

“DECIPHERING HMGB1: ACROSS A SPECTRUM OF DNA AND NUCLEOSOME DYNAMICS”

Ishu Gupta



**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

July 2025

© Indian Institute of Technology Delhi (IITD), New Delhi, 2025

“DECIPHERING HMGB1: ACROSS A SPECTRUM OF DNA AND NUCLEOSOME DYNAMICS”

by

Ishu Gupta

KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2025

Dedicated to my parents, sisters and nephew

For their endless love, support and encouragement

CERTIFICATE

This is to certify that the thesis titled “**Deciphering HMGB1: Across a spectrum of DNA and nucleosome dynamics**”, submitted by **Mr. Ishu Gupta** to the Indian Institute of Technology Delhi for the award of the degree of “**Doctor of Philosophy**” is a record of the Bonafide research carried out by him, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University for the award of any degree or diploma.

Dr. Ashok Kumar Patel, Ph.D.

Professor

Kusuma School of Biological Sciences

Indian Institute of Technology Delhi

New Delhi 110016, India

ACKNOWLEDGEMENTS

The completion of this thesis would not have been possible without the support, guidance, and encouragement of many individuals, to whom I am deeply grateful. First and foremost, I would like to express my heartfelt gratitude to my supervisor, **Dr. Ashok Kumar Patel**. Their exceptional expertise, constant support, and tireless commitment to my research have been instrumental in every step of this journey. From the earliest stages of shaping the research question to the final revisions, their thoughtful advice and insightful feedback have consistently pushed me to think critically and to refine my work. I am truly fortunate to have had the opportunity to work under their guidance.

I would also like to extend my sincere thanks to my thesis committee members, **Prof. Bishwajit Kundu, Dr. Manoj B. Menon, and Prof. Ritu Kulshreshtha**, for their valuable contributions to this work. Their suggestions, questions, and critiques have significantly enhanced the quality of this research, and their support has kept me focused and motivated during the challenging times. Their commitment to my academic development has been a constant source of inspiration.

A special note of thanks goes to my colleagues and fellow researchers in the Kusuma School of Biological Sciences and the administrative and technical staff at Indian Institute of Technology Delhi for their help in navigating the logistical aspects of my research. The collaborative environment we shared—whether through informal discussions, research seminars, or collaborative projects—has been enriching and made the academic journey more enjoyable. I have learned so much from each of you, and I will always value the camaraderie and intellectual exchange that characterized our time together. This research was made possible through the generous financial support of Department of Biotechnology, India.

I am also deeply grateful to my family, whose love and sacrifices have been a constant source of strength. To my parents, Mrs. Semita Gupta and Mr. Shyam Sunder Gupta, who have always encouraged me to follow my dreams and supported me in ways that words cannot fully express this achievement is as much yours as it is mine. To my sisters, Mrs. Nitika Gupta and Ms. Parul Gupta thank you for your unwavering patience, understanding, and emotional support. You have been my anchor through the many ups and downs of this journey, and I am forever grateful for your love and encouragement. To my nephew Mr. Aviraj Gupta, your innocence and joy have provided me with the motivation to persevere, and I am thankful for your love and understanding, especially during the challenging times.

Finally, this thesis is dedicated to my friend Ms. Ishitha, who has helped me a lot in the writing of my research paper and being the constant support and motivation in hard times. I would also like to thank Sidharth, Saadia and Priyanka for being my lab mates but from other labs. Vaibhav for being my metro travel buddy and discussion partner throughout my degree. I want to extend my appreciation to Dr. Sunil Kumar, Ananya, Shreya, Sunny, Mohit, Saloni, Adya, and Ghuncha for being wonderful lab mates.

Last but certainly not least, I would like to thank all of the individuals who contributed in ways both big and small to the completion of this thesis. From those who provided specific insights into my research to those who offered words of encouragement along the way—your influence has been immeasurable. Though I may not be able to name everyone individually, I want you to know how deeply I appreciate your help and support.

Ishu Gupta

ABSTRACT

HMGB1 is most abundant nonhistone nuclear protein that has been thoroughly studied for its functions beyond the nucleus, especially in the cytoplasm, where it serves as an autophagy mediator, and in the extracellular matrix, where it operates as an inflammatory molecule. Nonetheless, the complete range of its roles within the nucleus is still not well comprehended. This *in vitro* research sought to address this gap by examining the binding properties of HMGB1 with different types of DNAs, nucleosomes, and chromatin. The research discovered that HMGB1 shows varying binding affinities, displaying a particularly high affinity for modified DNA structures like triplex and bulge DNA. This implies that HMGB1 might be important in detecting and reacting to DNA damage or structural irregularities. Moreover, the study emphasized that HMGB1 binding disrupts nucleosome remodelling by the CHD7 chromatin remodeller, indicating that HMGB1 could impact chromatin accessibility and remodelling activities, potentially influencing transcriptional regulation. The research also showed that HMGB1 attaches to the linker DNA between nucleosomes, possibly affecting chromatin structure and function. These results offer fresh perspectives on the diverse functions of HMGB1, potentially going beyond its established roles in inflammation and autophagy to encompass significant contributions to gene expression, viral replication, and genetic disorders like CHARGE syndrome, where alterations in the CHD7 gene disrupt normal chromatin remodelling. This research consequently paves the way for investigating HMGB1's functions in the nucleus, possibly resulting in innovative treatment strategies for disorders associated with chromatin dysregulation.

सारांश

HMGB1 सबसे प्रचुर मात्रा में पाया जाने वाला नॉनहिस्टोन परमाणु प्रोटीन है जिसका नाभिक से परे, विशेष रूप से साइटोप्लाज्म में, जहां यह एक ऑटोफैगी मध्यस्थ के रूप में कार्य करता है, और बाह्य मैट्रिक्स में, जहां यह एक सूजन अणु के रूप में कार्य करता है, इसके कार्यों के लिए गहन अध्ययन किया गया है। बहरहाल, नाभिक के भीतर इसकी भूमिकाओं की पूरी श्रृंखला अभी भी अच्छी तरह से समझ में नहीं आई है। इस इन विट्रो शोध में विभिन्न प्रकार के डीएनए, न्यूक्लियोसोम और क्रोमैटिन के साथ एचएमजी1 के बंधन गुणों की जांच करके इस अंतर को संबोधित करने की कोशिश की गई। शोध से पता चला कि HMGB1 अलग-अलग बाध्यकारी समानताएं दिखाता है, जो ट्रिपलक्स और उभार डीएनए जैसी संशोधित डीएनए संरचनाओं के लिए विशेष रूप से उच्च समानता प्रदर्शित करता है। इसका तात्पर्य यह है कि HMGB1 डीएनए क्षति या संरचनात्मक अनियमितताओं का पता लगाने और उन पर प्रतिक्रिया करने में महत्वपूर्ण हो सकता है। इसके अलावा, अध्ययन में इस बात पर जोर दिया गया कि HMGB1 बाइंडिंग CHD7 क्रोमैटिन रीमॉडेलर द्वारा न्यूक्लियोसोम रीमॉडलिंग को बाधित करता है, यह दर्शाता है कि HMGB1 क्रोमैटिन पहुंच और रीमॉडलिंग गतिविधियों को प्रभावित कर सकता है, संभावित रूप से ट्रांसक्रिप्शनल विनियमन को प्रभावित कर सकता है। शोध से यह भी पता चला कि HMGB1 न्यूक्लियोसोम के बीच लिंकर डीएनए से जुड़ जाता है, जो संभवतः क्रोमैटिन संरचना और कार्य को प्रभावित करता है। ये परिणाम HMGB1 के विविध कार्यों पर नए दृष्टिकोण पेश करते हैं, संभावित रूप से सूजन और ऑटोफैगी में इसकी स्थापित भूमिकाओं से परे जाकर जीन अभिव्यक्ति, वायरल प्रतिकृति और चार्ज सिंड्रोम जैसे आनुवंशिक विकारों में महत्वपूर्ण योगदान शामिल हैं, जहां CHD7 जीन में परिवर्तन सामान्य क्रोमैटिन रीमॉडलिंग को बाधित करते हैं। . परिणामस्वरूप यह शोध नाभिक में HMGB1 के कार्यों की जांच करने का मार्ग प्रशस्त करता है, जिसके परिणामस्वरूप संभवतः क्रोमैटिन डिसरेगुलेशन से जुड़े विकारों के लिए नवीन उपचार रणनीतियाँ तैयार होती हैं।

HIGHLIGHTS

The principal findings of this study are summarized below:

- HMGB1 preferentially binds to the altered DNA structures
- HMGB1 has high affinity for DNA sequences that are AT-rich as compared to GC-rich
- The binding of HMGB1 increases as the length of the DNA increases
- Triplex DNA is destabilized by the HMGB1 upon its binding and leads to the formation of duplex DNA
- While in case of G-quadruplex structures, HMGB1 acts as a mild destabilizer
- HMGB1 binds to nucleosome with high affinity and this binding impedes the CHD7 mediated nucleosome remodelling
- HMGB1 also have affinity for chromatin, and it binds in the linker region DNA present between two nucleosomes.

Table of Contents

CERTIFICATE	iv
ACKNOWLEDGEMENTS	v
ABSTRACT.....	vii
सारांश.....	viii
HIGHLIGHTS	ix
LIST OF TABLES	xx
ABBREVIATIONS & SYMBOLS	1
Chapter 1.....	4
INTRODUCTION	4
1.1. Introduction.....	5
1.2. Rationale of the study.....	11
1.3. Objectives of the study.....	13
Chapter 2.....	14
REVIEW OF LITERATURE.....	14
2.1. HMGB1	15
2.2. Structure	18
2.2.1. AlphaFold and NMR structure comparison	20
2.2.2. Comparison between HMGB1 and its truncated forms	21

2.2.1.1.	Structural and Biophysical Differences	21
2.2.1.2.	DNA-Binding and Functional Variations.....	22
2.2.1.3.	Implications in Cellular Processes	22
2.3.	<i>Functions of the HMGB1</i>	24
2.3.1.	Nuclear HMGB1	24
2.3.1.1.	Interactions with the nucleosome and histone proteins	24
2.3.1.2.	Interaction with the DNA	25
2.3.2.	Cytoplasmic HMGB1	27
2.3.3.	Extracellular HMGB1	27
2.3.3.1.	Role in inflammation	28
2.3.3.2.	Role in cell proliferation and differentiation	28
2.3.3.3.	Role in cell death	29
2.4.	<i>Redox states of HMGB1</i>	31
2.4.1.	Reduced state of HMGB1	31
2.4.2.	Oxidized state of HMGB1	32
2.4.3.	Disulfide bond in HMGB1	32
2.5.	<i>HMGB1 release</i>	33
2.5.1.	Active secretion	33
2.5.2.	Passive release	34
2.6.	<i>HMGB1 receptor dependent function</i>	36
2.7.	<i>Dual role of HMGB1 in gene activation and repression</i>	38

2.7.1.	HMGB1 in gene activation	38
2.7.1.1.	Enhancement of transcription factor binding:	38
2.7.1.2.	Interaction with sterol regulatory element-binding proteins (SREBPs):	39
2.7.1.3.	Regulation of Ribosomal RNA (rRNA) Modification:	39
2.7.2.	HMGB1 in gene repression	39
2.7.2.1.	Endotoxin tolerance and inflammatory gene silencing:	39
2.7.2.2.	Cell-specific modulation of p53 family transcription	39
2.8.	<i>HMGB1 in diseases</i>	40
2.8.1.	Cancer	40
2.8.2.	Neurodegenerative diseases	41
2.8.3.	Inflammation	42
2.8.4.	Autoimmune diseases.....	43
2.9.	<i>Chromatin dynamics</i>	45
2.9.1.	Chromatin structure.....	45
2.9.2.	Mechanisms of chromatin remodelling	49
2.9.2.1.	Covalent modifications:	49
2.9.2.2.	Nucleosome movement and ATP-Dependent remodelling:.....	50
2.10.	<i>CHD7 chromatin remodeller</i>	52
2.10.1.	Functions of CHD7	53
2.10.1.1.	Chromatin remodelling:	53
2.10.1.2.	Development:.....	53
2.10.1.3.	Gene regulation:	53

2.10.1.4.	Interaction with other proteins:	53
2.11.	<i>HMGB1 and chromatin dynamics</i>	54
2.11.1.	HMGB1 and its interaction with chromatin remodelling complexes	55
2.11.2.	HMGB1 and chromatin remodelling complexes	56
2.11.2.1.	Enabling nucleosome remodelling:	56
2.11.2.2.	Inflammatory reaction and chromatin control:	56
2.11.2.3.	DNA repair:	57
2.11.2.4.	Transcription regulation:	57
2.11.3.	Chromatin remodeller complexes associated with HMGB1:	57
2.11.3.1.	SWI/SNF complex:	57
2.11.3.2.	ISWI complex:	58
2.11.3.3.	CHD complexes:	58
2.11.3.4.	INO80 complex:	58
2.12.	<i>Unresolved questions regarding HMGB1's chromatin remodelling mechanisms</i>	
	60	
Chapter 3		63
TO UNDERSTAND THE BINDING AFFINITY OF HMGB1 ON DIFFERENT TYPES OF DNA		63
3.1.	<i>Introduction</i>	64
3.2.	<i>Results</i>	66
3.2.1.	Recombinant HMGB1 overexpression and purification	66
3.2.2.	HMGB1 has no measurable affinity for ssDNA	68

3.2.3.	HMGB1 affinity for canonical dsDNA increases with length and has higher affinity towards AT-rich dsDNA	70
3.2.4.	Triplex DNA reconstitution	74
3.2.5.	HMGB1 binds triplex DNA and acts as a triplex DNA destabilizer	76
3.2.6.	HMGB1 binds and mildly alters G-quadruplexes	79
3.2.7.	Bulge DNA reconstitution	82
3.2.8.	HMGB1 has an affinity for bulge DNA and its affinity increases with an increase in the length of the bulge	83
3.3.	<i>Discussion</i>	85
<i>Chapter 4</i>		87
<i>To investigate the role of HMGB1 in nucleosome dynamics</i>		87
4.1.	<i>Introduction</i>	88
4.2.	<i>Results</i>	90
4.2.1.	Nucleosome core particle (NCP) reconstitution	90
4.2.1.1.	PCR amplification and purification of nucleosomal DNA	90
4.2.1.1.	Histone octamer preparation and purification	92
4.2.1.1.	Nucleosome reconstitution and purification	94
4.2.2.	Nucleosome characterization through TEM	97
4.2.3.	Δ CHD7 protein overexpression and purification	99
4.2.4.	HMGB1 hinders the remodelling activity of Δ CHD7 remodeller	102
4.3.	<i>Discussion</i>	106

Chapter 5.....	108
<i>To understand the role of HMGB1 in the context of chromatin.....</i>	108
5.1. Introduction.....	109
5.2. Results	111
5.2.1. Chromatin reconstitution	111
5.2.1.1. Preparation of chromatinized plasmid.....	111
5.2.1.2. MNase assay of reconstituted chromatin	113
5.2.2. DNA accessibility of HMGB1 in chromatin array	115
5.3. Discussion.....	119
Chapter 6.....	122
MATERIALS AND METHODS.....	122
6.1. Recombinant plasmids and E. coli strains	123
6.2. Recombinant protein overexpression and its purification.....	125
6.2.1. HMGB1	125
6.2.2. Δ CHD7	126
6.3. Western blot	127
6.4. Reconstitution of different DNA	128
6.4.1. dsDNA reconstitution.....	130
6.4.2. Triplex DNA reconstitution.....	130
6.4.3. G-quadruplex reconstitution	131

6.4.4.	Bulge DNA reconstitution	131
6.4.5.	HMGB1 and nucleic acid binding.....	131
6.5.	<i>Gel mobility shift assay</i>	132
6.6.	<i>Circular dichroism (CD) spectroscopy</i>	132
6.7.	<i>Nucleosome preparation</i>	133
6.7.1.	PCR amplification of 5' FAM labelled DNA/ nucleosomal DNA	133
6.7.2.	Histone octamer preparation and purification	134
6.7.3.	Reconstitution of nucleosome and its purification.....	134
6.8.	<i>Negative staining of NCP</i>	135
6.9.	<i>Nucleosome sliding assay</i>	136
6.10.	<i>Reconstitution of 34-mer chromatinized plasmid to form chromatin array</i>	136
6.11.	<i>DNA accessibility assay</i>	137
CONCLUSION AND FUTURE PROSPECTS.....		139
7.1.	<i>Conclusion</i>	140
7.2.	<i>Future prospects</i>	144
REFERENCES		146
APPENDICES		176
Appendix II Culture media and antibiotics stock solutions.....		182
1.	<i>Bacterial culture media</i>	182

1.1.	Luria Bertani broth	182
1.2.	Luria Bertani agar.....	182
1.3.	2XTY broth	182
2.	<i>Auto-induction Media</i>	182
2.1.	Composition of Auto-induction media (1L)	182
2.2.	ZY Media	183
2.3.	20x NPS	183
2.4.	50x 5052	183
3.	<i>Antibiotics stock solution</i>	183
3.1.	Ampicillin.....	183
3.2.	Kanamycin	183
3.3.	Chloramphenicol.....	184
<i>Appendix III Solutions and Buffers</i>		185
<i>AUTHOR'S RESUME</i>		193

LIST OF FIGURES

Figure 2.2.1 Structure of HMGB1 protein.....	18
Figure 2.2.2 The helical structure of HMGB1 without tail.	23
Figure 2.3.1 HMGB1 led binding of transcription factors.....	25
Figure 2.3.2 DNA bend produced as a result of HMGB1 and HMGB1 Δ C (without acidic tail) binding.	26
Figure 2.4.1 Different redox states of HMGB1.....	31
Figure 2.5.1 Different receptors for HMGB1 according to its redox states.....	34
Figure 2.9.1 Different structural modes of nucleosome.....	47
Figure 2.9.2 Schematic representation of different ATP dependent chromatin remodellers.	52
Figure 2.10.1 Schematic representation of CHD7 protein depicting all the domains.....	54
Figure 3.2.1 SDS PAGE of HMGB1 protein fractions from different chromatography.	67
Figure 3.2.2 HMGB1 has no measurable affinity for ssDNA	69
Figure 3.2.3 HMGB1 affinity for canonical dsDNA increases with length and has higher affinity towards AT-rich dsDNA	73
Figure 3.2.4 Native PAGE of reconstituted triplex DNA with increasing concentration of their respective TFO (Triplex Forming Oligonucleotides) as seen on gel mobility shift assay.	75
Figure 3.2.5 HMGB1 binds triplex DNA and acts as a triplex DNA destabilizer.....	78
Figure 3.2.6 HMGB1 binds and stabilizes G-quadruplexes.....	81
Figure 3.2.7 Agarose gel of bulge DNA reconstitution.	82
Figure 3.2.8 HMGB1 has affinity for bulge DNA and its affinity increases with an increase in the length of the bulge	84

Figure 4.2.1 Agarose gel of nucleosomal DNA.....	91
Figure 4.2.2 18% SDS PAGE of histones.	93
Figure 4.2.3 Schematic representation of nucleosome reconstitution.	95
Figure 4.2.4 Native PAGE of reconstituted NCP	96
Figure 4.2.5 A-B) TEM (Transmission Electron Microscope) images of nucleosome after negative staining with uranyl acetate.	98
Figure 4.2.6 SDS PAGE of ΔCHD7 protein fractions from different chromatography.	101
Figure 4.2.7 HMGB1 hinders the remodelling activity of ΔCHD7 remodeller.....	105
Figure 5.2.1 Agarose gel of 34-mer plasmid.	112
Figure 5.2.2 MNase assay of reconstituted chromatin at different MNase concentration and for different time points.....	114
Figure 5.2.3 DNA accessibility of HMGB1 in chromatin array	118
Figure 7.1 Illustrative overview of the study	143

LIST OF TABLES

Table 2.2.1 Role of HMGB1 amino acid residues.....	19
Table 2.2.2 Comparison between NMR and AlphaFold structure	20
Table 2.3.1 Location dependent function of HMGB1.....	30
Table 2.5.1 Post translational modifications of HMGB1	35
Table 2.6.1 HMGB1 and some of its major receptors with their respective function.....	36
Table 6.4.1 DNA oligonucleotides sequence used in this study	128

ABBREVIATIONS & SYMBOLS

%	Percent
~	Approximately
°C	Degree Celsius
β-ME	β-mercaptoethanol
μg	Microgram
μL	Microliter
μm	Micrometer
μM	Micromole
APS	Ammonium persulfate
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CaCl ₂	Calcium chloride
CD	Circular Dichroism
CHD	Chromodomain helicase DNA binding
DAMP	Damage assisted molecular pattern
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
DTT	1, 4-Dithiothreitol
EtBr	Ethidium Bromide
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic mobility shift assay

FAM	Fluorescein amidite
g	gram
GQ	G-quadruplexes
HCl	Hydrochloric Acid
HMGB1	High mobility group box 1
ISWI	Imitation switch
kb	Kilobase pair
KCl	Potassium chloride
kDa	Kilo Dalton
KH_2PO_4	Potassium dihydrogen phosphate
M	Molar
mg	Milligram
MgCl_2	Magnesium chloride
MgSO_4	Magnesium sulfate
mM	Millimolar
MNase	Micrococcal nucleas
Na_2HPO_4	Sodium phosphate dibasic
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCP	Nucleosome core particle
NER	Nucleotide excision repair
$(\text{NH}_4)_2\text{SO}_4$	Ammonium sulfate
nm	Nanometer

nM	Nanomole
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PMSF	Phenyl methyl sulfonyl fluoride
rpm	Revolutions per minute
RT	Room temperature
SD	Standard deviation
SDS	Sodium dodecyl sulfate
ssDNA	single stranded DNA
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TBS	Tris-buffered saline
TEM	Transmission Electron Microscopy
TEMED	N, N, N', N'-Tetramethylethylenediamine
TFO	Triplex forming oligonucleotide
TLR	Toll-like receptor