RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action

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RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action

by

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Submitted

in partial fulfillment of the requirements of the degree of Doctor of Philosophy

to the

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This work is dedicated to my beloved family

CERTIFICATE

This is to certify that the thesis entitled "**RyjB, a novel riboregulator in *Escherichia coli*: Regulation of its expression and mechanism of its action**" being submitted by **Ms. NAMRA SIDDIQUI** 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 her. Ms. Namra Siddiqui 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.

Date:

Place:

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Associate Professor

Department of Chemistry

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"The secret to success is not to try to avoid or get rid of or shrink from your problems; the secret is to grow yourself so that you are bigger than any problem."

- T. Harv Eker

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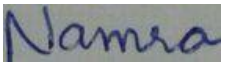

Namra Siddiqui

Abstract

Noncoding RNA molecules (ncRNAs) are transcripts, which directly act on their targets rather than coding for amino acids. These RNA molecules are present in all life forms and regulate diverse cellular functions. ncRNAs engage in a variety of mechanisms to regulate such as methylation of rRNA, inhibition of translation, and transcription and sequestration of regulatory proteins. Bacterial noncoding RNAs are generally signified as small RNAs (sRNAs). With the advancements in bacterial small RNA (sRNA) research, these sRNAs are found to be involved in many regulatory roles crucial for gene expression related to virulence in pathogenic bacteria, thus, controlling pathogenicity. Bacterial sRNAs are generally induced in response to changing environmental conditions and participate in numerous regulatory networks to fine tune the expressions of genes, which promote the cell survival in response to environmental cues. We have taken an insightful approach in the current study to investigate the regulation of expression of a novel sRNA RyjB. RyjB although was detected in all growth phases of *Escherichia coli* cells, but it was more prevalent in the exponential than the stationary phase of growth. RyjB was transcriptionally regulated neither by stationary phase sigma factor $E\sigma^S$ nor by $E\sigma^E$ under normal and stress conditions. RNA chaperones Hfq and ProQ are not necessary for the post-transcriptional abundance of RyjB, at least in the exponential phase of growth.

Interestingly, RyjB abundance appeared to be increased as the cells progressed through the exponential phase and decreased in the stationary phase, although RyjB was found to be acid stress-inducible small RNA. Nearly 2.5-fold upregulation was observed after 45 min of acid stress. The probability of RpoS involvement in *ryjB* expression under acid stress was eliminated as RyjB status was found to be identical in both wild-type and Δ*rpoS* cells. The mechanism how RyjB was upregulated to promote the cell viability under acid stress drew immense attention. PhoP, an integrant of the two-component regulatory system PhoPQ, has been reported to control many genes involved

in major cellular functions such as magnesium homeostasis, cell envelop composition, bacterial virulence, and acid resistance. Comparison of RyjB expression in wild-type and Δ*phoP* cells revealed that elimination of *phoP* allele from the chromosome resulted in a constant low-level expression of RyjB. Thus, *ryjB* appeared to be a PhoP-dependent acid stress-inducible sRNA.

RyjB is synthesized from a complementary strand to the one from which *sgcA* is synthesized and shares a 4-nt complementary region with *sgcA* transcript. This suggests that RyjB might act as an antisense RNA for *sgcA* expression. This finding supports the notion that RyjB:SgcA base pairing is a post-transcriptional event, thus, the potential role of RyjB is to increase the stability of *sgcA* transcript against intracellular ribonuclease-mediated degradation.

The final part of the thesis delineates that SgcA is a protein that is involved in a phospho-transfer reaction and has a potential role in cellular response to oxidative and osmotic stress. A remarkable growth difference observed between WT and Δ*sgcA* cells suggests that SgcA may have an important role in cellular growth and/or survival. SgcA plays a role in sugar transport within the cell, and its absence leads to decreased glucose uptake in the cell, which was confirmed by 2-NBDG assay. The observation that overexpression of RyjB in the cells leads to increased glucose uptake suggests that RyjB may be involved in regulating the expression of SgcA. Specifically, it has been postulated that RyjB may stabilize the mRNA of *sgcA*, enhancing the gene's expression and, subsequently, glucose uptake. The putative phosphotransferase protein SgcA lacks a crystal structure, making it challenging to comprehend its structural properties. To understand the secondary structure of SgcA, we performed CD and ICP-MS analysis, which allowed us to estimate the elements present in the protein. Overall, these findings suggest that SgcA is a protein that is involved in cellular growth, survival, and stress response, likely through its enzymatic activity in a phospho-transfer reaction. Further studies may be needed to fully elucidate SgcA's function and mechanisms of action.

सारांश

नॉनकोडिंग आरएनए अणु (एनसीआरएनए) प्रतिलेख हैं, जो अमीनो एसिड के लिए कोडिंग के बजाय सीधे अपने लक्ष्य पर कार्य करते हैं। ये आरएनए अणु सभी जीवन रूपों में मौजूद हैं और विविध सेलुलर कार्यों को नियंत्रित करते हैं। एनसीआरएनए विनियमित करने के लिए विभिन्न तंत्रों में संलग्न होते हैं जैसे कि आरआरएनए का मिथाइलेशन, अनुवाद का निषेध, और नियामक प्रोटीन का प्रतिलेखन और ज़ब्ती। बैक्टीरियल नॉनकोडिंग आरएनए को आम तौर पर छोटे आरएनए (एसआरएनए) के रूप में दर्शाया जाता है। बैक्टीरियल छोटे आरएनए (एसआरएनए) अनुसंधान में प्रगति के साथ, ये एसआरएनए रोगजनक बैक्टीरिया-रिया में विषाणु से संबंधित जीन अभिव्यक्ति के लिए महत्वपूर्ण कई नियामक भूमिकाओं में शामिल पाए जाते हैं, इस प्रकार, रोगजनकता को नियंत्रित करते हैं। बैक्टीरियल एसआरएनए आम तौर पर बदलती पर्यावरणीय परिस्थितियों के जवाब में प्रेरित होते हैं और जीन की अभिव्यक्तियों को ठीक करने के लिए कई नियामक नेटवर्क में भाग लेते हैं, जो पर्यावरणीय संकेतों के जवाब में कोशिका अस्तित्व को बढ़ावा देते हैं। हमने एक नए sRNA RyjB की अभिव्यक्ति के नियमन की जांच के लिए वर्तमान अध्ययन में एक व्यावहारिक दृष्टिकोण अपनाया है। RyjB हालांकि एस्चेरिचिया कोली कोशिकाओं के सभी विकास चरणों में पाया गया था, लेकिन यह विकास के स्थिर चरण की तुलना में घातीय में अधिक प्रचलित था। RyjB को सामान्य और तनाव की स्थिति में न तो स्थिर चरण सिग्मा कारक EσS और न ही EσE द्वारा ट्रांसक्रिप्शनल रूप से विनियमित किया गया था। आरएनए चैपरोन एचएफक्यू और प्रोक्यू आरवाईजेबी की पोस्ट-ट्रांसक्रिप्शनल बहुतायत के लिए आवश्यक नहीं हैं, कम से कम विकास के घातीय चरण में।

दिलचस्प बात यह है कि जैसे-जैसे कोशिकाएं घातीय चरण के माध्यम से आगे बढ़ीं, RyjB बहुतायत में वृद्धि हुई और स्थिर चरण में कमी आई, हालांकि RyjB को एसिड तनाव-प्रेरित छोटा आरएनए पाया गया। 45 मिनट के एसिड तनाव के बाद लगभग 2.5 गुना अपनियमन देखा गया। एसिड तनाव के तहत ryjB अभिव्यक्ति में RpoS की भागीदारी की संभावना समाप्त हो गई क्योंकि RyjB स्थिति जंगली-प्रकार और

ΔrpoS कोशिकाओं दोनों में समान पाई गई। एसिड तनाव के तहत सेल व्यवहार्यता को बढ़ावा देने के लिए RyjB को कैसे अपग्रेड किया गया, इस तंत्र ने अत्यधिक ध्यान आकर्षित किया। PhoP, दो-घटक नियामक प्रणाली PhoPQ का एक अभिन्न अंग है, जो मैग्नीशियम होमियोस्टैसिस, सेल आवरण संरचना, जीवाणु विषाणु और एसिड प्रतिरोध जैसे प्रमुख सेलुलर कार्यों में शामिल कई जीनों को नियंत्रित करने की सूचना दी गई है। जंगली-प्रकार और ΔphoP कोशिकाओं में RyjB अभिव्यक्ति की तुलना से पता चला कि गुणसूत्र से phoP एलील के उन्मूलन के परिणामस्वरूप RyjB की निरंतर निम्न-स्तरीय अभिव्यक्ति हुई। इस प्रकार, ryjB एक PhoP-निर्भर एसिड तनाव-प्रेरक sRNA प्रतीत होता है।

RyjB को एक पूरक स्ट्रैंड से संश्लेषित किया जाता है जिससे sgcA संश्लेषित होता है और sgcA प्रतिलेख के साथ 4-एनटी पूरक क्षेत्र साझा करता है। इससे पता चलता है कि RyjB sgcA अभिव्यक्ति के लिए एक एंटीसेंस RNA के रूप में कार्य कर सकता है। यह खोज इस धारणा का समर्थन करती है कि RyjB: SgcA बेस पेयरिंग एक पोस्ट-ट्रांसक्रिप्शनल घटना है, इस प्रकार, RyjB की संभावित भूमिका इंट्रासेल्युलर राइबोन्यूक्लिज़-मध्यस्थता गिरावट के खिलाफ sgcA ट्रांसक्रिप्ट की स्थिरता को बढ़ाना है।

थीसिस का अंतिम भाग बताता है कि एसजीसीए एक प्रोटीन है जो फॉस्फो-ट्रांसफर प्रतिक्रिया में शामिल होता है और ऑक्सीडेटिव और ऑस्मोटिक तनाव के लिए सेलुलर प्रतिक्रिया में इसकी संभावित भूमिका होती है। WT और ΔsgcA कोशिकाओं के बीच देखा गया एक उल्लेखनीय वृद्धि अंतर बताता है कि सेलुलर विकास और/या अस्तित्व में SgcA की महत्वपूर्ण भूमिका हो सकती है। SgcA कोशिका के भीतर शर्करा परिवहन में एक भूमिका निभाता है, और इसकी अनुपस्थिति से कोशिका में ग्लूकोज की मात्रा कम हो जाती है, जिसकी पुष्टि 2-NBDG परख द्वारा की गई थी। यह अवलोकन कि कोशिकाओं में RyjB की अधिक अभिव्यक्ति से ग्लूकोज की मात्रा बढ़ जाती है, यह पता चलता है कि RyjB SgcA की अभिव्यक्ति को विनियमित करने में शामिल हो सकता है। विशेष रूप से, यह माना गया है कि RyjB sgcA के mRNA को स्थिर कर सकता है, जीन की अभिव्यक्ति को बढ़ा सकता है और, परिणामस्वरूप, ग्लूकोज ग्रहण कर सकता

है। कल्पित फॉस्फोट्रांसफेरेज प्रोटीन एसजीसीए में क्रिस्टल संरचना का अभाव है, जिससे इसके संरचनात्मक गुणों को समझना चुनौतीपूर्ण हो जाता है। एसजीसीए की द्वितीयक संरचना को समझने के लिए, हमने सीडी और आईसीपी-एमएस विश्लेषण किया, जिससे हमें प्रोटीन में मौजूद तत्वों का अनुमान लगाने में मदद मिली। कुल मिलाकर, इन निष्कर्षों से पता चलता है कि एसजीसीए एक प्रोटीन है जो सेलुलर विकास, अस्तित्व और तनाव प्रतिक्रिया में शामिल है, संभवतः फॉस्फो-ट्रांसफर प्रतिक्रिया में इसकी एंजाइमेटिक गतिविधि के माध्यम से। SgcA के कार्य और क्रिया के तंत्र को पूरी तरह से स्पष्ट करने के लिए आगे के अध्ययन की आवश्यकता हो सकती है।

Contents

List of Figures

List of Tables

List of Abbreviations

BLAST	Basic Local Alignment Search Tool
Nt	Nucleotide
WT	Wild type
Amp	Ampicillin
Cat	Chloramphenicol acetyl transferase
Kan	Kanamycin
PFU	Plaque-forming units
RNA	Ribonucleic acid
sRNA	Small RNA
RNase	Ribonuclease
DEPC	Diethyl Pyro carbonate
rRNA	Ribosomal RNA
mRNA	Messenger RNA
tRNA	Transfer RNA
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
DNase	Deoxyribonuclease
RT-qPCR	Quantitative Real-time PCR
RT-PCR	Reverse Transcriptase PCR
PAGE	Poly acrylamide gel electrophoresis
DTT	Dithiothreitol
TEMED	Tetramethyl ethylenediamine
IPTG	Isopropyl β- d-1-thiogalactopyranoside
PMSF	Phenyl Methyl Sulfonyl Fluoride
PVDF	Polyvinylidene fluoride
DTT	Dithiothreitol
CFU	Colony Forming Units
pH	Negative logarithm of hydrogen ion
$MgCl_2$	Magnesium chloride

KCl	Potassium chloride
$CaCl_2$	Calcium chloride
mAbs	Monoclonal antibodies
MB	Molecular Grade
LB	Luria-Bertani
OD	Optical Density
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
Tm	Melting Temperature
SD	Standard deviation
Δ	Deletion
bp	Base pair