DETERMINATION OF SECONDARY STRUCTURE IN *adhE* mRNA REQUIRED FOR RNase G-DEPENDENT DEGRADATION

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Introduction. RNase G is a homologue of the essential *Escherichia coli* ribonuclease RNase E. RNase E plays a major role in the degradation of mRNA and the processing of tRNA and rRNA in *E. coli.* On the other hand, the biological function of RNase G is more limited. RNase G is required for the maturation of the 5'-end of 16S rRNA (1). It is also involved in the degradation of several specific mRNA such as *adhE* and *eno* (2, 3). Consequently, proteins encoded by these genes are overproduced in the RNase G mutant cells. Previous study showed that the 5'-untranslated region (5'-UTR) of *adhE* mRNA confers RNase G-dependent regulation on the *lacZ* mRNA when lagged at its 5'-end (2). However, it has not been elucidated yet how RNase G recognizes and cleaves the *adhE* mRNA.
In this study, we determined the cleavage site of *adhE* mRNA by RNase G and the secondary structure in the 5'-UTR required for the RNase G-dependent regulation.

Methods. The cleavage site of *adhE* mRNA by RNase G was determined by primer extension analysis. To determine the recognition sequence of RNase G, deletion analysis of the 5'-UTR of *adhE-lacZ* fusion was carried out.

Results. Primer extension analysis showed that RNase G cleaved a phosphodiester bond between -18 and -19 in the *adhE* 5'-UTR (the first nucleotide A of the *adhE* coding region is defined as +1). To examine whether RNase G recognizes the sequence around the cleavage site, nucleotides at -18 and -19 were substituted by site-directed mutagenesis. It was found that RNase G did not recognize the sequence around the cleavage site. Then, to determine the recognition sequence of RNase G, a random deletion analysis of *adhE-lacZ* fusion was done. It was shown that the -145 ~ -125 region in the 5'-UTR was required for RNase G-dependent degradation (Fig.1). Further analysis revealed that a unique stem-loop structure having a bubble in its stem was required for RNase G-dependent cleavage (Fig.2).

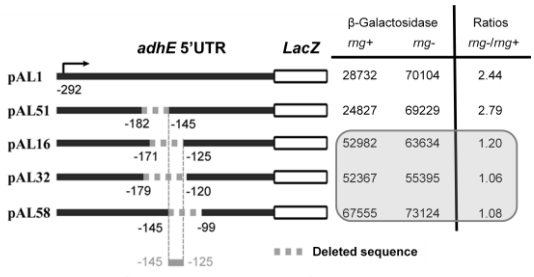

Fig.1 Deletion analysis of *adhE-lacZ* fusions.

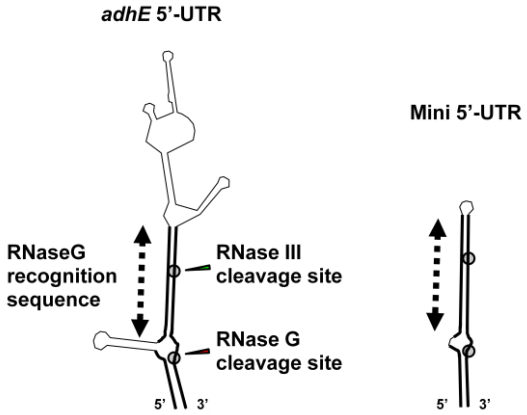

Fig.2 Predicted secondary structure of the *adhE* 5'-UTR (A) and a minimum stem-loop structure required for RNase G-dependent cleavage (B).

Conclusions. RNase G does not recognize the nucleotides around the cleavage site. Instead, the stem-loop structure having a bubble in its stem is required for the RNaseG-dependent degradation of *adhE* mRNA.

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