Abstract

Bacillus subtilis is a gram-positive sporulating bacterium. Under unfavorable conditions, it initiates the sporulation process that results in a resistant spore. The transcription factor Spo0A is a master regulator of sporulation initiation. The hallmark of sporulation is the formation of an asymmetric septum near a cell pole, which divides the cell into the larger mother cell and smaller prespore. The asymmetric septum is localized at 1/6 of the cell length relative to the nearer pole. One of the players involved in this localization is the RefZ protein, referred to as the FtsZ regulatory protein, which forms the Z-ring. The Z-ring is important for the formation of both the vegetative (mid-cell) and asymmetric septa. RefZ facilitates the relocalization of the Z-ring from midcell to the poles at an early stage of sporulation. RefZ also binds DNA (RefZ binding motifs [RBMs]) near the ori site of the chromosome, thereby promoting precise positioning of the chromosome arms during sporulation.

The entire sporulation process is controlled by a cascade of compartment-specific sporulation σ factors that recognize specific consensus sequences in the promoter regions of genes, thereby allowing RNA polymerase to initiate transcription of sporulation-specific genes. These σ factors ensure spatially and temporally controlled expression of genes in the cell. Although this process has been intensively studied, many aspects of sporulation are still enigmatic.

The overall aim of this Thesis was to provide detailed insights into selected aspects of sporulation. The first main aim of this Thesis was to characterize expression and localization of RefZ in the model organism *Bacillus subtilis*. Bioinformatic analysis was used to identify potential promoters of the *refZ* gene. This analysis identified σ^{H}, $\sigma^{F/G}$ dependent promoters and binding sites for the transcription factor Spo0A. *In vitro* transcriptional experiments were then used to test the activity of the predicted promoters and their dependence on Spo0A. By SIM microscopy, *in vivo* expression and localization of RefZ (RefZ-GFP) was characterized in three developmental stages of sporulation in strains with *refZ-gfp* at the ectopic locus and with *refZ-gfp* at the native locus. In the second part of the Thesis, the interaction partners (interactome) of the RefZ protein during sporulation were identified. Taken together, these results contributed to the spatio-temporal picture of RefZ expression, localization, and interactions during sporulation.

Keywords: transkripction, promoter, sporulation sigma factors, sporulation, Spo0A, RefZ.