**„Translocon SEC as a mediator of the cellular response to disruption of the extracytoplasmic homeostasis in the bacterium *Helicobacter pylori*"**

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Protein export is a key process in bacteria, ensuring proper functioning of cells, including membrane biosynthesis, formation of surface structures, and interactions with the host organism in both pathogenic and symbiotic species. Most bacterial proteins destined for export are translocated from the cytoplasm via the SEC system. This system comprises two main components: the cytoplasmic SecA protein and the SecYEG channel located in the inner membrane. SecA plays a dual role in the translocation process: (1) it acts as an ATPase and, through ATP hydrolysis, provides the energy required for translocating protein substrates across the SecYEG channel, and (2) it participates in the recruitment and delivery of protein substrates to the SecYEG channel. The functioning of the SEC system has been studied most extensively in the model bacterium *Escherichia coli*, whereas in the case of *Helicobacter pylori*, one of the most common bacterial pathogens in humans, knowledge in this respect is limited. One of the major virulence factors of *H. pylori* is the HtrA protein, which is also an important component of the extracytoplasmic protein quality control system in Gram-negative bacteria. Previous studies on *H. pylori* HtrA demonstrated that the lethal effect of the absence of a functional HtrA protease can be suppressed by mutations in the *secA* gene in the region encoding the C-terminal domain of SecA. This observation led to the hypothesis that the activity of the SEC translocon may be regulated in response to extracytoplasmic stress. Such a mechanism could involve both alterations in the efficiency of individual SEC components and modulation of the abundance of translocon components, resulting in changes in the rate of protein export from the cytoplasm.

The aim of this thesis was to verify this hypothesis by (1) performing a biochemical characterization of *H. pylori* SecA and its mutated C-terminal variants, (2) determining the impact of the *secA* mutations on the bacterial phenotype, and (3) analyzing the expression levels of genes encoding major SEC system components under conditions of membrane and periplasmic stress.

The first part of the study involved a biochemical characterization of *H. pylori* SecA, which has not been previously reported in the literature. The analyses included ATPase activity, structural properties, and thermal stability. The results enabled a comparison between *H. pylori* SecA and its *E. coli* homologue. Significant differences in quaternary structure and molecular stability were observed: *H. pylori* SecA was present mainly as a monomer, whereas *E. coli*

SecA predominantly formed dimers. Moreover, *H. pylori* SecA displayed higher thermal stability than its *E. coli* homologue. These findings suggest that the two species employ different strategies to regulate SEC system activity.

Subsequently, the phenotypes of *H. pylori* strains carrying mutations in the C-terminal region of SecA were analyzed. It was found that such mutations can alter cell surface properties and decrease bacterial sensitivity to the antibiotic tetracycline. The most pronounced effects were associated with mutations disrupting the metal-binding site (C841Y, C852Y), which resulted in elevated ATPase activity of SecA and increased autoagglutination of *H. pylori* cells. However, no disturbances in the export of the secreted virulence factor, gGT protein, were observed.

In the next stage, the effect of stress factors targeting the membranes and the periplasmic space on the expression of genes encoding SEC translocon components was examined. The analyses revealed transcriptional changes, particularly for *secY*, *secE*, and *secG*, which encode the structural elements of the SecYEG channel.

In summary, this study demonstrates that the SEC system in *H. pylori* plays an important role in the bacterial response to membrane and periplasmic stress. Mutations in *secA* affect the catalytic activity of SecA and influence bacterial phenotypes, while the expression of genes encoding SEC components is regulated by environmental factors. These findings support the hypothesis that modulation of protein translocation via the SEC system is part of the adaptive response of *H. pylori* to cellular stress. Furthermore, this work provides the first biochemical characterization of *H. pylori* SecA, uncovering significant differences from its *E. coli* homologue and highlighting unique features of this pathogen.