5 Summary

The main aim of this work was the determination of the three dimensional structure of the δ-protein encoded by low-copy-number plasmid pSM19035 of Gram-positive bacteria *Streptococcus pyogenes*. The crystal structures of the proteins (ε and ζ) involved in the toxin-antitoxin system (TA system) and their transcription regulator, the ω-repressor protein, were already known. However, the concomitance of a second plasmid segregation system like a partitioning system and the presence of its main components (ParA, ParB and *parS* DNA) on this plasmid were not well clarified. The primary sequence alignment of the ORF *delta* (*δ*) located upstream of *ω* gene classified δ as a ParA-homologous Walker-type ATPase and it was suggested to be involved in the faithful DNA segregation in prokaryotic microorganisms.

The gene *δ* (ORF *delta*) encoding the potential partitioning protein δ was cloned in *E. coli* cloning strain DH5α and transformed into ER2566 for overproduction. A protocol for the purification of the protein (298 residues) was developed and optimised. The purity level of the protein has been verified and it was finally co-crystallised in complex with ATPγS.

The X-ray diffraction data were collected with a max. resolution of 1.83 Å at beam line BL1 of BESSY. The three dimensional structure of the protein was determined via molecular replacement (MR) using a homology model of δ based on Soj protein structure of *T. thermophilus* (PDB-Id 2BEJ) as search model. Following the solution of the phase problem with MR, a structural model was built and refined. The asymmetric unit contains one δ•ATPγS complex, however, packing analysis revealed a crystallographic two fold axis relating two δ•ATPγS that contact each via a large hydrophobic interface. The crystal structure shows δ_2 as a “V-shaped” homodimer featuring a deep cleft between the two monomers. One ATP binding site on each arm of the “V” is occupied by an ATPγS molecule and a Mg^{2+} atom. Furthermore, electrostatic surface potential calculations indicate that δ_2 is bipolar with a negatively charged bottom of the “V” and positively charged tips. The bipolarity of the δ_2 dimer indicates that it might be involved in DNA binding or may interact with the negatively charged bottom of a subsequent dimer when forming polymers with two poles. The described structural model is the first reported structure of a plasmid encoded ParA-type protein in the ATPγS- and Mg^{2+}-binding state $(\delta\bullet ATP\gamma S/Mg^{2+})_2$.

Despite moderate sequence identities, a structure similarity search for δ monomer using the Dali server identified best matches for the ParA family proteins Soj of *T. thermophilus* and MinD of *A. fulgidus* and also for another Walker-type ATPase like NifH. By contrast, the structure of the homodimer $(\delta\bullet ATP\gamma S)_2$ exhibits significant differences

compared to the dimers formed by Soj and MinD. They are monomeric in absence of ATP, but form compact dimers upon ATP binding.

The results of ATPase-Assay indicate that δ_2 is able to hydrolyse ATP. The enzyme activity of the protein seems to be stimulated in the presence of wt-ω_2 protein (ParB) and the centromer like DNA-sites (*parS1, parS2 and parS3*). However, this stimulation could not be observed in the presence of the N-terminal deletion mutant $\omega_2\Delta$N19, indicating that the residues involved in the stimulation of ATPase activity of δ_2 have to be located among these deleted 19 residues. The dependence of these three components, omega, delta and DNA, to form nucleoprotein filaments, has been confirmed by dynamic light scattering- and sedimentationassays as well as by electronmicroscopy. The results of recent experiments indicate that δ_2 seems to be able to form dynamically nucleoprotein filaments in the presence of wt-ω_2, *parS* DNA and ATP. These possibly bipolar nucleoprotein filaments might elucidate the movement of the nucleoid from the midcell position to the cell poles observed by the *in vivo* localisation of δ_2 -GFP-fusion in *B. subtilis*.

The presented structure model and the additionally performed functional analysis are making a contribution to the clarification of the whole segregation mechanism of plasmid pSM19035 of the human pathogenic bacterium *S. pyogenes*.