Molecular recognition of threonine tRNA by threonyl-tRNA synthetase from an extreme thermophilic archaeon, *Aeropyrum pernix* K1

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ABSTRACT
To investigate the recognition sites of tRNA Thr for threonyl-tRNA synthetase (ThrRS) from an extreme thermophilic and aerobic archaeon, *Aeropyrum pernix* K1, threonylation experiments using various *in vitro* mutant transcripts of tRNA Thr were examined. The results indicated that *A. pernix* ThrRS did recognize the first three base pairs of acceptor stem in addition to the second and the third letters of anticodon of tRNA Thr, in spite of its N-terminal truncated unique structure. Discriminator base was not involved in recognition by *A. pernix* ThrRS. These determinants were confirmed by the identity switching experiments from the *in vitro* mutants of *A. pernix* tRNA Pro and tRNA Asn.

INTRODUCTION
Aminoacyl-tRNA synthetases (ARSs) are key enzymes at translation process of protein biosynthesis. Correct recognition and discrimination of tRNA by each cognate ARS is essential to the maintenance of accurate translation. Molecular mechanism of tRNA recognition and discrimination by ARS is called tRNA identity, and the research of this tRNA identity has drastically progressed in recent decade (1). Recognition sites of all amino acid species tRNAs by cognate ARS have been already determined in *Escherichia coli* system. Although information about tRNA identity of archaea which is classified into the third domain is still a little, some interesting aspects of molecular recognition mechanism by archaeal enzymes such as transamidation pathway, a dual-specificity of Pro-CysRS and class I type LysRS are recently found (2-4).

Here, we report the molecular recognition of tRNA Thr by ThrRS from an extreme thermophilic and aerobic archaeon, *A. pernix*, first archaeon whose complete genome sequence has been determined as a crenarchaeota (5).

RESULTS AND DISCUSSION
It has been reported that *A. pernix* possesses two ORFs encoding ThrRS consisting of 406 and 484 amino acids, respectively (5). Sequence homology analysis of ThrRSs from various organisms showed that shorter ThrRS does not possess conserved catalytic motifs of class Ia ARS. On the other hand, the longer ThrRS has almost all active sites that are expected to be involved in substrate binding and catalytic activity. As a striking feature, it was found that the sequence of the longer ThrRS was unique in its compact N-terminal domain. These two possible ThrRS genes from *A. pernix* were cloned and expressed. Aminoacylation activity with expressed these possible ThrRSs were studied and only the longer ThrRS having conserved motifs showed threonylation activity. Thus, the shorter ThrRS itself appears to be pseudogene-like, and the longer one appears to be the only active ThrRS of *A. pernix*.

It is known that tRNA Thr was the only substrate in which discriminator base A73 was not recognized by the cognate ARS in the *E. coli* system (6). To examine the importance of the discriminator base of archaeal tRNA Thr in threonylation by ThrRS, cross-species aminoacylation were investigated. Halophilic archaeon, *Haloferax volcanii*...
ThrRS threonylated only archaeal tRNA^{Thr}s having U73, but did not threonylate E. coli tRNA^{Thr} having A73 (7). In contrast to H. volcanii, expressed ThrRS from A. pernix threonylated not only archaeal tRNA^{Thr}s but also E. coli tRNA^{Thr}. These findings indicate that the importance of the discriminator base of archaeal tRNA^{Thr} differ among the archaea. To confirm these observations, threonylation experiments using in vitro mutant transcripts of E. coli tRNA^{Thr} were examined. Though H. volcanii ThrRS threonylated the only mutant tRNA^{Thr} having U73, N-terminal truncated A. pernix ThrRS did not recognize the discriminator base of tRNA^{Thr}.

To investigate the identity elements of tRNA^{Thr} by a unique ThrRS from A. pernix, threonylation experiments using in vitro mutant transcripts were studied. It was found that A. pernix ThrRS did recognize the first base pair of the acceptor stem of tRNA^{Thr} as well as other specie ThrRSs (6, 8, 9), in spite of N-terminal truncated unusual structure. Furthermore, threonylation experiments indicated the second and the third base pairs of acceptor stem were also strong recognition sites by A. pernix ThrRS. This enzyme lacks corresponding amino acid residues which are specified to contact with the acceptor stem region of tRNA^{Thr} in E. coli system (10). These findings show that the molecular mechanism of acceptor stem recognition by A. pernix ThrRS must be so different from it in the E. coli system. Substitution of U73 with G73, but not A73 or C73, impaired the threonine accepting activity drastically, indicating that the discriminator base U73 is not involved in recognition by A. pernix ThrRS, as described above cross-species threonylation experiments. The recognition sites of acceptor stem vicinity by A. pernix ThrRS was similar to that of E. coli that the second base pair of acceptor stem was a strong recognition site, whereas the discriminator base was not involved in recognition (6, 8). Identity switching experiments using tRNA^{pro} and tRNA^{aan} supported that the major identity elements of tRNA^{Thr} by A. pernix ThrRS were the first three base pairs of the acceptor stem in addition to the second and the third letters of anticodon.

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