UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE
E BIOMOLECOLARI "RODOLFO PAOLETTI" - DiSFeB
*Prof.ssa Alessandra Polissi*

PhD Thesis evaluation
Candidate: Akshay Maniyeri Suresh

Akshay Maniyeri Suresh performed his doctoral studies in the Faculty of Chemistry at the Gdansk University of Technology (Poland) under the supervision of Prof. Satish Raina, who is leading a group internationally recognised for research on envelope biogenesis and envelope stress responses in Gram-negative bacteria.
In this context, Akshay Maniyeri Suresh thesis work focuses on the molecular mechanisms that regulate cell envelope homeostasis in *Escherichia coli*, with a particular focus on the functions of the Lap proteins, LapD and LapC. The work addresses how bacterial cells coordinate the biosynthesis and transport of lipopolysaccharides (LPS), phospholipids (PLs), and fatty acids to maintain envelope integrity.
The thesis manuscript is well structured and includes a comprehensive and reference rich Introduction, Method and Result sections followed by a well thought Discussion and Conclusion chapters. The last part of the thesis manuscript includes the four manuscripts published by the candidate during his PhD thesis work. Notably, the candidate is first or co-first author in two of them.

**Scholarly Merit**

Introduction

The introduction chapter gives the readers the sufficient background and contextualization of the research carried out.
This section provides a comprehensive and well-structured overview of our current knowledge on Gram-negative bacterial cell envelope biogenesis. In the context of outer membrane (OM) lipid asymmetry, the biogenetic pathways of LPS, PL, and fatty acid metabolism in *Escherichia coli* are detailed discussed. Integration of these pathways is crucial for a proper growth of the OM. In *E. coli* the heart of lipid homeostasis lies in the first committed step of LPS biosynthesis which is catalysed by the LpxC deacetylase. Importantly, the levels of LpxC are tightly controlled in the cell via FtsH-mediated proteolysis by the IM proteins LapB and LapC in response to LPS levels. These findings establish the rationale for investigating the functions of Lap proteins, especially LapD and LapC, as important actors establishing the proper LPS and PL flux, essential for overall envelope homeostasis.

LapC and LapD are factors identified in the laboratory of Prof. Raina whose role in modulating FtsH activity is illustrated in detail in the Result section.
The Introduction chapter provides also details on outer membrane proteins (OMP) and Lipoprotein biogenesis pathways and on the Mla system that operates the retrograde transport of PL from OM outer leaflet to IM and that plays a crucial role in maintaining OM asymmetry.
The final paragraphs of the Introduction discuss the $\sigma^E$ regulon and the Rcs phosphorelay system in the context of envelope homeostasis and specifically in their coordinated response to LPS and OM stress.

Results

The central body of the thesis consists of a result chapter where the candidate summarizes the main findings he obtained around the LapC and LapD proteins. The results included in this section are all published in the four articles included in the thesis manuscript. Through a combination of genetic, biochemical, and molecular approaches, the candidate demonstrates that LapD plays a central role in coordinating LPS, phospholipid, and fatty acid biosynthesis. First, the candidate shows by co-purification experiments that LapD interacts with proteins implicated in LPS and PL biosynthesis. Notably, *lapD* is required to maintain proper LpxC levels at elevated temperature and its deletion is conditional synthetic when combined with deletion of *clsA* (cardiolipin synthase) or *waaC* (LPS core biosynthesis). Furthermore, suppressor analyses of the synthetic lethal phenotype of the *lapD waaC* deletion mutant can be suppressed by mutations in *lptD* which encode the essential OMP implicated in LPS assembly at the OM. Overall, these data support a role of LapD in coordinating LPS and PL biogenetic pathways. The parallel investigation of LapC further strengthens the work by uncovering functional overlap and hierarchy between LapC and LapD. Infact, *lapD* overexpression suppresses the Ts phenotype of allele, and moreover *lapD* cannot be deleted in a *lapC190* genetic background. Finally, the candidate identifies additional synthetic lethal pair (including *marA* and *pldA*) in the *lapC190* genetic background leading to the identification of new factors involved in LpxC regulation and envelope integrity.
The results are clearly presented and discussed. The writing of the document is of a professional standard.

Discussion and Conclusions

The discussion is thoughtful, critical, and effectively integrates the experimental findings with existing literature. The candidate successfully develops a coherent model in which the LapB/C/D complex functions as an assembly and surveillance unit coordinating LPS, phospholipid, and fatty

acid biosynthesis. The conclusions are fully supported by the presented data and highlight the broader biological significance of Lap proteins in maintaining bacterial envelope integrity. Importantly, the work extends current understanding of envelope homeostasis and identifies several novel regulatory connections that may have implications for future antimicrobial strategies targeting Gram-negative bacteria.

**Overall Evaluation**

This doctoral dissertation represents an original contribution to the field of bacterial cell envelope biology. The research addresses important scientific questions using appropriate and methodologies, and the results provide significant new insights into the regulation of envelope homeostasis by Lap proteins in *Escherichia coli*. The findings are of clear scientific value and contribute meaningfully to our understanding of bacterial physiology.

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