Importance of the G27–A43 mismatch at the anticodon stem of *Escherichia coli* $tRNA^{Thr_2}$

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The $tRNA^{Thr2}$ isoacceptor of *E. coli* has a G–A mismatch at positions 27–43. When the anticodon of this tRNA was converted to an amber anticodon (CUA), this tRNA showed suppressor activity in *E. coli*. Moreover, introduction of the base pair (G–C or U–A) at positions 27–43 of this suppressor tRNA reduced its suppressor activity. These results indicate that the G27–A43 mismatch is necessary for full function of $tRNA^{Thr2}$.

E. coli; $tRNA^{Thr}$; Amber suppressor; G27–A43 mismatch

1. INTRODUCTION

Most tRNAs can be folded into a well-defined secondary structure called a 'cloverleaf' with four (or five) base-paired stems, though a few exceptions are found, such as some tRNAs in mitochondria. Usually, tRNAs have an anticodon stem constructed from five base pairs, but for the $tRNA^{Thr2}$ (anticodon CGU) of *E. coli*, only four base pairs can be formed. Positions 27 and 43, which usually make the first base pair of the anticodon stem, are occupied by G and A, respectively [1]. Previously, we have made an exhaustive survey of tRNA genes in the *E. coli* genome by using Kohara's library of the *E. coli* genome [2], and have revealed that there are 79 genes for 46 tRNA species on the *E. coli* K12 genome [3]. Among all the tRNAs encoded by *E. coli*, $tRNA^{Thr2}$ is the only tRNA species that has a mismatch at positions 27–43, although G27–A43 mismatches are found in the $tRNA^{Thr}$(CGU) of *Pseudomonas putida* (the tRNA corresponding to the *E. coli* $tRNA^{Thr2}$) [1], $tRNA^{Ile}$(GUU) of bacteriophage T4 [4] and $tRNA^{Asn}$(NAU) of T5 [5]; moreover other mismatches at positions 27 and 43 are reported for some tRNAs of eukaryotic cells [5].

The $tRNA^{Lys}$ encoded by the chloroplast genome of *Marchantia polymorpha* has a C27–C43 mismatch [6]. Even when this gene was introduced into *E. coli*, after changing its anticodon (UUU) to an amber anticodon (CUA) and deleting its intron by in vitro mutagenesis, the amber suppressor activity was not observed. However, mutants which showed amber suppressor activity could be isolated. By sequence analysis, it was shown that their $tRNA^{Lys}$ genes have a C–G pair at positions 27–43, altering C43 to G43 [6,7]. Therefore, it is possible that $tRNA^{Thr2}$ of *E. coli* may not work as a usual tRNA because of its G–A mismatch at positions 27–43. It is noteworthy that $tRNA^{Thr2}$ would be dispensable in the *E. coli* translation system, because $tRNA^{Thr4}$(UGU) could recognize the threonine codon ACG as well as ACA by wobbling.

We wanted to know whether or not the $tRNA^{Thr2}$ of *E. coli* is functional. To test this, we have changed the anticodon (CGU) to an amber anticodon (CUA) and determined the suppression of amber mutations in *E. coli*. It is possible that mutations at anticodon positions may cause a decrease in the efficiency of aminoacylation by the aminoacyl-tRNA synthetase. However, in the case of $tRNA^{Thr}$, this effect may be small since it was reported that the $tRNA^{Thr4}$ derivative with anticodon CUA is recognized by threonyl-tRNA synthetase at least partially and shows suppressor activity when expressed from multicopy plasmids [8].

2. MATERIALS AND METHODS

Plasmid pTH2 was constructed by sub-cloning of the 0.5 kb *HpaI–PstI* fragment containing *thrW* from λ clone 128 of Kohara's library [2] into pUC118 [9] by the methods described by Maniatis et al. [10]. Primers for in vitro mutagenesis (ACGACCTTCGCATTTAGAATGCGCTGCTCT for pTH2-1, CTACGACCTGCGCATTTA for pTH2-2 and pTH2-4, GAATGCGATGCTCTACCA for pTH2-3 and pTH2-4) were synthesized using ABI 380B DNA synthesizer, and mutagenesis was done using MUTA-GENE kit (Bio-Rad). To check amber suppressor activity of tRNA derivatives, plasmids carrying their genes were introduced into *E. coli* CA274 (HfrC, $lacZ_{am125}$, trp_{am}, Sup^-) [11] and the transformants were grown on a minimal plate containing lactose as a single carbon source and ampicillin (50 μg/ml). β-Galactosidase activities were measured by the method of Miller [12].

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3. RESULTS AND DISCUSSION

The *thrW* gene coding for $tRNA^{Thr2}$ had been detected near the *proBA* genes [13] by data base searching [1], and we have revealed that it is on the λ phage clone 128 (8G4) of Kohara's library [3]. Southern hybridization of this phage DNA with *E. coli* total tRNA as a probe showed that the 3.1 kb *Pst*I-*Pst*I fragment contained tRNA gene(s) (data not shown), which corresponded to the one carrying *proBA* and *thrW* sequenced by Deutch et al. [13]. These data allowed us to define the exact location of *thrW* and to construct a plasmid containing the *thrW* gene (Fig. 1, pTH2). Two bases (GT) in the anticodon of *thrW* on pTH2 were converted to TA by in vitro mutagenesis. The resulting plasmid (pTH2-1) carries a gene coding for a $tRNA^{Thr2}$ derivative with anticodon CUA (Fig. 2). These two plasmids were tested for their amber suppressor activity in *E. coli* CA274. The results in Fig. 3 show that pTH2-1, carrying $tRNA^{Thr2}$ gene in which the anticodon was changed to an amber anticodon (CUA), can suppress $lacZ_{am125}$ and trp_{am} of CA274. This indicates clearly that $tRNA^{Thr2}$ when converted to an amber suppressor is a functional tRNA.

We then wanted to test whether the presence of a base pair at position 27–43 affects the activity of the amber suppressor derived from $tRNA^{Thr2}$.

We constructed a set of plasmids carrying mutant $tRNA^{Thr2}$ genes, with the CUA anticodon and mutations at positions 27–43. The plasmids were pTH2-2, pTH2-3 and pTH2-4 having G27–C43, U27–A43 and C27–U43, respectively (Fig. 2). Suppressor activities in CA274 transformed with these plasmids were compared with pTH2-1. Judging from their colony growth rate on a minimal lactose plate, the suppressor activities are following order: pTH2-1 (G27–A43) = pTH2-2 (G27–C43) > pTH2-3 (U27–A43) > pTH2-4 (U27–C43). Interestingly, pTH2-4 showed very low activity, but higher than the basal level of pTH2 (Fig. 3). To confirm these results, we measured β-galactosidase activity of bacteria CA274 carrying the various plasmids (Table I). Relative β-galactosidase activity of pTH2-2, pTH2-3 and pTH2-4 are about 60%, 20% and 8%, compared with pTH2-1, respectively. It is possible

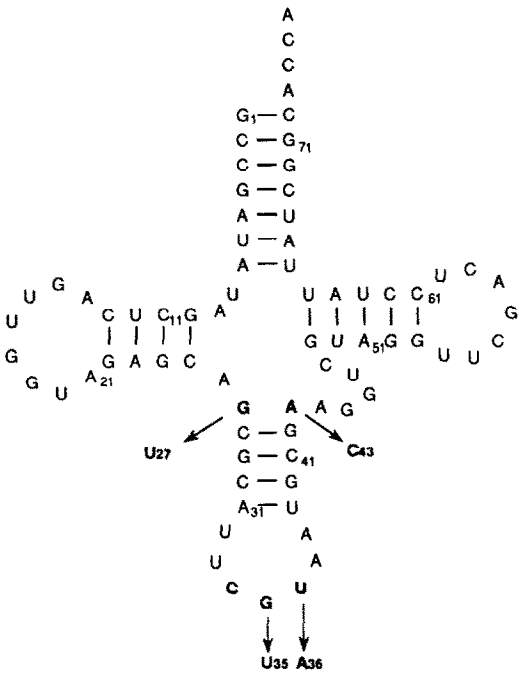

Fig. 2. Cloverleaf structure of $tRNA^{Thr2}$ and its derivatives. All nucleotides are shown in unmodified form. Anticodon sequence and the mismatched pair are shown in bold. Arrows indicate mutagenized nucleotides in each plasmid: pTH2-1, U35, A36; pTH2-2, U35, A36, C43; pTH2-3, U35, A36, U27; pTH2-4, U35, A36, U27, C43.

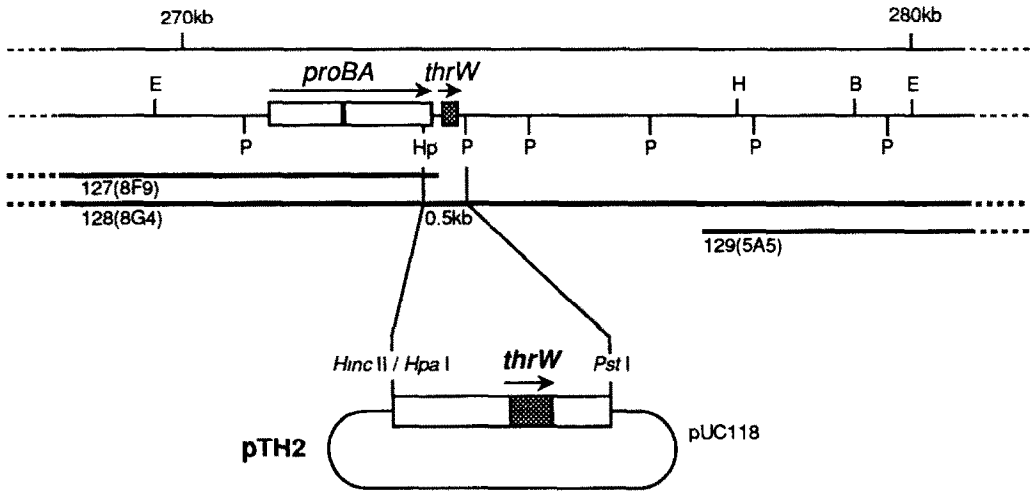

Fig. 1. Physical map of the *thrW* region of *E. coli* (above), and the plasmid pTH2 (below). Recognition sites for *Bam*HI (B), *Hin*dIII (H), *Eco*RI (E) and *Pst*I (P) are from Kohara et al. [3]. A *Hpa*I site is from Deutch et al. [12], though other *Hpa*I sites have not been determined. Bold lines indicate clones of Kohara's library. Arrows indicate the orientation of transcription.

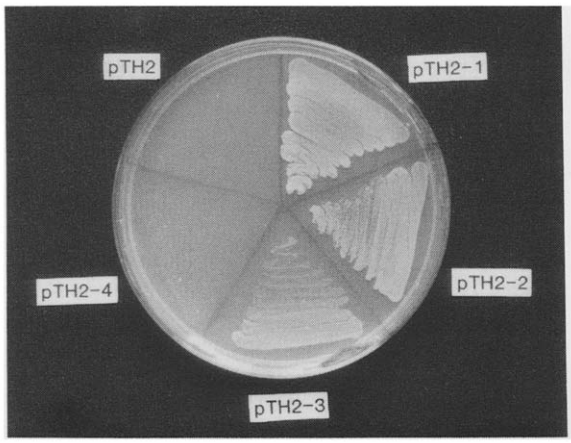

Fig. 3. Suppression of amber mutations in *E. coli* by $tRNA^{Thr2}$ derivatives. CA274 ($lacZ_{am125}$, trp_{am}) was transformed with plasmids pTH2 (wild type $tRNA^{Thr2}$), pTH2-1 (anticodon CUA, G27–A43), pTH2-2 (CUA, G27–C43), pTH2-3 (CUA, U27–A43), pTH2-4 (CUA, U27–C43). Each transformant was grown on a minimal lactose plate containing ampicillin at 37°C for 2 days.

that the differences in suppressor activity of these plasmids were due to the differences in tRNA levels, because some mutations in tRNA are known to cause an effect on its levels [14]. We compared tRNA levels expressed from pTH2 and its derivatives by Northern hybridization with a synthetic probe, and revealed that the mutations did not affect the level of tRNA (data not shown). These results indicate that both G27 and A43 (especially G27) of $tRNA^{Thr2}$ suppressor are necessary for its full function.

We do not know why the G27–A43 mismatched pair is optimal for the function of amber suppressor $tRNA^{Thr2}$, but possible explanations are: (i) G27 and/or A43 of $tRNA^{Thr2}$ are modified to be available for base pairing to each other, although the only modified base found at position 27 is pseudouridine, and no modified base at position 43 is known [5]. (ii) G27 and A43 are important for recognition by some other factor(s) including aminoacyl tRNA synthetase. Recently, it was shown that amber suppressor $tRNA^{Thr2}$ inserts lysine rather than threonine into suppressed protein [15]. The wild-type $tRNA^{Lys}$ of *E. coli* has a G27–U43 mismatch. This position might be somewhat important for recognition by lysyl-tRNA synthetase, and G27–A43 in $tRNA^{Thr2}$ could substitute for G27–U43 of $tRNA^{Lys}$. (iii) G27 and/or A43 might interact with bases on the other positions of this tRNA, and the tertiary structure of $tRNA^{Thr2}$ is slightly different from the other tRNAs.

Table I

Suppression of $lacZ_{am125}$ by $tRNA^{Thr2}$ derivatives

Plasmids	$tRNA^{Thr2}$			β-Galactosidase activity	Relative activity
	Anticodon	27	43		
pTH2-1	CUA	G	A	54.0	100.0
pTH2-2	CUA	G	C	33.7	62.4
pTH2-3	CUA	U	A	11.6	21.5
pTH2-4	CUA	U	C	4.4	8.1
pTH2	CGA (w.t.)	G	A	1.5	2.5

In any case, this is the first example to be shown that a mismatched pair (except G–U pairing) in a stem of a tRNA is important for its function.

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