This thesis describes the cloning and characterization of the genes encoding the proteins which constitute the 75 kilodalton (kDa), competence specific, protein complex of Bacillus subtilis. This membrane-bound complex consists of two subunits of the 17 kDa DNA-entry nuclease and two subunits of an 18 kDa protein.

Chapter I gives a brief general introduction of several aspects concerning natural competence and transformation of Gram positive bacteria.

Chapter II describes the isolation of transformation-deficient mutants by insertional mutagenesis with plasmid pHV60. This plasmid is able to replicate in E. coli, but not in B. subtilis. It contains a chloramphenicol resistance (Cm^R) marker which is expressed in both organisms. Chapter II also describes an unexpected phenomenon which was observed when chromosomal DNA of these mutants was used to transform a transformation-proficient strain. As expected, almost all the transformants had the mutant phenotype. However, atypical transformants with the wild-type phenotype were produced with a frequency of approximately 3×10^{-4}. Data concerning the amplification of the DNA containing the Cm^R marker and duplication of DNA sequences in these atypical transformants are presented that suggest that they are the result of a Campbell-like integration of the chromosomal DNA containing the integrated plasmid. A model based on circularization of single-stranded vector-containing chromosomal DNA through fortuitous bases paring with an independently entered single-stranded molecule and subsequent integration by a Campbell-like mechanism is presented to explain the production of these atypical transformants.

Chapter III describes the cloning in E. coli of the

gene specifying the DNA-entry nuclease of B. subtilis. Analysis of the mutants obtained by insertional mutagenesis had shown that the mutants, mapping in the AroI region, lacked DNA-entry nuclease activity. Plasmid pHV60 derivatives, containing flanking chromosomal DNA fragments, were isolated from these mutants and used to screen a library of B. subtilis chromosomal DNA prepared in phage λ EMBL4. In E. coli lysates prepared with phages that hybridized to the pHV60-based probe, a prominent nuclease activity could be detected. The DNA fragment encoding the nuclease was subcloned in plasmid pGV1. Analysis of the nuclease showed that it had the same M_r as the B. subtilis DNA-entry nuclease and that its activity was strongly stimulated by Mn^{2+}, which is also characteristic for the B. subtilis DNA entry nuclease. Subsequently it was shown that the nuclease activity was specified by a 700 base pairs EcoRI-PstI fragment, and that the nuclease had been cloned without its own promoter. The gene was expressed under the control of the lacZ promoter.

Chapter IV describes the cloning of a 3100bp DNA fragment which encodes the 14 and 17 kDa nucleases as well as the 18 kDa protein. In this construct the expression of the genes is governed by their own promoter. The DNA fragment carrying the promoter was obtained from the λEMBL4 bank referred to above, by a search for sequences upstream of the nuclease gene, with the 700 base pairs EcoRI-PstI fragment as probe.

Using the cloned gene, a B. subtilis mutant was constructed by insertion of a Cm^R marker into the gene encoding the nuclease. This mutant lacked both nuclease activities and the 18 kDa protein. The polar effect of mutation suggests that the genes are organized in an operon. The mutant showed 5% residual transformation. DNA-binding was higher than in the wild-type cells, and DNA-entry was reduced to 30% of the wild-type level. These results suggest that an alternative pathway may exists for

the internalization of transforming DNA.

The effects of the mutation could be complemented with plasmids, carrying the cloned genes.

In **Chapter V** the nucleotide sequences of the genes encoding both the nuclease and the 18 kDa protein are presented. The genes are encoded by a 904 basepairs PstI-HindIII fragment. The open reading frames encoding both proteins are partly overlapping.

A mutant, exclusively deficient for the 18 kDa protein, was constructed by insertion of a kanamycin resistance gene into the coding sequence of the gene. The results suggest that the 18 kDa protein is probably not involved in the binding of donor DNA to the competent cell, since the mutant showed wild-type DNA-binding activity. The transforming activity of the mutant was reduced to 25 % of the wildtype level, indicating that the 18 kDa protein has a function in the transformation process. In vitro experiments showed that the 18 kDa protein is capable of inhibiting the activity of the competence specific nuclease. A possible role of the 18 kDa protein in transformation might be related to this phenomenon, and is discussed.