ABSTRACT

Rhomboid proteases are a class of serine intramembrane proteases, a large family of enzymes that catalyze the proteolytic cleavage of membrane proteins within their transmembrane regions, in the hydrophobic environment of cellular lipid membranes. Rhomboid proteases were discovered in 2001 in *Drosophila*. In their pioneering study, Lee *et al*. identified the essential role of Rhomboid-1 protein (Rhom-1), which proteolytically activates the epidermal growth factor (EGF) receptor signaling pathway, in the early stages of fly eye development. Members of the rhomboid superfamily – active proteases (rhomboids) as well as their catalytically-dead counterparts (rhomboid-like proteins, including iRhoms and Derlins) - are widely conserved, implying their biological significance. Rhomboids are present in all kingdoms of life from archea to humans, while proteolytically inactive rhomboid-like proteins are present in eukaryotes only. Rhomboid superfamily proteins play roles in a wide range of processes, as diverse as signaling in metazoan development, mitochondrial biogenesis in yeast, host-cell invasion by protozoan parasites, protein quality control in the endoplasmic reticulum (ER) or bacterial quorum sensing. Rhomboids are the best understood intramembrane proteases from a structural and mechanistic points of view. Most of the work has been done on the rhomboid protease GlpG from the Gram-negative bacterium *Escherichia coli.*

The thesis focuses on the mechanistic characterization of the intramembrane rhomboid protease GlpG from the Gram-negative bacterium *Escherichia coli* and on the identification of biological role of YqgP from the Gram-positive bacterium *Bacillus subtilis.* Based on genetic analyses, GlpG-like and YqgP-like proteases are highly populated among bacterial rhomboid proteins and are also present in several pathogens such as Gram-negative *Salmonella* or *Shigella* and Gram-positive *Listeria* or *Staphylococcus.* To refine our knowledge of the mechanistic principles covering substrate specificity, we have mapped the amino acid preferences of the GlpG rhomboid protease and developed its substrate-derived inhibitors. The tools that we developed for GlpG rhomboid, were subsequently used to characterise the biological function of YqgP. We analysed the degradome and the interactome of YqgP in *Bacillus subtilis in vivo* and identified MgtE, the main magnesium transporter in *Bacillus subtilis*, as the natural substrate of YqgP. Finally, we showed that YqgP cooperates with the membrane embedded AAA+ protease FtsH during membrane protein quality control in *Bacillus subtilis*, representing an ancestral membrane protein degradation system conceptually similar to the eukaryotic ER associated degradation.