EVOLUTION AND tRNA RECOGNITION OF THREONYL-tRNA SYNTHETASE FROM AN EXTREME THERMOPHILIC ARCHAEON, *Aeropyrum pernix* K1

Yoshiyuki Nagaoka, Kazuhide Ishikura, Atsushi Kuno and Tsunemi Hasegawa

Department of Material and Biological Chemistry, Faculty of Science,
Yamagata University, Yamagata 990-8560, Japan
Fax/Phone: +81-23-628-4604
E-mail: hasegawa@sci.kj.yamagata-u.ac.jp
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Abstract

An extreme thermophilic archaeon, *Aeropyrum pernix* K1 possesses two possible threonyl-tRNA synthetase genes. Sequence homology analysis of these genes with other species threonyl-tRNA synthetase showed that the shorter gene did not possess motif-2 and motif-3 of catalytic core that were conserved in class II aminoacyl-tRNA synthetases. On the other hand, the longer gene had almost all amino acids that were expected to be involved in substrate binding and catalytic activity. As a striking feature, it was found that the sequence of the longer threonyl-tRNA synthetase was unique in its quite compact N-terminal domain. This peculiar structure of *A. pernix* threonyl-tRNA synthetase may suggest one of the hints that can decipher not only the evolutionary position of this archaeon but also the evolutionary process for threonyl-tRNA synthetase. Cross-species aminoacylation experiments showed that threonyl-tRNA synthetase from *A. pernix* threonylated not only *Escherichia coli* threonine tRNA having A73 as a discriminator base, but also an extreme halophilic archaeon *Haloferax volcanii* threonine tRNA possessing U73. These results indicate that *A. pernix* threonyl-tRNA synthetase does not recognize the discriminator base like *E. coli* system.

Key Words: *Aeropyrum pernix* K1, *Haloferax volcanii*, *Escherichia coli*, archaea, threonyl-tRNA synthetase, threonine tRNA, cross-species aminoacylation, tRNA identity, discriminator base

Introduction

Correct recognition of tRNA by aminoacyl-tRNA synthetase (ARS) is essential to maintain accurate translation. To distinguish the cognate tRNA from a pool of various tRNAs consisting of a similar L-shaped tertiary structure, it was found that the ARS recognized a relatively small number of nucleotides of tRNA including anticodon bases and the discriminator base N73 (fourth nucleotide from the 3'-end of tRNA) together with the base pair(s) of the acceptor stem [1]. The tRNA identity elements of all kinds of amino acid tRNA have already been elucidated in the *Escherichia coli* system. However, little is known about tRNA recognition sites for ARS from archaea, which is classified as a third kingdom [1-3].

The discriminator base N73 of tRNA plays generally a crucial role in recognition by cognate ARS. However, A73 of *E. coli* $tRNA^{Thr}$ is not involved in threonylation by threonyl-tRNA synthetase (ThrRS) [4]. This is the only case of a discriminator base not contributing to recognition by the cognate ARS in the *E. coli* system [1]. On the other hand, it has been shown that the discriminator base of $tRNA^{Thr}$ from an extreme thermophilic eubacterium *Thermus thermophilus* and *Saccharomyces cerevisiae* was involved in recognition by the cognate ThrRS [5, 6]. To examine the importance

of the discriminator base of archaeal $tRNA^{Thr}$, cross-species aminoacylation between *E. coli* and archaea, an extreme halophilic archaeon *Haloferax volcanii* and an extreme thermophilic and aerobic archaeon, *Aeropyrum pernix* K1 in which the whole genome sequence was determined recently [7], was studied.

Materials and Methods

Sequence homology analysis of ThrRS genes

A search for the sequence of ThrRS from various organisms was made of the Aminoacyl Synthetase Database (http://rose.man.poznan.pl/aars/index.html). Comparisons of sequence homology among species were conducted by the BLAST program [8] and LALIGN program (http://www.ch.embnet.org/software/LALIGN_form.html). The origins of ThrRSs are *Homo sapiens*, *Saccharomyces cerevisiae* (Eukaryote), *Escherichia coli*, *Thermotoga maritima* (Prokaryote), *Methanococcus jannaschii and Pyrococcus horikoshii* (Archaea).

Bacterial growth and preparation of tRNA and ThrRS

E. coli Q13 was cultivated in 1L of medium (pH7.5) containing 10g of trypton, 8g of NaCl, 1g of glucose and 1g of yeast extract at 37℃. *A. pernix* K1 was cultivated in 1L of medium (pH7.0-7.2) containing 1g of yeast extract, 1g of trypton and 1g of $Na_2S_2O_3 \cdot 5H_2O$ in synthetic seawater Jamarin S (Jamarin Laboratory, Osaka, Japan) at 90℃. *H. volcanii* was cultivated in 1L of medium (pH6.8) containing 125g of NaCl, 51g of $MgCl_2$, 5g of K_2SO_4, 5g of yeast extract and 5g of trypton at 37 ℃. Unfractionated tRNA from each organism was isolated from cells by the standard phenol extraction method. ThrRS was partially purified using DEAE-TOYOPEARL (TOSOH, Japan) chromatography from S100 fraction except *H. volcanii* cells. ThrRS fraction from *H. volcanii* cells was prepared by the method of Gupta [9].

Aminoacylation assay

The aminoacylation assay was performed in a 50 μL reaction mixture containing 100mM HEPES-NaOH (pH 7.5), 10mM $MgCl_2$, 2mM ATP, 10mM KCl, 10 μM L-[U-^{14}C] threonine (208 mCi/mmol, NEN), unfractionated tRNA and partially purified ThrRS. For the aminoacylation assay of *H. volcanii* system, KCl concentration of the reaction mixture was adjusted to 3.8 M. The reaction was proceeded at 37℃ for *E. coli* and *H. volcanii*, and at 50℃ for *A. pernix* respectively.

Results and Discussion

Sequence homology analysis of ThrRS genes from A. pernix and other organisms

ThrRS belongs to class II ARS which possesses conserved motif-1, motif-2 and motif-3 as a catalytic core, and diverged from the other subclass IIa enzymes through its peculiar N-terminal extensions. ThrRS is organized into four structural domains; two N-terminal domains consisting of N-1 and N-2, a catalytic core and a C-terminal anticodon binding domain. ThrRSs are classified based on the length of the N-terminal domains, eukaryote having a specific N-terminal extension domain in addition to N-1 and N-2, eubacteria possessing standard N-terminal domains consisting of N-1 and N-2 and archaea with a short N-2 domain compared to the other kingdoms (Figs. 1 and 4). It has been reported that *A. pernix* possesses two possible ThrRS genes encoding a polypeptide chain of 406 and 484 amino acids, respectively [7]. Both genes are relatively shorter than the other kingdoms' ThrRSs (Fig. 1). Sequence homology analysis of the shorter gene (ThrRS-1) with the other ThrRSs from eukaryote, eubacteria and archaea indicates some similarities in the C-terminal anticodon binding domain, but ThrRS-1 did not possess motif-2 and motif-3 of the catalytic core which are strongly conserved in class II ARS (Fig. 1). On the other hand, the longer one (ThrRS-2) had all conserved motifs, which were expected to be involved in substrate binding and catalytic activity. Striking feature is that ThrRS-2 has a unique structure in its quite

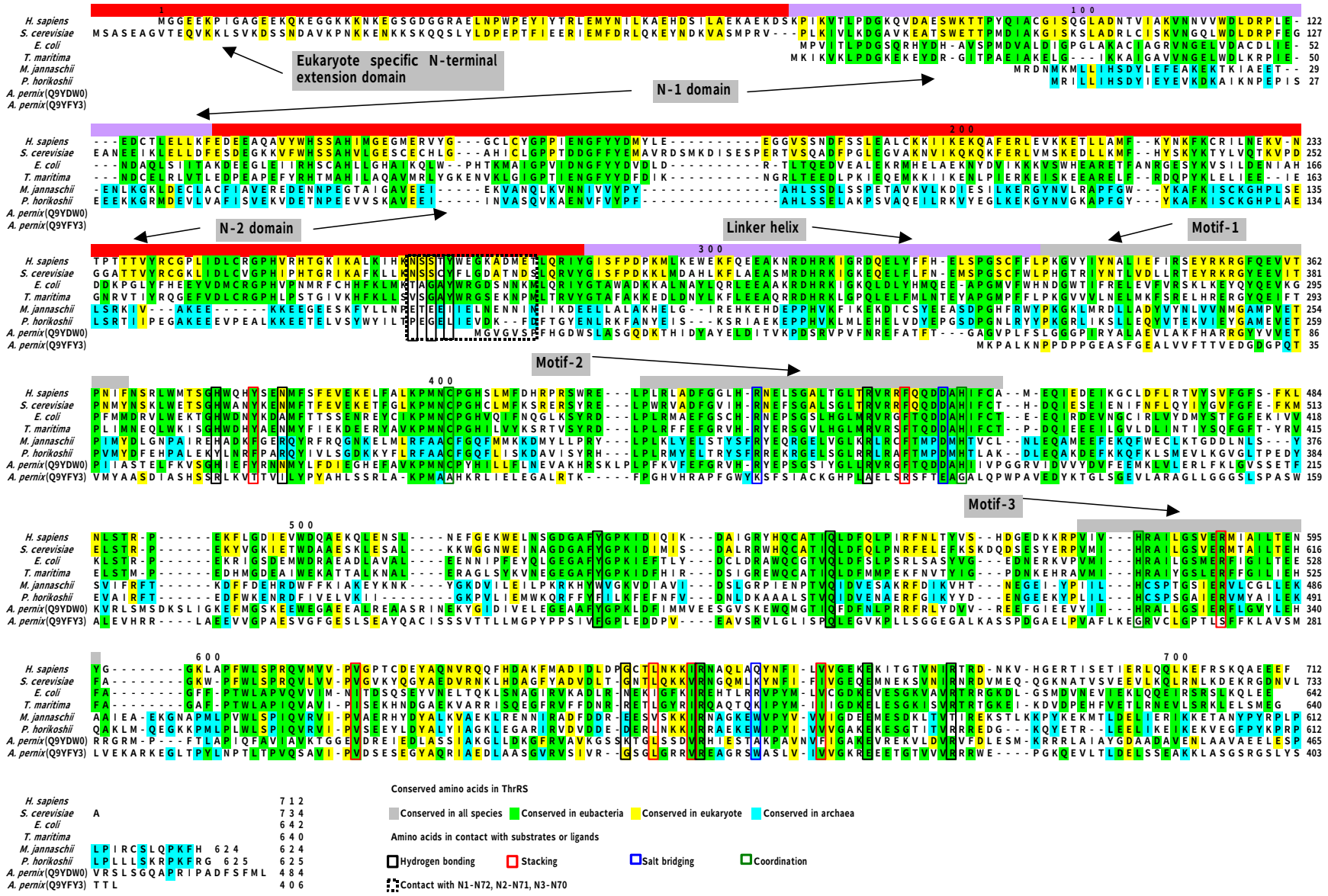

Fig. 1. Sequence comparison of threonyl-tRNA synthetases.

The alignment of the eight known ThrRS sequences is summarized. The origin of the ThrRSs is as follows: *H. sapiens* (*Homo sapiens*), *S. cerevisiae* (*Saccharomyces cerevisiae*), *E. coli* (*Escherichia coli*), *T. maritima* (*Thermotoga maritima*), *M. jannaschii* (*Methanococcus jannaschii*), *P. horikoshii* (*Pyrococcus horikoshii*), *A. pernix* ThrRS-1 (*Aeropyrum pernix*, Genebank accession number Q9YFY3), *A. pernix* ThrRS-2 (*Aeropyrum pernix*, Genebank accession number Q9YDW0).

Table 1. Amino acid residues of threonyl-tRNA synthetases from *E. coli* and *A. pernix* in contact with substrates or ligands.

Substrates and ligands	Nature of interaction	*E. coli*	*A. pernix* ThrRS-2	*A. pernix* ThrRS-1
Threonyladenylate				
α-Phosphate	Salt bridging	R363(motif-2)	R157(motif-2)	
Adenine	Stacking	F379(motif-2)	F173(motif-2)	
		R520(motif-3)	R332(motif-3)	
α-NH2	Salt bridging	D383(motif-2)	D177(motif-2)	E121
Zinc	Coordination	H385(motif-2)	H179(motif-2)	
		H511(motif-3)	H323(motif-3)	
		C334	C125	
Acceptor stem				
A76(cycle)	Stacking	R363(motif-2)	R157(motif-2)	
		Y313	Y104	
A76(N6)	Hydrogen bonding	A316	N107	
A76(O2')	Hydrogen bonding	H309	H100	
		Y462	Y270	F210
A76(O3')	Hydrogen bonding	Q484	Q295	Q231
A76(O4')	Hydrogen bonding	Y313	Y104	
C75	No contact			
C74	Two hydrogen bondings	R375(motif-2)	R169(motif-2)	
A73	No contact	No contact	-	
G1-C72,C2-G71 and C3-G70		Residue201-214	Residue1-7(*Lacking*)	
G71(N2)	Hydrogen bonding	G203	*Lacking*	
G1(N2)	Hydrogen bonding	Y205	*Lacking*	
C3(O2)	Hydrogen bonding	G203	*Lacking*	
G70(N2)	Hydrogen bonding	T201	*Lacking*	
Anticodon loop				
C34	Hydrogen bonding	N575	K394	G336
G35,U36	Stacking	I547,I578,I582,V595	V365,L397,V401,F415	V309,L339,V343,V356
G35(N1)	Hydrogen bonding	E600	E420	E361
U36(O2)	Hydrogen bonding	R609	R429	R370
A37(N3)	Hydrogen bonding	R583	R402	R344
A37(mt6)	Salt bridging	R589	A408	W350
A38(cycle)	Stacking	R583	R402	R344
Conserved in ThrRSs				

The interactions in the *E. coli* system were determined by crystal structure analysis of the enzyme complexed with threonine tRNA [10]. The residues of *A. pernix* ThrRSs in contact with substrates or ligands were predicted by sequence homology comparison with *E. coli.*

compact N-terminal domain, different from the other species. In a recent study of the cocrystal structure of *E. coli* ThrRS and $tRNA^{Thr}$, the active sites of ThrRS involving in contact with substrates or ligands were determined (Table 1) [10]. Based on these results, the active sites of ThrRS-1 and ThrRS-2 were conjectured by comparing the amino acid sequences to those of *E. coli* (Table 1). It is shown that ThrRS-1 is missing the most active sites except amino acids that might make contact with the anticodon of $tRNA^{Thr}$. It therefore seems likely that ThrRS-1 is a pseudogene. In contrast to ThrRS-1, ThrRS-2 possessed almost all the active sites that were expected to be involved in substrate binding and catalytic activity (Table 1). In the *E. coli* threonylation system, the formation of the most important catalytic activity for amino acid activation has been studied, and the zinc ion was found to be involved in direct threonine recognition by making a pentacoordinate intermediate with both an amino group and a hydroxyl group of the side chain [11, 12]. It would be possible for *A. pernix* ThrRS-2 to form the same catalytic environment as *E. coli* ThrRS (Figs. 2 and 3). Based on these results, we proposed a possible model of *A. pernix* ThrRS-2 (Fig. 4). It is of interest that ThrRS has diverged from other species in the particular length of the N-terminal domains. Studying aminoacylation with *A. pernix* ThrRS-2 may provide a hint which can decipher not only the evolutionary position of *A. pernix* but also the evolutionary process for ThrRS because this enzyme is characterized by the specific length of its N-terminal domains among species (Fig. 1).

Cross-species threonylation activity

To speculate on the recognition sites of amino acid specific tRNA for cognate ARS, cross-species aminoacylation experiments between different species may be effective. *E. coli* ThrRS threonylated $tRNA^{Thr}$ from *A. pernix* and *H. volcanii*. This result can be explained by the previous report that *E. coli* ThrRS does not recognize the discriminator base of $tRNA^{Thr}$ [4].

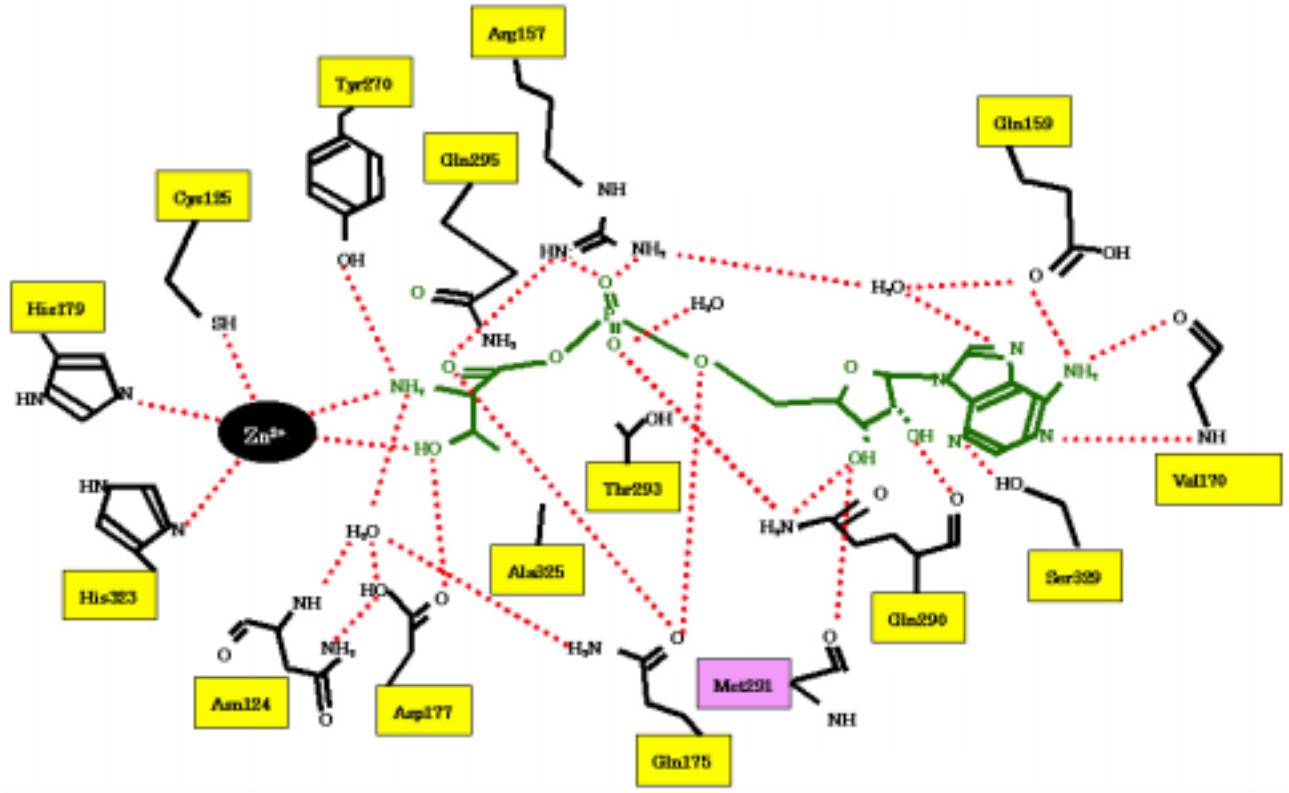

Fig. 2. The interaction network model of *A. pernix* threonyl-tRNA synthetase with zinc ion in threonylation.

Interaction network of the threonine-AMP molecule with zinc ion and the enzyme residues was found in the catalytic domain of *E. coli* ThrRS [11, 12]. A possible model of zinc ion binding area in *A. pernix* threonylation was predicted by alignment comparison with *E. coli*. The amino acids except Met291 are homologous to *E. coli* ThrRS.

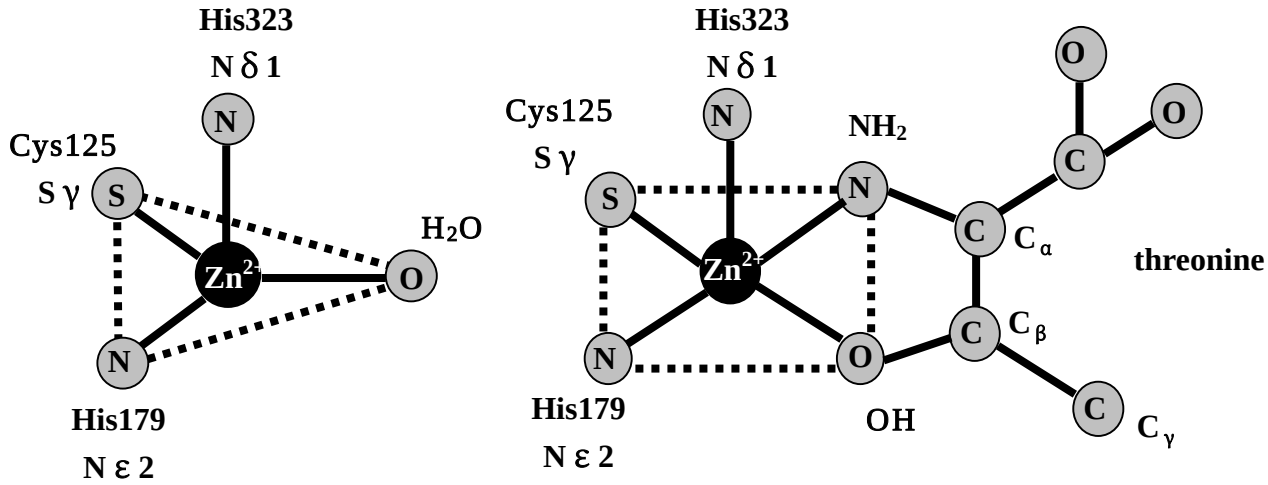

Fig. 3. Zinc ion-mediated discrimination model in *A. pernix* threonylation.

In the *E. coli* threonylation system, it was indicated that the zinc ion was involved in direct threonine recognition by forming a pentacoordinate intermediate with both the amino group and the hydroxyl group of the side chain [11, 12]. A possible discrimination model in *A. pernix* threonylation was predicted by sequence homology comparison with *E. coli*. (A) Before binding to threonine, (B) after binding to threonine.

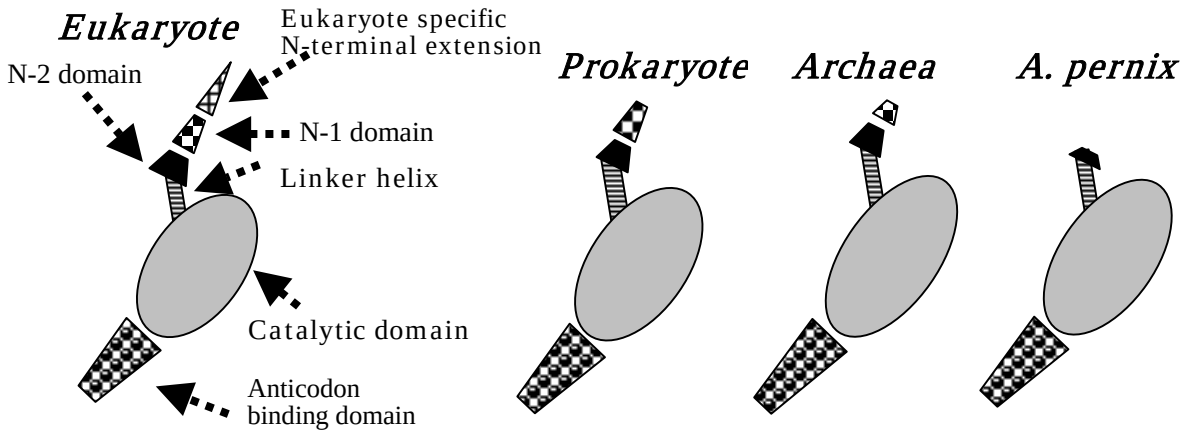

Fig. 4. Possible model of *A. pernix* threonyl-tRNA synthetase.

This model of *A. pernix* ThrRS was predicted by sequence comparison with other species. It was found that the N-terminal domain of *A. pernix* ThrRS was much shorter than the other species reported before.

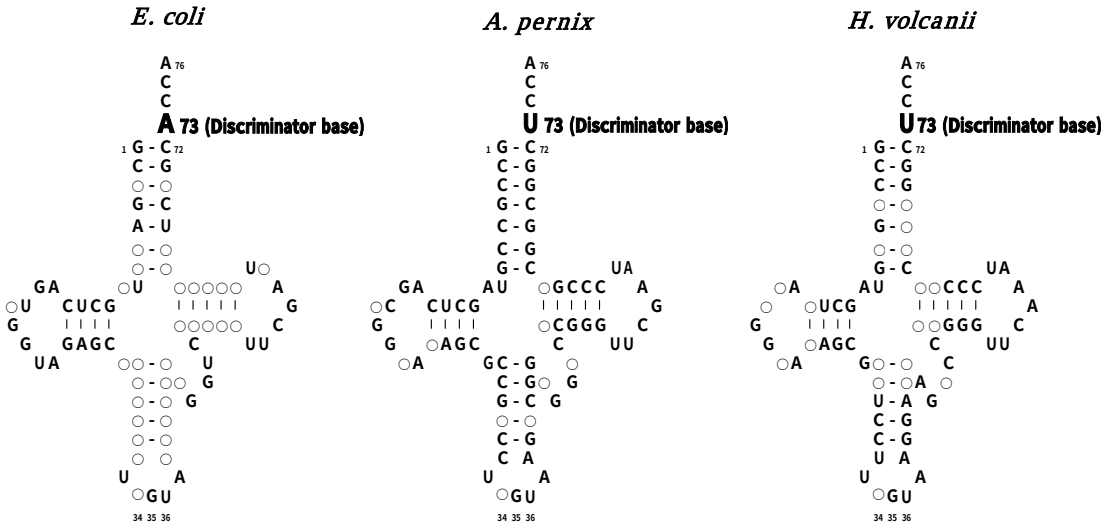

Fig. 5. Cloverleaf secondary structure of threonine tRNAs from *E. coli, A. pernix* and *H. volcanii.*

Open circles indicate the non-conserved nucleotides among isoacceptor $tRNA^{Thr}$s.

H.volcanii ThrRS threonylated archaeal $tRNA^{Thr}$, but not *E. coli* $tRNA^{Thr}$. These findings indicate that the discriminator base U73 of *H. volcanii* $tRNA^{Thr}$ is a strong determinant in the threonylation by *H. volcanii* ThrRS (Fig. 5). In contrast to *H. volcanii* ThrRS, the enzyme from *A. pernix* threonylated not only archaeal threonine tRNA but also *E. coli* $tRNA^{Thr}$. These results indicate that the discriminator base U73 of *A. pernix* $tRNA^{Thr}$ is not involved in threonylation by the *A. pernix* ThrRS like *E. coli* enzyme [4]. Results of cross-species threonylation experiments among *E. coli*, *A. pernix* and *H. volcanii* were shown in Fig. 6.

Different importance of the discriminator base of archaeal $tRNA^{Thr}$

In previous studies, the identity nucleotides of $tRNA^{Thr}$ from *E. coli*, *T. thermophilus* and *S. cerevisiae* were already determined [4-6]. According to these reports, the recognition sites of *E. coli* $tRNA^{Thr}$ in threonylation by ThrRS were the first two base pairs of the acceptor stem

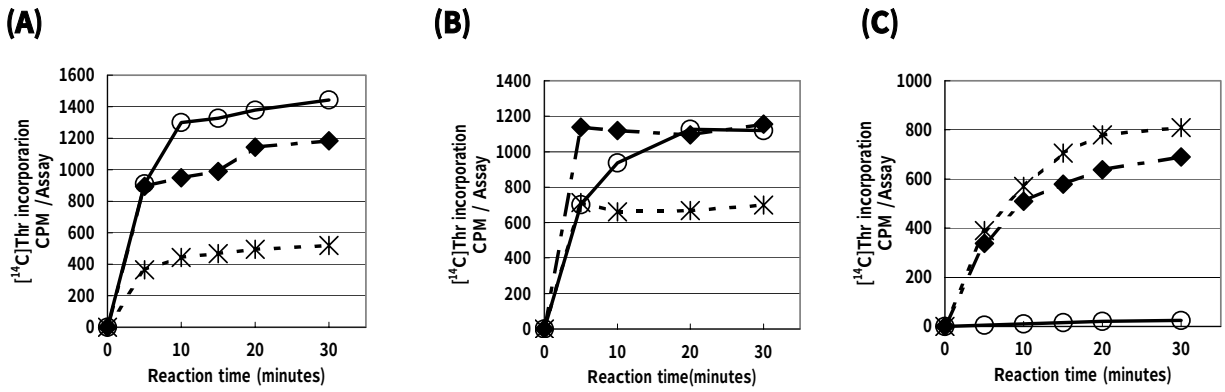

Fig. 6. Cross-species threonylation activity.

Cross-species threonylation activity was examined using (A) *E. coli* ThrRS, (B) *A. pernix* ThrRS, (C) *H. volcanii ThrRS*, and (◆) *A. pernix* tRNA, (◯) *E. coli* tRNA, (✳) *H. volcanii* tRNA, respectively. The assay conditions were described in Materials and Methods.

in addition to the second and the third position of the anticodon. Of interest, the discriminator base A73 is not involved in threonylation by ThrRS in the *E. coli* system [4]. This is the only case in which a discriminator base is not recognized by the cognate ARS in the *E. coli* system [1]. On the other hand, it also has been shown that the discriminator base of $tRNA^{Thr}$ from *T. thermophilus* and *S. cerevisiae* is involved in threonylation by their cognate ThrRS [5, 6]. To understand the importance of the discriminator base of archaeal $tRNA^{Thr}$ in threonylation by ThrRS, cross-species aminoacylation was examined as described above. The extreme halophilic archaeon *H. volcanii* ThrRS threonylated archaeal $tRNA^{Thr}$ having U73, but not *E. coli* $tRNA^{Thr}$ possessing A73. However, the extreme thermophilic archaeon *A. pernix* ThrRS threonylated not only archaeal $tRNA^{Thr}$ but also *E. coli* $tRNA^{Thr}$. These findings indicate that the importance of the discriminator base of archaeal $tRNA^{Thr}$ differ among archaeal organisms (Fig. 5).

Possible role of N-terminal domains of ThrRS

It has been shown that ThrRS diverged from a common subclass IIa ARS in its peculiar N-terminal extensions, and the enzyme from each species diverged further in the length of N-terminal domains [13]. In this study, it was postulated that only ThrRS-2 was a possible threonyl-tRNA synthetase of the extreme thermophilicarchaeon *A. pernix*. The sequence of ThrRS-2 is unique in its quite compact N-terminal domain, lacking almost all N-terminal domains. ThrRS having such a unique structure has not been reported previously. It was shown that N-1 domain of the eubacterium *T. thermophilis* ThrRS was not essential for threonine charging in an experiment with N-1 domain-truncated ThrRS [13]. Furthermore, it was shown that neither N-1 domain nor N-2 domain was not essential for charging by ThrRS in the *E. coli* system [11, 12]. However, the results of sequence analysis and the threonylation experiments in this study suggest strongly that the N-terminal domains of *A. pernix* ThrRS are not necessary for threonine charging to $tRNA^{Thr}$. It was reported that N-2 domain of *E. coli* ThrRS contained not only a similar sequence to the C-terminal moiety of alanyl-tRNA synthetase but also several residues that were expected to make contact with the acceptor stem vicinity of $tRNA^{Thr}$ (Table 1) [11, 12]. In the *E. coli* system, it was also revealed that specific interactions occurred between a hairpin motif (amino acids 201-214) from N-2 domain and the first two base pairs of the acceptor stem of $tRNA^{Thr}$ on the minor groove side [11, 12]. However, as shown in Fig. 1 and Table 1, *A. pernix*

ThrRS-2 truncated most of these residues at N-2 domain which were determined to contact with the acceptor stem of $tRNA^{Thr}$ in the *E. coli* system. Does *A. pernix* ThrRS-2 recognize the acceptor stem of $tRNA^{Thr}$ in the threonylation reaction? This possibility is currently under investigation. Furthermore it is predicted that N-2 domain of ThrRS plays an important role in discriminating similar amino acid serine in the threonylation process. It has been reported that the N-2 domain-truncated *E. coli* ThrRS lost the editing function of mischarged serine with $tRNA^{Thr}$ [11]. The most obvious structural element in the *E. coli* system responsible for tRNA-mediated editing would be N-2 domain of ThrRS [11, 13]. Thus, it would be very interesting to study the molecular mechanism of *A. pernix* ThrRS in the threonylation because it may reveal different molecular mechanism in recognition and discrimination from other organisms.

Conclusion

In this study, it was found that the sequence of ThrRS from the extreme thermophilic archaeon *A. pernix* was unique in its quite compact N-terminal domain. Such a ThrRS truncating the N-terminal domains drastically was not reported except *A. pernix* ThrRS. It was also indicated that the importance of the discriminator base of $tRNA^{Thr}$ differed among archaeal organisms.

Acknowledgments

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