Hypothetical protein Spr1962 as a new substrate of the signalling pathway of the Ser/Thr protein kinase StkP and phosphatase PhpP

The extracellular human pathogen *Streptococcus pneumoniae* encodes in its genome only a eukaryotic-type serine/threonine protein kinase (StkP) and the corresponding PP2C phosphatase (PhpP) in its genome. This makes it a unique model organism for the studying of signaling pathways in bacteria. To date, several substrates of this signaling protein have been identified and characterized, including DivIVA, KhpB, FtsA, FtsZ, MacP, GlmM, GpsB, ComE and others. These proteins are involved in various cellular processes, including cell division and peptidoglycan biosynthesis. In a global phosphoproteome study based on the LC-MS approach, one of the potential new substrates was the hypothetical protein Spr1962, whose phosphorylation was detected only in the hyperphosphorylated strain with deleted PhpP phosphatase.

The aim of this work was to characterize this new substrate Spr1962 and to clarify its possible function. Based on the significant structural similarity to Spr1962 the FloT flotillin protein of *Bacillus subtilis*, it was suggested that it might be a protein with an analogous function in pneumococci. Deletion of this gene in different genetic backrounds was found to cause an enlarged cell phenotype. Monitoring of the GFP-tagged fusion protein Spr1962 by fluorescence microscopy revealed a localization correspondings to spots at the cell edge and signal analysis showed that it is enriched poles and subsequently in the cell septa during the late phase of cell division. Similar to FloT in *B. subtilis* the pneumococcal protein Spr1962was ahown to associate with the cytoplasmatic membrane and to affect protein secretion. In addition, immunochemical methodsdemonstrated that the Spr1962 protein is a substrate of the protein kinase StkP, and is phosphorylated *in vivo* on threonine at position 273 *in vivo*. Microscopic observations of morphology and test of growth ability under various physiological conditions led to an approximation of a possible function of Spr1962 protein phosphorylation.

The results of this work contribute to the clarification of the function of the Spr1962 protein and to the overall characterization of the signaling cascade controlled by the protein kinase StkP and the phosphatase phpP.

Key words:

Streptococcus pneumoniae, StkP protein kinase, phosphorylation, Spr1962 protein, PhpP phosphatase, flotillin, functional membrane microdomains