Karolinska Institutet

Institutionen för Mikrobiologi, Tumör- och Cellbiologi (MTC)

Oxidoreductases and RNA degradosome controlling virulence-associated traits of *Salmonella enterica* serovar Typhimurium

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i **Föreläsningssal Atrium, Nobels Väg 12b.**

Torsdagen den 26 September 2013, kl 13.00

av

Naeem Anwar

Huvudhandledare:
Professor Mikael Rhen
Karolinska Institutet
Institutionen för Mikrobiologi, Tumör- och Cellbiologi

Bilhandledare:
Docent, Katrin Pütsep
Karolinska Institutet
Institutionen för Mikrobiologi, Tumör- och Cellbiologi

Fakultetsopponent:
Professor Mikael Skurnik
University of Helsinki, Finland
Department of Microbiology and Immunology
The Haartman Institute, Finland

Betygsnämnd:
Professor Arne Holmgren
Karolinska Institutet
Institutionen för Medicinsk Biokemi och Biofysik

Professor Ann-Beth Jonsson
Stockholm Universitet
Institutionen för Genetik, Mikrobiologi och Toxikologi
Wenner-Grens Institut

Docent Åsa Sjöling
Göteborgs Universitet
Institutionen för Biomedicin

Stockholm 2013

ABSTRACT

The oxidative stress response is a fundamental and primitive mode of innate immune defense in nearly all forms of life. The host oxidative stress response becomes truly important and effective especially against intracellular pathogens such as *Salmonella*. Pathogens have evolved diverse mechanisms to withstand the oxidative responses. These include the use of oxidoreductases to neutralize oxidative products and repair oxidative damages, rapid alteration in their transcriptome and diversion of such oxidative products in cellular signaling, for their survival and successful infection.

In the first study in this thesis, we have introduced the putative ScsABCD oxidoreductases of the thioredoxin superfamily for their role in oxidative stress tolerance and in virulence of *S.* Typhimurium. We demonstrated that ScsABCD proteins are dispensable for invasion in cultured epithelial cells under normal invasive conditions, although ScsABCD acts as a suppressor of SPI-1 mediated invasion upon oxidative stress. Our results have further shown a functional association between ScsABCD and thiroredoxin 1 (TrxA) oxidoreductase of *S.* Typhimurium. In this, we demonstrated that absence of ScsABCD restored the invasiveness of a *trxA* mutant in epithelial cells and its virulence in *C. elegans*. **(Paper I).**

Next, we present the analyses on the role of periplasmic Dsb oxidoreductase system in *S.* Typhimurium's biofilm-development, specifically under redox stress. In this, we show that DsbA and DsbB act as suppressors of *rdar*-morphotype development and affect biofilm-regulation using either Csg-dependent or -independent mechanism, respectively. Our results further reveal that oxidative stress abrogates *rdar*-morphotype of *S.* Typhimurium, whereas reductive stress reduces *rdar*-morphotype with concomitant plentiful release of extracellular slimy material containing, notably, the extracellular DNA (eDNA). Furthermore, we have demonstrated the oxidative recovery of swimming motility defects of a *dsbA* mutant. **(Paper II).**

Finally, we have demonstrated that exoribonuclease; PNPase and its genetic associate membrane lipoprotein NlpI constitute an operon and are functionally connected **(Paper III and IV).** PNPase was required for *rdar*-morphotype development whereas, NlpI suppresses the biofilm formation. In addition, we established the association of PNPase with c-di-GMP metabolism in biofilm regulation. Moreover, we showed that both PNPase and NlpI are required, independently, for cold adaptation of *S.* Typhimurium.

ISBN 978-91-7549-285-8