UNRAVELLING THE FUNCTION AND THE REGULATORY MECHANISM OF SMALL NONCODING RNAS IN *DEINOCOCCUS RADIODURANS*

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SEPTEMBER 2024

Unravelling the function and the regulatory mechanism of small noncoding RNAs in *Deinococcus radiodurans*

By

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in Fulfilment of the Requirements of the Degree of

Doctor of Philosophy

to the

Indian Institute of Technology Delhi

September 2024

CERTIFICATE

This is to certify that the thesis entitled "**Unravelling the function and the regulatory mechanism of small noncoding RNAs in *Deinococcus radiodurans***" being submitted by Mr. **Shiv Narayan Rai** 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. Shiv Narayan Rai** has worked under my guidance and supervision and has fulfilled the requirements for the thesis submission, which has reached the requisite standard to my knowledge.

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.

Dr. Tanmay Dutta
Professor of Department of Chemistry
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Date: September 21, 2024,
Place: IIT Delhi

Acknowledgments

First and foremost, I would like to express my deepest gratitude to my supervisor, **Dr. Tanmay Dutta**, for his invaluable guidance, constant encouragement, and insightful discussions throughout the course of my PhD journey. His expertise and mentorship have greatly shaped my approach to scientific inquiry and have been instrumental in the successful completion of this work.I would also like to extend my sincere thanks to **Dr. Sunil K Khare** and **Dr. Pramit K Chowdhury** for their advice, support, and constructive feedback during various stages of my research. Their expertise has been a valuable resource, and I am deeply grateful for their input.

I am extremely thankful to the Indian Institute of Technology Delhi for providing me with the necessary facilities and an enriching academic environment. My heartfelt appreciation goes to all my **lab members** and colleagues for their collaboration, support, and camaraderie. Special thanks to my friends, who have stood by me through challenges and celebrated the small victories with me. I am also profoundly grateful to my family, particularly my parents, for their unwavering love, patience, and belief in me. Their support has been my constant source of strength and motivation throughout this academic endeavor. Lastly, I acknowledge the financial support from the Government of India, which made this research possible. I dedicate this work to everyone who has contributed to this journey, directly or indirectly.

Shiv Narayan Rai

Abstract

Small regulatory RNAs (sRNAs) have emerged as critical regulators of gene expression in response to environmental stress in bacteria. This study investigates the role of sRNAs in the extremophilic bacterium *Deinococcus radiodurans*, particularly in regulating manganese (Mn) homeostasis under stress conditions. *D. radiodurans* is known for its exceptional resistance to ionizing radiation and desiccation, largely attributed to its ability to maintain Mn (II) homeostasis. However, the molecular mechanisms driving this regulation remain largely unexplored.

Through RNA-sequencing (RNA-seq), we identified a set of sRNAs responsive to Mn stress, which were further characterized by gene knockout and overexpression strategies. Our analysis revealed that these sRNAs regulate key genes involved in Mn transport and oxidative stress response. The use of binding assays and in vitro transcription provided mechanistic insights into the RNA-RNA interactions involved in this regulation. Additionally, RT-qPCR and Northern blotting confirmed the differential expression of these sRNAs under varying Mn concentrations.

This study highlights the significance of sRNAs in the adaptive stress response of *D. radiodurans*, with potential implications for understanding Mn homeostasis in other extremophiles. The findings contribute to a deeper understanding of the role of post-transcriptional regulation in microbial stress responses, offering new perspectives on the resilience mechanisms of extremophilic organisms.

सार

छोटे नियामक आर. एन. ए. (एस. आर. एन. ए.) बैक्टीरिया में पर्यावरणीय तनाव के जवाब में जीन अभिव्यक्ति के महत्वपूर्ण नियामकों के रूप में उभरे हैं। यह अध्ययन एक्सट्रिमोफिलिक बैक्टीरिया डीनोकोकस रेडियोड्यूरन्स में एस. आर. एन. ए. की भूमिका की जांच करता है, विशेष रूप से तनाव की स्थिति में मैंगनीज (एम. एन.) होमियोस्टेसिस को नियंत्रित करने में। डी. रेडियोड्यूरान्स आयनीकरण विकिरण और निर्जलीकरण के लिए अपने असाधारण प्रतिरोध के लिए जाना जाता है, जिसका मुख्य कारण एमएन (II) होमियोस्टेसिस को बनाए रखने की इसकी क्षमता है। हालांकि, इस विनियमन को चलाने वाले आणविक तंत्र काफी हद तक अज्ञात हैं।

आर. एन. ए.-अनुक्रमण (आर. एन. ए.-एस. ई. क्यू.) के माध्यम से हमने एम. एन. तनाव के प्रति उत्तरदायी एस. आर. एन. ए. के एक समूह की पहचान की, जिसे आगे जीन नॉकआउट और अति-अभिव्यक्ति रणनीतियों द्वारा चिह्नित किया गया। हमारे विश्लेषण से पता चला है कि ये एसआरएनए एमएन परिवहन और ऑक्सीडेटिव तनाव प्रतिक्रिया में शामिल प्रमुख जीनों को नियंत्रित करते हैं। आर. एन. ए. बाइंडिंग परख और इन विट्रो प्रतिलेखन के उपयोग ने इस विनियमन में शामिल आर. एन. ए.-आर. एन. ए. अंतःक्रियाओं में यांत्रिक अंतर्दृष्टि प्रदान की। इसके अतिरिक्त, आरटी-क्यूपीसीआर और नॉर्दर्न ब्लॉटिंग ने अलग-अलग एमएन सांद्रता के तहत इन एसआरएनए की अंतर अभिव्यक्ति की पुष्टि की।

यह अध्ययन डी. रेडियोड्यूरन्स की अनुकूली तनाव प्रतिक्रिया में एस. आर. एन. ए. के महत्व पर प्रकाश डालता है, जिसमें अन्य चरमपंथियों में एम. एन. होमियोस्टेसिस को समझने के लिए संभावित निहितार्थ हैं। निष्कर्ष सूक्ष्मजीव तनाव प्रतिक्रियाओं में प्रतिलेखन के बाद के विनियमन की भूमिका की गहरी समझ में योगदान करते हैं, जो चरम जीवों के लचीलापन तंत्र पर नए दृष्टिकोण प्रदान करते हैं।

Certificate

Acknowledgments

Abstract (Hindi/English)

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