# Ribonuclease mediated regulation of small RNA expression in *Escherichia coli*

There are several ribonucleases (RNase) in *Escherichia coli* and presumably, each one has an important role in RNA metabolism. After an individual RNA has been fully transcribed, the fate of that RNA is determined by its sequence, structure, and availability of RNases, which may be determined by conditions inside and outside of the cell. During growth periods, the majority of RNA degradation is carried out by RNase II, RNase R, and polynucleotide phosphorylase, which lead to the generation of small RNA fragments. Small RNAs (sRNAs) play a central role in regulating almost all physiological processes in bacteria. Majority of those sRNAs base pair with their targets and modulate their expressions. RyeA, previously known as SraC in *Escherichia coli*, is transcribed from a DNA strand complementary to the one from which another stationary phase induced sRNA RyeB/SdsR is synthesized. RyeA and RyeB in the stationary phase constitute a toxin-antitoxin system where RyeA normalizes the accumulation of RyeB toxin by acting as RNA sponge. Ectopic expression of RyeA was reported to diminish the RyeB accumulation by serving as a RNA trap. Aside from that, no more information is known about the regulation of RyeA expression. In the present work, we have systematically investigated the regulation of RyeA expression in different growth phases and identified that RyeA expression is regulated neither by stationary phase-specific σ-factor nor by RNA chaperone Hfq. A dual function ribonuclease RNase BN mitigates its expression in the exponential phase and stabilizes the RyeA expression upon the deletion of *rbn* gene. Thus, the lower abundance of RyeA in the early exponential growth phase turned out to be the outcome of its degradation by RNase BN/Z.

RyeA is an acid stress inducible sRNA, and global stress responsive factor RpoS was not found to regulate RyeA induction. RyeB under low pH condition was also stimulated by ~4-fold in the form of *ryeB-pphA* dicistronic transcript. However, RyeB population was found to be decreased by > 50% under the same condition by the decoy action of enhanced RyeA accumulation. Investigation of the mechanism-why is RyeA induced at low pH in the exponential phase, revealed that RNase BN/Z, which catabolizes RyeA in the exponential phase, appeared to be highly sensitive to low pH. Both mRNA and protein levels of RNase BN transpired to be decreased to < 10% of their initial population. GcvB, a sRNA which protects *rbn* mRNA in the exponential phase, emerged as acid stress sensitive sRNA in this study. Thus, the expression of RyeA under acid stress is regulated by a feed-forward mechanism to normalize the RyeB profusion. These regulatory mechanisms will help identify the primary role of RyeA in *E. coli*.

Furthermore, *E. coli* adaptation at elevated pH (9.0) has been extensively studied the alkaline stress response of RNase BN expression profile in relation to *pspF* transcription factor regulation. Phage shock protein F (*pspF*) protects the bacteria during stress, which encodes a transcriptional activator named for its role in phage shock protein response and it activates the target operons (*pspABCDE* and *pspG*) by $\sigma^{54}$-dependent transcription. It was observed that at high pH, the production of RNase BN has been increased. Simultaneously the expression of *pspF* was also increased after stress, while at low pH, it was completely degraded. This study showed the stress (pH) specific expression of RNase BN and observed that the RNase BN up-regulated the transcription of *pspF* by up-regulating itself.

The final part of the thesis covers the regulatory role of ribonuclease D (RNase D) on small RNA expression. Based on the result obtained, it was elucidated that RNase D was identified as an important regulator, which modulates the expression of RyeA during the

exponential phase, simultaneously the RyeB expression, which is σ-factor dependent also increase its expression in exponential phase upon deletion of *rnd* gene. This part also covers the regulation of RNase D on CsrB, McaS, and IsrC expression. The expression of CsrB, and McaS are increased in wild type, while this expression remains constant in RNase D mutant cells. Thus, it confirms that there was no regulation of CsrB, and McaS by RNase D. But in the case of IsrC, expression was increased in the stationary phase in the wild-type. However, IsrC expression was increased in the early exponential phase upon the deletion of *rnd* gene. It indicates that the *rnd* also regulates IsrC expression. RNase D also plays a role in the regulation of the CsrA gene. The expression of CsrA was increased in the stationary phase in RNase D mutant cells. However, it was almost constant in wild type cells.

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