

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

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

Submitted by

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Dedicated to the Almighty

*Failure will never overtake me if any determination to succeed
is strong enough*

APJ Abdul Kalam.

CERTIFICATE

This is to certify that the thesis entitled “**Ribonuclease mediated regulation of small RNA expression in *Escherichia coli***” being submitted by **Mr. AMIT KUMAR GUPTA** to the Indian Institute of Technology Delhi for the award of the degree of ***Doctor of Philosophy*** in chemistry is a record of bonafide research work carried out by him. Mr. Amit Kumar Gupta has worked under my guidance and supervision, and has fulfilled the requirements for the submission of the thesis, which, to my knowledge, has reached the requisite standard. The results contained in this dissertation have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

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Abstract

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 σ^{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.

सार

एस्चेरिचिया कोलाई में कई राइबोन्यूक्लियूसेस (RNase) हैं और संभवतः, आरएनए चयापचय में प्रत्येक की महत्वपूर्ण भूमिका है। किसी व्यक्ति के RNA को पूरी तरह से स्थानांतरित करने के बाद, उस RNA के भाग्य को RNases के अनुक्रम, संरचना और उपलब्धता द्वारा निर्धारित किया जाता है, जो सेल के अंदर और बाहर की स्थितियों द्वारा निर्धारित किया जा सकता है। विकास की अवधि के दौरान, आरएनए क्षरण का अधिकांश भाग RNase II, RNase R, और पॉली न्यूक्लियोटाइड फॉस्फोराइलेज द्वारा किया जाता है, जो छोटे RNA अंशों की पीढ़ी का नेतृत्व करते हैं। छोटे आरएनए (sRNAs) बैक्टीरिया में लगभग सभी शारीरिक प्रक्रियाओं को विनियमित करने में एक केंद्रीय भूमिका निभाते हैं। उन sRNAs बेस पेयर के अधिकांश अपने लक्ष्यों के साथ और उनके भावों को संशोधित करते हैं। RyeA, जिसे पहले *Escherichia coli* में SraC के रूप में जाना जाता है, को एक डीएनए स्ट्रैंड सप्लिमेंट्री से प्रेषित किया जाता है जिसमें से एक और स्थिर चरण प्रेरित sRNA RyeB / SdsR संश्लेषित होता है। स्थिर चरण में RyeA और RyeB एक विष-रोधी प्रणाली का निर्माण करते हैं जहाँ Rye RNA स्पंज के रूप में कार्य करके RyeB विष के संचय को सामान्य करता है। राई के एक्टोपिक अभिव्यक्ति को आरएनए जाल के रूप में सेवा करके राईबी संचय को कम करने के लिए सूचित किया गया था। इसके अलावा, राईए अभिव्यक्ति के नियमन के बारे में अधिक जानकारी नहीं है। वर्तमान कार्य में, हमने अलग-अलग विकास चरणों में RyeA अभिव्यक्ति के नियमन की व्यवस्थित रूप से जांच की है और पहचाना है कि RAA अभिव्यक्ति को न तो स्थिर चरण-विशिष्ट σ - कारक द्वारा और न ही RNA चैप्टर Hfq द्वारा नियंत्रित किया जाता है। एक दोहरे कार्य राइबोन्यूक्लाइज RNase बीएन घातीय चरण में अपनी अभिव्यक्ति को कम कर देता है और आरबीएनए जीन को हटाने पर आरईएए अभिव्यक्ति को स्थिर करता है। इस प्रकार, प्रारंभिक घातीय वृद्धि चरण में RAA की निचली बहुतायत RNase BN / Z द्वारा इसके क्षरण का परिणाम निकला। RyeA एक एसिड स्ट्रेस इंड्यूसबल sRNA है, और Rye इंडक्शन इंडक्शन को रेगुलेट करने के लिए ग्लोबल स्ट्रेस रेस्पॉन्सिव फैक्टर RpoS नहीं पाया गया। कम पीएच स्थिति के तहत RyeB भी ~ 4 गुना ryeB-ppha

dicistic transcript के रूप में उत्तेजित किया गया था। हालांकि, RyeB की आबादी में वृद्धि हुई RyeA की क्षय कार्रवाई द्वारा समान स्थिति के तहत > 50% तक कम होना पाया गया संचय। तंत्र की जांच-क्यों RyeA घातीय चरण में कम पीएच पर प्रेरित है, ने खुलासा किया कि RNase BN / Z, जो घातीय चरण में RyeA को catabolizes करता है, कम पीएच के प्रति अत्यधिक संवेदनशील दिखाई दिया। आरएनएस बीएन के एमआरएनए और प्रोटीन स्तर दोनों को उनकी प्रारंभिक आबादी के <10% तक घटा दिया गया। GcvB, एक sRNA जो घातीय चरण में *rbn* mRNA की सुरक्षा करता है, इस अध्ययन में एसिड तनाव संवेदनशील sRNA के रूप में उभरा। इस प्रकार, एसिड तनाव के तहत राई की अभिव्यक्ति को एक फ्रीड-फॉरवर्ड तंत्र द्वारा विनियमित किया जाता है, ताकि राईब की गड़बड़ी को सामान्य किया जा सके। ये विनियामक तंत्र ई कोलाई में RyeA की प्राथमिक भूमिका की पहचान करने में मदद करेंगे।

इसके अलावा, उन्नत पीएच (9.0) पर ई कोलाई अनुकूलन का व्यापक रूप से pspF प्रतिलेखन कारक विनियमन के संबंध में RNase BN अभिव्यक्ति प्रोफाइल के क्षारीय तनाव प्रतिक्रिया का अध्ययन किया गया है। फेज शॉक प्रोटीन एफ (pspF) तनाव के दौरान बैक्टीरिया की रक्षा करता है, जो फेज शॉक प्रोटीन प्रतिक्रिया में अपनी भूमिका के लिए नामित एक ट्रांसक्रिप्शनल एक्टिवेटर को एनकोड करता है और यह σ 54-निर्भर ट्रांसक्रिप्शन से टारगेट ऑपरेशंस (pspABCDE और pspG) को सक्रिय करता है। यह देखा गया कि उच्च पीएच में, RNase BN का उत्पादन बढ़ा दिया गया है। इसके साथ ही तनाव के बाद pspF की अभिव्यक्ति में भी वृद्धि हुई थी, जबकि कम पीएच में, यह पूरी तरह से नीचा था। इस अध्ययन ने RNase BN के तनाव (pH) विशिष्ट अभिव्यक्ति को दिखाया और देखा कि RNase BN ने pspF के प्रतिलेखन को स्वयं-विनियमित करके नियंत्रित किया।

थीसिस का अंतिम भाग छोटे आरएनए अभिव्यक्ति पर राइबोन्यूक्लेज डी (आरएनएस डी) की नियामक भूमिका को कवर करता है। प्राप्त परिणाम के आधार पर, यह स्पष्ट किया गया था कि RNase D को एक महत्वपूर्ण नियामक के रूप में पहचाना गया था, जो घातीय चरण के दौरान RyeA की अभिव्यक्ति को नियंत्रित करता है, साथ ही RyeB अभिव्यक्ति, जो कि σ - कारक पर निर्भर है, विलोपन पर घातीय चरण

में भी इसकी अभिव्यक्ति है। *rnd* जीन की। यह भाग CsrB, McaS, और IsrC अभिव्यक्ति पर RNase D के विनियमन को भी कवर करता है। CsrB, और McaS की अभिव्यक्ति जंगली प्रकार में बढ़ जाती है, जबकि RNase D उत्परिवर्ती कोशिकाओं में यह अभिव्यक्ति स्थिर रहती है। इस प्रकार, यह पुष्टि करता है कि RNase डी। द्वारा लेकिन CsrB, और McaS का कोई विनियमन नहीं था IsrC के मामले में, अभिव्यक्ति को जंगली-प्रकार में स्थिर चरण में बढ़ाया गया था। हालाँकि, *rnd* जीन के विलोपन पर प्रारंभिक घातीय चरण में IsrC अभिव्यक्ति को बढ़ाया गया था। यह इंगित करता है कि रैंड IsrC अभिव्यक्ति को भी नियंत्रित करता है। RNase D भी CsrA जीन के नियमन में भूमिका निभाता है। RNase D उत्परिवर्ती कोशिकाओं में स्थिर अवस्था में CsrA की अभिव्यक्ति बढ़ गई थी। हालांकि, यह जंगली प्रकार की कोशिकाओं में लगभग स्थिर था।

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List of Abbreviations

RNase	Ribonuclease
mRNA	Messenger RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA
sRNA	Small RNA
cDNA	Complementary DNA
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
PCR	Polymerase chain reaction
RT-PCR	Reverse Transcriptase PCR
qPCR	Quantitative PCR
bp	Base-pair
SDS	Sodium dodecyl sulfate
PAGE	Poly acrylamide gel electrophoresis
IPTG	Isopropyl β - d-1-thiogalactopyranoside
DTT	Dithiothreitol
Tris-HCl	Tris hydrochloride
mAbs	Monoclonal antibodies
CFU	Colony Forming Units
KCl	Potassium chloride
MB	Molecular Grade
PFU	Plaque-forming units
CaCl ₂	Calcium chloride
MgCl ₂	Magnesium chloride
PVDF	Polyvinylidene fluoride
T _m	Melting Temperature
LB	Luria-Bertani
TB	Terrific broth
WT	Wild Type

EDTA	Ethylenediaminetetraacetic acid
TEMED	Tetramethyl ethylenediamine
ATR	Acid tolerance receptor
rpm	Revolution per minute
Tris	Tris(hydroxymethyl)aminomethane
pH	Negative logarithm of hydrogen ion concentration
SD	Standard deviation
SE	Standard error
Δ	Deletion
nt	Nucleotide
asRNA	Anti-sense RNA