## Analysis of signaling cascade of protein kinase StkP in *Streptococcus pneumoniae*

*Streptococcus pneumoniae* is not only an important human pathogen but also an appropriate model organism to investigate cell division in ovoid bacteria. This bacterium lacks both, NO and Min systems for selection of cell division site. Thus, the mechanism which determines the site of cell division is unknown. Additionally, the genome of *S. pneumoniae* encodes a single gene for eukaryotic-like serine/threonine protein kinase StkP and a single gene for eukaryotic-like serine/threonine protein phosphatase of PP2C type called PhpP. StkP is one of the main regulators of cell division. Cell division is probably affected by the phosphorylation of its substrates, which include, among others, cell division proteins FtsZ, FtsA, DivIVA, MacP, Jag/KhpB/EloR, and LocZ/MapZ.

The aim of the first project of this dissertation thesis is determination of the function of protein LocZ in the cell division. In summary, *locZ* is not essential, however, it is involved in proper septum placement in *S. pneumoniae* and our data suggest that it is a positive regulator of Z-ring placement. Cells lacking LocZ are able to form Z-ring, but the Z-ring is spatially misplaced resulting in cell division defects, shape deformation, and generation of unequally sized, occasionally anucleated daughter cells. LocZ has a unique localization pattern. It arrives early at midcell, before FtsZ and FtsA. Likewise, it also leaves the septum early, apparently moving along with the equatorial rings that mark the future division sites. We propose that the extracellular domain of the protein LocZ is responsible for this localization. Interestingly, homologs of LocZ are found only in streptococci, enterococci and lactococci, indicating that these phylogenetically closely related bacteria evolved a unique mechanism to find their middle.

The second project of this thesis is concerned with the essentiality of protein phosphatase PhpP in the *S. pneumoniae* Rx1 unencapsulated strain, in which the gene *phpP* was previously postulated as essential. We prepared a viable Δ*phpP* strain and excluded selection of suppressor mutation by transformation kinetic analysis, thus confirming that *phpP* gene is not essential. We demonstrate that PhpP negatively controls the level of protein phosphorylation in *S. pneumonaie* both by direct dephosphorylation of its substrates and by dephosphorylation of its cognate kinase, StkP. Absence or catalytic inactivation resulted in the hyperphosphorylation of StkP substrates and specific phenotypic changes, including sensitivity to certain environmental stresses. Morphologically is depletion PhpP manifested by the formation of smaller spherical cells that reflect phenotype of StkP overexpression. Conversely, the overproduction of PhpP

resulted in cell elongation mimicking the *stkP* null phenotype. Our results suggest that PhpP and StkP cooperatively regulate cell division of *S. pneumoniae.*

**Keywords:** LocZ, MapZ, Spr0334, serine/threonine protein kinase, StkP, serine/threonine protein phosphatase, PhpP, cell division, *Streptococcus pneumoniae*, phosphorylation.