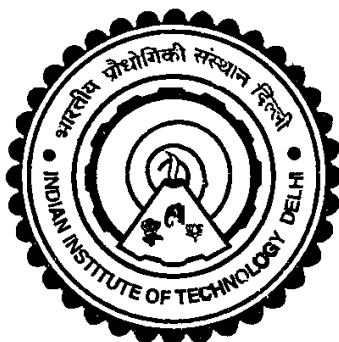


**ELUCIDATING THE PROTEIN FOLDING FUNCTION OF THE  
*MYCOBACTERIUM TUBERCULOSIS* & *MYCOBACTERIUM MARINUM*  
CHAPERONIN SYSTEMS**

**BAKUL PIPLANI**



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

**OCTOBER 2022**

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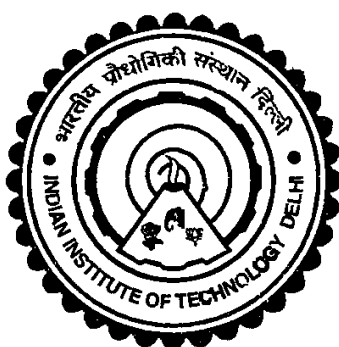
*by*

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Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy  
to the



**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**OCTOBER 2022**

## CERTIFICATE

This is to certify that the thesis titled “**Elucidating the protein folding function of the *Mycobacterium tuberculosis* & *Mycobacterium marinum* chaperonin systems**”, being submitted by **Ms. Bakul Piplani** to the **Indian Institute of Technology Delhi** for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research work carried out by her, and prepared under my supervision in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The research reported and the results described in this thesis have not been submitted to any other University or Institute for the award of any Degree or Diploma.

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## ACKNOWLEDGMENTS

*“Dubito ergo cogito, ergo sum”*

– Antoine-Léonard Thomas, in *Éloge de René Descartes* (1765)

*What started as a statement by the French philosopher, mathematician and scientist René Descartes in his *Principles of Philosophy* (1644), eventually became a fundamental element of modern philosophy. Originally articulated in Latin, in English this statement translates to “I doubt, therefore I think, therefore I am”, emphasising that human beings are thinking entities capable of building knowledge on the foundation of reason. Although arisen out of Descartes’ scepticism, this quote precisely expresses my notion about the scientific process of research. If asked about my reasons for pursuing a PhD, this quote sums up my answer just fine. The process of research fascinates me for the very reason that it challenges me to doubt everything around me, to think about complex phenomenon in great detail, to ask questions and find their answers, to solve puzzles, and to steadily keep working towards solving a problem. Now, almost at the end of the PhD, when I look back at my journey, I see it as a period of metamorphosis. This journey, although long and arduous, has influenced me in more ways than one. It would have been much more strenuous without the support of my family and the company of my friends and colleagues. Many people have helped me traverse the ragged path in countless ways on several occasions, often by going out of their way to help me.*

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To me, PhD seemed like a far-fetched dream, had it not been for my late grandfather, **Shri Vir Bhan Arora**. He encouraged me to dream, believed in my dreams and helped me turn them into reality. He taught me how to remain calm and composed in the face of adversity. He taught me the importance of patience and persistence. Had he been with me today, I'm sure he would have been the happiest to see me navigate towards the end of this journey. My grandmother, **Smt. Santosh Arora**, taught me to keep moving forward. She was the first to encourage me and motivate me when the going got tough. She has patiently stood by me and provided unconditional support. Determination is the key to success in any field, and more so in research. My first lesson in determination came from my mother, **Smt. Sapna Arora**. She inspires me

*every day with her resilience and her refusal to bow down in front of challenges, no matter how difficult the circumstances. On days when I dreaded going to the laboratory, after repeated failures, she motivated me and reminded me of my reasons to start this journey. I have looked up to her words and teachings, more so in times of frustration. Moreover, she always patiently heard me out, stood by me and supported me. My family are the pillars that I have been standing on since I can remember. Thank you, Maa, Naani and Daddy for always being with me.*

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***Bakul Piplani***

## ABSTRACT

The Chaperonins (HSP60) constitute a family of essential molecular chaperone proteins that function as key players in the cellular proteostasis network. They are responsible for the folding and function of ~10 – 15% of the cellular proteins under normal conditions, which increases to ~ 30% under conditions of stress, such as heat shock. Group I chaperonins, that are predominantly found in the bacterial cytosol, mitochondrial lumen and in chloroplasts, function in association with the HSP10 (HSP20 in chloroplasts) family of proteins, also known as the co-chaperonins. The bacterial homologs of HSP60 and HSP10 are denoted by Cpn60 and Cpn10 respectively, to distinguish them from the organellar homologs. Most of the studies investigating the structure and function of bacterial chaperonin systems have been centred around the *E. coli* chaperonin system consisting of GroES (Cpn10) and GroEL (Cpn60). Until recently, the GroES-GroEL system was deemed as the prototypical bacterial chaperonin systems *vis-à-vis* structure, function and mechanisms of action. However, recent investigation of diverse chaperonin systems have illustrated that the biochemical and biophysical properties of chaperonins may not be universal. Mycobacteria, a genus containing some notorious pathogens, curiously possess two chaperonin genes, *cpn60.1* and *cpn60.2*, but only one co-chaperonin gene, *cpn10*. The protein products of the Mycobacterial *cpn* genes are particularly enigmatic in that they possess multiple moonlighting functions, while their molecular chaperone functions have not been illustrated with certainty. In this thesis, we sought to explore the molecular chaperone function of the chaperonin systems, Cpn10-Cpn60.1 and Cpn10-Cpn60.2, from two pathogenic *Mycobacterium* species, *M. tuberculosis* and *M. marinum*.

We began by analysing the similarities and differences between the *E. coli* and Mycobacterial chaperonin and co-chaperonin proteins at the sequence and structural levels. We specifically probed the presence of conserved motifs and domains critical for chaperonin function in the Mycobacterial homologs. Thereafter, we evaluated the ability of the Mycobacterial chaperonin

systems to reinstate intracellular chaperonin function in an *E. coli* experimental strain depleted of endogenous GroES and GroEL. We initially investigated the chaperonin function by evaluating the ability of the Mycobacterial chaperonin systems to restore cell viability and proliferation in the *E. coli* model. We found that the Cpn10-Cpn60.1 systems could only support cell survival, while the Cpn10-Cpn60.2 systems could also augment cell proliferation. Our findings suggest that, in Mycobacteria, both the chaperonin systems possess intracellular chaperonin function but with subtle differences amongst the two systems. We subsequently proceeded with chaperonin-assisted protein-folding assays to probe the ability of the chaperonin systems in preventing the aggregation and augmenting the folding of MetK, an obligate GroEL client and an essential *E. coli* protein. The *in vivo* functional assays illustrated that the Cpn10-Cpn60.1 systems are adept at preventing MetK aggregation, while Cpn10-Cpn60.2 are proficient at supporting both aggregation prevention and productive folding. Furthermore, in line with a previous report on phosphorylation-induced oligomerisation of Cpn60.1, we decided to evaluate the impact of Cpn60.1 phosphorylation on its chaperonin function. Our findings suggest that phosphorylation at Ser75 and Ser393 augments the chaperonin function of the *M. tuberculosis* Cpn60.1, allowing it to support cell proliferation in the *E. coli* model. This appears to be an adaptive mechanism to regulate the overall intracellular chaperonin capacity under normal conditions, which may be enhanced by Cpn60.1 phosphorylation to improve cellular fitness under conditions of stress.

In conclusion, our findings illustrate the intracellular molecular chaperone function of the Mycobacterial chaperonin systems. We hereby establish that both the chaperonin systems of *M. tuberculosis* and *M. marinum* (and by extrapolation, possibly of all Mycobacteria) are adept at canonical chaperonin function. On the basis of our findings, we propose that Cpn10-Cpn60.2 represents the housekeeping chaperonin system in Mycobacteria, with Cpn10-Cpn60.1 supplementing the intracellular chaperonin capacity as per the cellular requirements.

## सार

चैपरोनिन (HSP60) आवश्यक आणविक चैपरोन प्रोटीन के एक परिवार का गठन करते हैं जो सेलुलर प्रोटियोस्टेसिस नेटवर्क में प्रमुख खिलाड़ियों के रूप में काम करते हैं। वे सामान्य परिस्थितियों में ~ 10-15% सेलुलर प्रोटीन के तह और कृत्य के लिए जिम्मेदार हैं, जो गर्मी के झटके जैसे तनाव की स्थितियों में ~ 30% तक बढ़ जाता है। ग्रुप I चैपरोनिन, जो मुख्य रूप से बैक्टीरियल साइटोसोल, माइटोकॉन्ड्रियल लुमेन और क्लोरोप्लास्ट में पाए जाते हैं, HSP10 (क्लोरोप्लास्ट में HSP20) प्रोटीन परिवार के साथ मिलकर काम करते हैं, जिन्हें को-चैपरोनिन भी कहा जाता है। HSP60 और HSP10 के जीवाणु समरूपों को क्रमशः Cpn60 और Cpn10 द्वारा निरूपित किया जाता है, ताकि उन्हें ऑर्गेनेल होमोलॉग से अलग किया जा सके। बैक्टीरियल चैपरोनिन सिस्टम की संरचना और कार्य की जांच करने वाले अधिकांश अध्ययन *एस्चेरिचिया कोलाई* चैपरोनिन सिस्टम के प्रोटीन, GroES (Cpn10) और GroEL (Cpn60), पर केंद्रित हैं। कुछ समय पहले तक, चैपरोनिन संरचना, कार्य और क्रिया के विषय में GroES-GroEL सिस्टम को आदर्श बैक्टीरियल चैपरोनिन सिस्टम के रूप में समझा जाता था। हालांकि, विविध चैपरोनिन प्रणालियों की हालिया जांच ने स्पष्ट किया है कि चैपरोनिन के जैव-रासायनिक और जैव-भौतिक गुण सार्वभौमिक नहीं हो सकते हैं। उत्सुकता से, माइकोबैक्टीरिया में दो चैपरोनिन जीन, *cpn60.1* और *cpn60.2*, है लेकिन केवल एक को-चैपरोनिन जीन, *cpn10*, है। माइकोबैक्टीरियल *cpn60* जीन्स के प्रोटीन उत्पाद विशेष रूप से रहस्यपूर्ण हैं क्योंकि उनके पास कई मूनलाइटिंग कृत्य हैं, जबकि उनके आणविक चैपरोन कृत्यों को निश्चितता के साथ चित्रित नहीं किया गया है। इस थीसिस में हमने दो रोगजनक *माइकोबैक्टीरियम* प्रजातियों, *एम. ट्यूबरकुलोसिस* और *एम. मेरिनम* के चैपरोनिन सिस्टम, Cpn10-Cpn60.1 और Cpn10-Cpn60.2, के आणविक चैपरोन फ़ंक्शन का पता लगाने का प्रयास किया है।

हमने *ई. कोलाई* और माइकोबैक्टीरियल चैपरोनिन और सह-चैपरोनिन प्रोटीन के बीच समानता और अंतर का विश्लेषण किया। हमने विशेष रूप से माइकोबैक्टीरियल होमोलोग्स में चैपरोनिन फ़ंक्शन के लिए महत्वपूर्ण संरक्षित रूपांकनों और डोमेन की उपस्थिति की जांच की। इसके बाद, हमने अंतर्जात

GroES और GroEL से हीन *ई. कोलाई* में इंटरसेल्युलर चैपरोनिन फ़ंक्शन को बहाल करने के लिए माइकोबैक्टीरियल चैपरोनिन सिस्टम की क्षमता का मूल्यांकन किया। हमने शुरू में *ई. कोलाई* में जीवन क्षमता और वृद्धि को बहाल करने के लिए माइकोबैक्टीरियल चैपरोनिन सिस्टम की क्षमता का मूल्यांकन किया। हमने पाया कि Cpn10-Cpn60.1 सिस्टम केवल सेल अस्तित्व का समर्थन कर सकते हैं, जबकि Cpn10-Cpn60.2 सिस्टम सेल प्रसार को भी बढ़ा सकते हैं। हमारे निष्कर्ष बताते हैं कि, माइकोबैक्टीरिया में, दोनों चैपरोनिन प्रणालियों में इंटरसेल्युलर चैपरोनिन फ़ंक्शन होता है, लेकिन दोनों प्रणालियों के बीच सूक्ष्म अंतर होता है। हम ने चैपरोनिन-सहायता प्राप्त प्रोटीन-फोल्डिंग एसेज़ के साथ MetK, एक बाध्य GroEL क्लाइंट और एक आवश्यक *ई. कोलाई* प्रोटीन, के फोल्डिंग को बढ़ाने में चैपरोनिन सिस्टम की क्षमता की जांच की। *इन वीवो* फ़ंक्शनल एसेज़ ने सचित्र किया कि Cpn10-Cpn60.1 सिस्टम MetK एकत्रीकरण को रोकने में माहिर हैं, जबकि Cpn10-Cpn60.2 एकत्रीकरण की रोकथाम और उत्पादक फोल्डिंग दोनों का समर्थन करने में कुशल हैं। इसके अलावा, Cpn60.1 के फॉस्फोराइलेशन-प्रेरित ओलिगोमेराइजेशन पर पिछली रिपोर्ट के अनुरूप, हमने इसके चैपरोनिन फ़ंक्शन पर Cpn60.1 फॉस्फोराइलेशन के प्रभाव का मूल्यांकन किया। हमारे निष्कर्ष बताते हैं कि Ser75 और Ser393 पर फॉस्फोराइलेशन *एम. ट्यूबरकुलोसिस* Cpn60.1 के चैपरोनिन फ़ंक्शन को बढ़ाता है, जिससे यह *E. coli* मॉडल में सेल प्रसार का समर्थन करता है। यह सामान्य परिस्थितियों में समग्र इंटरसेल्युलर चैपरोनिन क्षमता को विनियमित करने के लिए एक अनुकूली क्रियाविधि प्रतीत होती है, जिसे तनाव की स्थिति में सेलुलर फिटनेस में सुधार के लिए Cpn60.1 फॉस्फोराइलेशन द्वारा बढ़ाया जा सकता है।

अंत में, हमारे निष्कर्ष माइकोबैक्टीरियल चैपरोनिन सिस्टम के इंटरसेल्युलर आणविक चैपरोन फ़ंक्शन का वर्णन करते हैं। हम एतद्वारा स्थापित करते हैं कि *एम. ट्यूबरकुलोसिस* और *एम. मेरिनम* (और एक्सट्रप्लेशन द्वारा, संभवतः सभी माइकोबैक्टीरिया) के दोनों चैपरोनिन सिस्टम कैनोनिकल चैपरोनिन फ़ंक्शन में माहिर हैं। हमारे निष्कर्षों के आधार पर, हम प्रस्ताव करते हैं कि Cpn10-Cpn60.2 माइकोबैक्टीरिया में हाउसकीपिंग चैपरोनिन सिस्टम का प्रतिनिधित्व करता है, Cpn10-Cpn60.1 सेलुलर आवश्यकताओं के अनुसार इंटरसेल्युलर चैपरोनिन क्षमता को पूरक करता है।

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## ABBREVIATIONS AND SYMBOLS

%	Percentage
~	Approximately
°C	Degree Celsius
Å	Angstrom
µg	Microgram
µl	Microlitre
µM	Micromolar
ADP	Adenosine Diphosphate
APS	Ammonium Persulphate
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
Cpn	Chaperonin
Cpn10	10 kDa Chaperonin protein (Co-chaperonin)
Cpn60	60 kDa Chaperonin protein
CV	Column Volumes
DTT	Dithiothreitol
EBI	European Bioinformatics Institute
EDTA	Ethylene diamine tetra acetic acid
g	Gram
HSP	Heat Shock Protein
IPTG	Isopropyl- β- D-1-thiogalactopyranoside
kb	Kilobase pair
kDa	KiloDalton
LA	Luria Agar
LB	Luria Broth (Lysogeny Broth)

mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
M <sub>r</sub>	Molecular weight
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometre
OD <sub>600</sub>	Optical Density measured at 600 nm
PAGE	Polyacrylamide Gel Electrophoresis
PDB	Protein Data Bank
PIC	Protease Inhibitor Cocktail
RCSB	Research Collaboratory for Structural Bioinformatics
RMSD	Root Mean Square Deviation
rpm	Revolutions per minute
SDS	Sodium Dodecyl Sulphate
s	Second
TAE	Tris-Acetate-EDTA
TEMED	N, N, N', N'-Tetramethyl ethylenediamine
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultra-violet
Vis	Visible