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Analysis of tRNA modification system in halophilic archaea

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Research aims

Among over 80 modified nucleosides identified in tRNAs, two remarkable nucleosides have an unusual 7-deazaguanosine structure, namely, queuosine and archaeosine. Archaeosine is exclusively found in archaea, and present at position 15 in the D-loop of the tRNA. This position plays a role in interacting with the D-loop and T-stem in the tertiary structure of the tRNA, suggesting that archaeosine function in the stabilization of the tRNA structure. Queuosine is found in bacteria and eucarya, and present at position 34, the wobble position, in a subset of the tRNAs possessing a GUN anticodon (specific for amino acids Asp, Asn, His, and Tyr). The wobble position suggests a role in modulating translational fidelity and/or efficiency. Disruption of a gene involved in queuosine biosynthetic pathway of the pathogenic bacterium *Shigella flexneri* results in a significantly reduced virulence[1,2]. This is due to the poor expression of VirF, which is the most upstream regulator protein of the virulence cascade. Therefore, the enzymes in queuosine biosynthesis pathway are putative targets for anti-shigellosis drugs[3]. The preQo is an intermediate of archaeosine as well as queuosine, suggested that the biosynthetic pathway of archaeosine is closely related to that of queuosine. Biochemical and genetic studies in bacteria (*Escherichia coli*, *Bacillus subtilis*, and *Streptomyces rimosus*) have shown that Guanosine 5′-triphosphate (GTP) is converted to preQo in four steps[4] (Fig. 1). In the biosynthetic pathways to deazapurine-containing metabolites from GTP, four enzymes (GCHI, QueD, QueE, and QueC) are necessary for production of preQo. Although the genes of these enzymes involved in preQo biosynthesis are found in most archaea, the activity of each enzyme has not been confirmed yet. Moreover, nothing is presently known about the final step of biosynthesis of archaeosine. In order to clarify the whole biosynthetic pathway of archaeosine and to infer its function, gene disruption experiments were attempted.

Methods and Results

Halophiles grow in extremely saline environments, and therefore, its intracellular KCl concentration is at high levels to maintain osmotic balance. The computational analysis suggests that archaeosine function in the stabilization of the tRNA structure[5]. However, it is still unclear whether archaeosine stabilizes tRNA structure in high KCl concentration *in vitro*. Halophilic archaeon *Halobacterium* sp. NRC-1 was utilized in this study because many genetic tools have been developed and its genome information is available. Although ArcX protein concerned with the final step of archaeosine biosynthetic pathway should catalyze the unique reaction converting nitrile [R–C≡N] into amidine [R–C(=NH)NH_2], its gene has not been identified yet. The genome search for *arcX* gene was performed by using the amino acid sequences of the PUA and C2 domains in tRNA-guanine transglycosylase (TGT), and the possible candidate gene was found. Both of the PUA and C2 domains are present in ArcX. The PUA domain is highly conserved among tRNA modification enzymes, and the C2 domain supposedly stabilizes the λ-formed tRNA in which the D-loop is accessible to the catalytic domain[6].

In order to obtain each DNA fragment encoding *queC, queD, queE, tgt*, and *arcX* gene involved in archaeosine biosynthesis, polymerase chain reaction (PCR) was per-

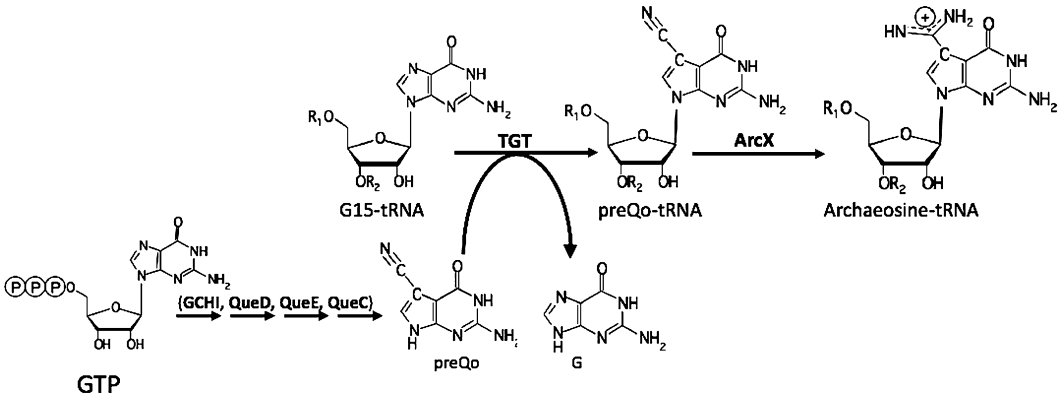

Fig. 1. The biosynthesis pathway of archaeosine. Abbreviation used: preQo, 7-cyano-7-deazaguanine; GCHI, GTP cyclohydrolase; TGT, tRNA-guanine transglycosylase; ArcX, unknown enzyme(s); archaeosine, 7-formamidino-7-deazaguanosine.

formed by using two primers based on the genome information of *Halobacterium* sp. NRC-1. A reaction mixture (25 μl) comprising 100 ng of genomic DNA, 7.5 pmol of each primer, and KOD plus ver. 2 DNA polymerase (Toyobo Co., Ltd., Japan) was subjected to PCR (98°C for 10 sec, 61°C for 30 sec, and 68°C for 3 min; 25 cycles), and the amplified DNA fragment (approximately 2.5 kbp) was gel-purified and successfully cloned into vector pUC19 digested with *Hin*cII. To construct the plasmids for gene disruption, the mevilolin (competitive inhibitor of HMG-CoA reductase) resistant (Mev^r) marker was inserted into the open reading frame of each target gene. The Mev^r marker was obtained by PCR amplification using the shuttle vector pWL102[7] as a template. Transformations of *Halobacterium* sp. NRC-1 were performed by the PEG method described by Cline *et al*.[8] The gene disruption mutants were attempted to obtain by spreading transformation mixture on the selective plate medium with simvastatin (a derivative of mevinolin) at a final concentration of 25 μg/ml.

Conclusion

Each DNA fragment encoding *queC, queD, queE, tgt*, and *arcX* gene involved in archaeosine biosynthesis was cloned into plasmid pUC19, and the plasmids for disruption of target genes were successfully constructed by inserting Mev^r marker into the respective open reading frame of them.

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