that the pol III transcription can be blocked by their repressor proteins[2,3]. Recently. Drabkin et al. found that the expression of tRNAs could be indirectly regulated by aminoacyl tRNA synthetases[4]. We previously inserted a bacterial $tRNA^{Sec}$ gene into the site of downstream of yeast pol II promoter, and demonstrated that the $tRNA^{Sec}$ gene could be transcribed by RNA polymerase II but the transcripts could not be processed into mature molecules[5]. But how an eukaryotic tRNA gene can be transcribed by RNA pol II and whether the pol II transcripts can be processed correctly? The research was conducted to answer these questions.

1 Materials and methods

1.1 Oligos

All oligos used in this work are listed in table 1. They were synthesized by a Beckman oligo

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1000 DNA synthesizer, labeled with (γ-^{32}P) ATP (Yahui Company, China), and purified through denaturing polyacrylamide gel electrophoresis.

Table 1 Oligo sequences

Oligos	Sequences	Comments
Tyr1	^{32}P-gatcctcgca agaaatcgac tctcggtagc caagttggtt	
Tyr2	^{32}P-taaggcgcaa gactctaatt t(x)atcactac gaaatcttga g	tRNA gene assembly.
Tyr3	^{32}P-atcgggcgtt cgactcgccc ccgggagatt tttttatgtc g	(x represents -,t,tt,ttt,
Tyr4	^{32}P-gccttaaacc aacttggcta ccgagagtcg atttcttgcg ag	or ctt; y
Tyr5	^{32}P-gatttcgtag tgat(y)aaatt agagtcttgc	represents -,a,aa,aaa,
Tyr6	^{32}P-aattcgacat aaaaaaatct cccgggggcg agtcgaacgc ccgatctcaa	or aag)
1	^{32}P-cgcaagaaat cgactctcgg t	RT-PCR to determine pre-
2	^{32}P-caagatttcg tagtgataaa ttag	tRNAs. Oligos 1 + 2 for
3	^{32}P-caagatttcg tagtgataaa atta	WT; oligos 1 + 3 for MT;
4	^{32}P-caagatttcg tagtgataaa aatt	oligos 1 + 4 for MT2;
5	^{32}P-caagatttcg tagtgataaa aaat	oligos 1 + 5 for MT3;
6	^{32}P-caagatttcg tagtgataag aaat	oligos 1 + 6 for MCT2.
7	^{32}P-agtcttgcgc cttaaaccaa cttggctacc gag	oligo ligation to assay
8	^{32}P-ctcccggggg cgagtcgaac gcccgatctc aagatttag	amber suppressor tRNA^{Tyr}
9	^{32}P-gatccgtcga ccctgaagag ctcggttacg agcgaaactc cttggggagt cggtc	assembly DNAs encoding
10	^{32}P-gatcgaccga ctccccaagg agtttcgctc gtaaccgagc tcttcagggt cgacg	5′-hammerhead structure
11	^{32}P-aattggtcag gaccaattgg tcctctgatg aggcgcgtta gcgccgaaac cac	assembly DNAs encoding
12	^{32}P-tcgagttggt ttcggcgcta acgcgcctca tcagaggacc aattggtcct gacc	3′-hammerhead structure

1.2 Gene construction

tRNA genes (WT, MT, MT2, MT3 and MCT2) were constructed by assembling oligos Tyr 1—6 as described in ref. [6] and were further cloned into plasmid Bluescripts KS(+). DNAs encoding 3′-hammerhead structures were prepared through annealing oligos 11 and 12, and further inserted between restriction sites *Eco*RI and *Sal* I at the downstream of the tRNA gene. DNAs encoding 5′-hammerhead structure were constructed through annealing oligos 9 and 10, and further inserted at *Bam*H I site (at the upstream of tRNA gene) in correct direction. All constructs were confirmed by DNA sequencing and were further cloned into yeast plasmid Ycp 50.

1.3 Selection of positive clone and extraction of total RNAs

Yeast YPH 499(α: ura3-52, lys2-801^{amber}, ade 2-101^{ochre}, trp1$\triangle$63, his3$\triangle$200, leu2$\triangle$1) was transformed with the recombinant Ycp50 plasmids carrying different types of tRNA genes and spread on the plates of selective media SD (glucose as the only carbon source) or SG (galactose as the only carbon source) lacking lysine (Lys) to determine whether the amber suppresser tRNA gene was expressed. SD and SG include 0.17% nitrogen base without amino acids (Difco company, USA), 0.5% ammonium sulfate and 2% glucose or galactose. Yeast total RNAs were extracted with the method described in reference [7].

1.4 Transcription of suppressor tRNA^{Tyr} genes and maturation of the transcripts

Using yeast total RNAs as templates and oligos 1—6 as primers, RT-PCR was conducted according to the protocol provided by Promega company to analyze the transcription of the amber suppressor tRNA^{Tyr} genes. To determine whether matured suppressor tRNA^{Tyr} existed in the transformed yeast cells, oligos 7 and 8 were annealed with yeast total RNAs and then ligated. In the reaction, 2 μg yeast total RNAs, 1.8 pmole oligo 7, 1.8 pmole oligo 8, 10 mmol/L Tris-HCl and 0.1 mmol/L EDTA (pH7.0) were contained in the 15 ul annealing mixture. The annealing mixture was incubated at 95℃ for 5 min and then was allowed to cool naturally to room temperature. Oligos were ligated with T4-DNA ligase (Takara Biotechnical Company, Japan) according to the manufacturer's instruction. The ligated products were analyzed through denaturing polyacrylamide gel electrophoresis.

2 Results

2.1 tRNA gene construction

The constructed tRNA genes are shown in fig. 1. A total of 8 constructs of tRNA genes were obtained by oligo assembling: (i) wild type tRNA^{Tyr}(amber suppressor, WT); (ii) a single T insertion at position 3 of the intervening sequence (IVS), (MT1); (iii) a TT insertion at position 3 of IVS (MT2); (iv) a TTT insertion at position 3 of IVS (MT3); (v) a CTT insertion at position 3 of IVS (MCT2); (vi) the 5′-hammerhead structure was inserted at the upstream of MT3 construct (HH5′-MT3); (vii) the 3′-hammerhead structure was inserted at the downstream of MT3 (MT3-HH3′); and (viii) MT3 construct was modified with the 5′-hammerhead structure at the upstream and the 3′-hammerhead structure at the downstream (HH5′-MT3-HH3′). These constructs were cloned into Ycp 50 directly, or ligated with GAL 1—10 promoter at first (Gal 1—10 promoter was obtained by cutting plasmid pGT554, kindly offered by Dr. Kerstin Straby, Umea university, Sweden, with restriction enzymes *Eco*R I and *Bam*H I. The promoter was located at the upstream in the recombinant constructs, then cloned into Ycp 50. Yeast YPH 499 was transformed with Ycp 50 derivatives. The predicated transcripts and processing sites were shown in figure 2.

*Bam*H I ⟶

ggatcc tcgca agaaatcgac **tctcggtagc caagttggtt taaggcgcaa gactctaa**ttt ⬡

atcactacgaa **atcttgag atcgggcgtt cgactcgccc ccgggaga** tt tttttatgtc gaattc

Eco RI

Fig. 1. The chemically synthesized tRNA genes. Exons are written in bold letters, the nucleotide insertion between the position 3 and 4 of IVS is indicated with a hexagon (⬡). Underlined g is the start site of pol III transcription and the arrow represents the direction of pol III transcription.

2.2 Pol III transcription and processing

The pol III transcription and *in vivo* processing were not affected by inserting 1—3 nucleotide(s) at IVS, but the pol III transcription could stop at IVS if three nucleotides inserted formed a termina-

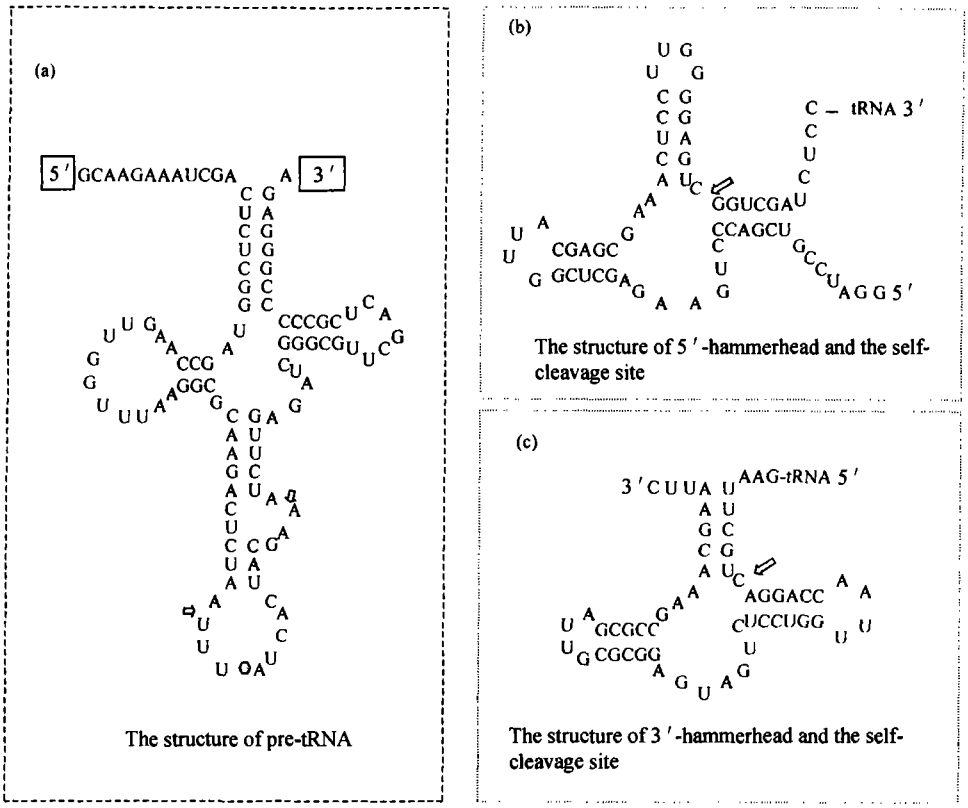

Fig. 2. The transcripts from different tRNA gene constructs. Pre-tRNA is shown on the left and splicing sites in pre-tRNA are indicated with arrows (a); the self-cleavage hammerhead structures used to modify pre-tRNA ends are shown on the right ((b) and (c)), cleavage sites in hammerhead structures are indicated with arrows.

tor. The yeast transformants with WT, MT and MCT2 could survive well on the medium SD without lysine, indicating that these constructs could be expressed *in vivo* and suppress yeast amber mutation. However, the transformants with MT3 could not survive on the medium SD without lysine, and as well as the transformants with MT2 (fig. 3(a)). RT-PCR with different combinations of the primers showed that no products appeared with MT3 RNAs primed with oligos 1 and 5, and a very faint band appeared with MT2 RNAs primed with oligos 1 and 4. The rest three constructs gave similar strong signals to one another (figure 3(b)).

2.3 Pol II transcription

The tRNA gene flanked by GAL 1 promoter and a terminator for pol II transcription could be transcribed efficiently by pol II in galactose medium, but the transcripts could not be processed into the mature tRNAs. The yeast transformants with GAL 1p-WT or GAL 1p-MCT2 could survive on both SD and SG media without lysine. However, the transformants with GAL 1p-MT3 could not survive on the synthetic media lacking lysine (fig. 4(a)), indicating that no mature amber suppressor $tRNA^{Tyr}$ existed in these transformants. The RT-PCR results indicated that GAL1p-MT3 in SD did not produce the intact tRNA gene transcripts (fig. 4(b), lane 2), but produced the intact transcripts in SG (fig. 4(b), lane 4).

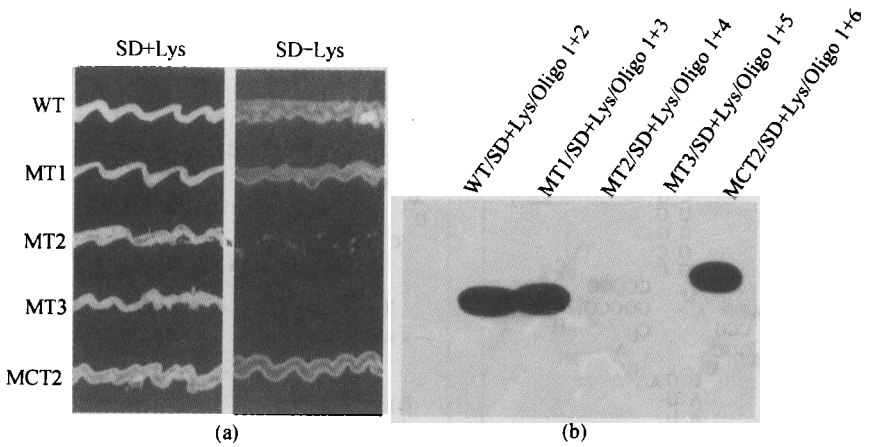

Fig. 3. Amber suppression by different $tRNA^{Tyr}$ constructs. (a) Genetic experiments. (b) RT-PCR with the templates RNAs (isolated from the transformants with Drabkin method) plus oligos 1 and 2,3, 4,5, or 6 and then analyzed on 20% polyacrylamide gel electrophoresis (containing 8 mol/L urea).

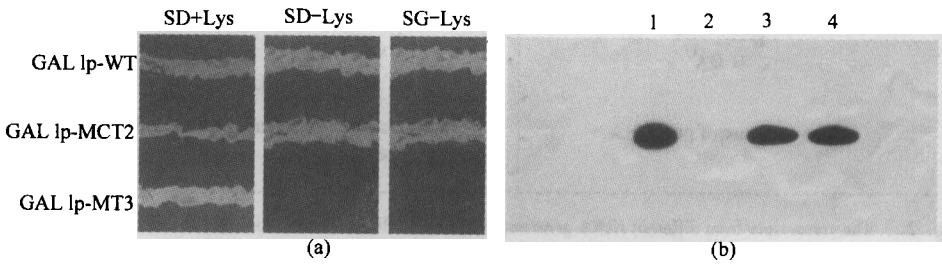

Fig. 4. The maturation of pol II transcripts of tRNA gene. (a) Genetic experiments. (b) RT-PCR with the templates RNAs (isolated from the transformants with Drabkin method) plus oligos 1 and 5 or 6 and then analyzed on 20% polyacrylamide gel electrophoresis (containing 8 mol/L urea). Lane 1: RNA templates were extracted from transformants with GAL1p-MCT2 grown in SD + lysine, primers for RT-PCR were oligos 1 and 6. Lane 2: RNA templates were extracted from transformants with GAL1p-MT3 grown in SD + lysine, primers for RT-PCR were oligos 1 and 5. Lane 3: RNA templates were extracted from transformants with GAL1p-MCT2 grown in SG + lysine, primers for RT-PCR were oligos 1 and 6. Lane 4: RNA templates were extracted from transformants with GAL1p-MT3 grown in SG + lysine, primers for RT-PCR were oligos 1 and 5.

2.4 The effect of modifications at the ends and Mg^{2+} concentration on the maturation of pol II transcripts

GAL1p-MT3, GAL1p-HH5′-MT3, GAL1p-HH5′-MT3-HH3′ and GAL1p-MT3-HH3′ could not suppress amber mutations in SG medium (fig. 5(a), left). When the concentration of Mg^{2+} in the medium increased to 50 mmol/L, some suppression was observed with Gal1p-HH5′-MT3-HH3′ and GAL1p-MT3-HH3′ (fig. 5(a), middle). Apparent suppression was observed with the last two constructs in the medium containing 100 mmol/L Mg^{2+} (fig. 5(a), right). Oligo ligations with oligos 7 and 8 also indicated that maturation was improved when the concentration of Mg^{2+} increased (fig. 5 (b)). With the modification of a self-cleaved hammerhead structure at 3′-end of tRNA, pol II transcripts could be processed into mature tRNAs if the medium contains 100 mmol/L Mg^{2+}.

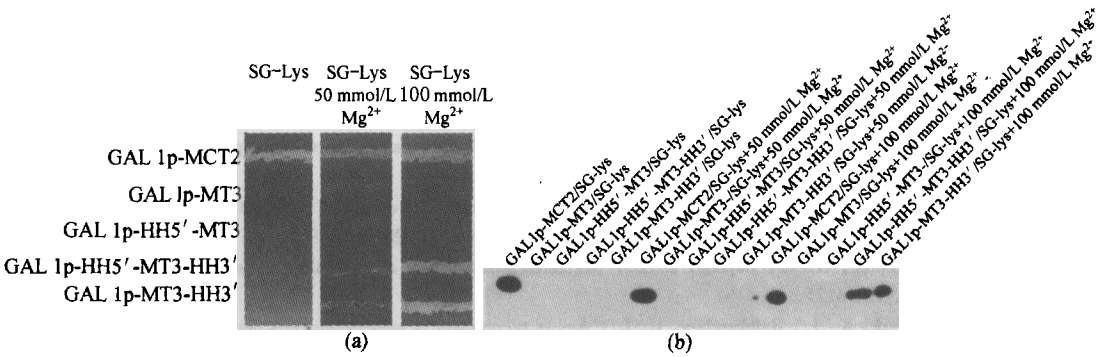

Fig. 5. The effect of modifications at the ends and Mg^{2+} concentration on the maturation of pol II transcripts. (a) Genetic results; (b) oligo ligation.

3 Discussion

When both pol II and pol III transcribed the same tRNA gene simultaneously, they did not interfere with each other. RT-PCR showed that GAL1p-MT3 could be transcribed efficiently in the medium SG (fig. 4(b)), indicating GAL1p-MT3 can be efficiently transcribed by pol II in galactose medium, because GAL1p-MT3 has a terminator of pol III in the intron and pol III transcripts should be halves. However GAL1p-MCT2 could suppress amber mutation both in galactose and glucose medium while GAL1p-MT3 could not, indicating that the mature tRNAs are from the pol III transcripts of GAL1p-MCT2. GAL1p-MCT2 would not suppress the amber mutation in galactose medium if pol III transcription was inhibited by pol II, or GAL1p-MT3 would not be transcribed efficiently in galactose medium if pol III transcription inhibited pol II transcription. Therefore, no interfering occurs when both pol II and pol III transcribe the same tRNA gene simultaneously.

3′-long trailer sequence blocks the maturation of 3′-end of tRNA transcript. In our previous study[5], a bacterial $tRNA^{Sec}$ gene was cloned at the downstream of an yeast pol II promoter, and Northern blot demonstrated that tRNA gene could be transcribed by pol II, but the transcripts could not be processed into the mature ones. In this work, an eukaryotic tRNA gene, which contains an authentic pol III promoter, was used. Although it is an eukaryotic tRNA gene, it can also be transcribed efficiently by pol II. No matured tRNAs were obtained in the transformants with GAL1p-HH5′-MT3, demonstrating that 5′-leading sequence has little effect on the maturation of the transcripts. The modification with a self-cleavage hammerhead structure at 3′-end is enough for the maturation of pol II transcripts. These results confirm that only the 3′-long trailer blocks tRNA maturation. The processing of pol II transcripts also suggest that tRNA transcription and processing *in vivo* are not a channel process.

High concentration of Mg^{2+} is required for the functions of the self-cleavage hammerhead structure. Our hammerhead structures function very well *in vitro* transcription, almost all T 7-transcripts were self-cleaved (data not shown). However, *in vivo* pol II transcripts can not be processed into mature tRNAs in SG medium without additional Mg^{2+}. Very high concentrations of Mg^{2+} are required to force the transcripts be processed into the mature ones. 100 mmol/L of Mg^{2+} is much higher than the concentration required *in vitro*, suggesting that the yeast cells can actually exclude Mg^{2+}. We also

observed that a lot of yeast cells could not survive in such high concentrations of Mg^{2+}.

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