Summary and concluding remarks

The development of genetic competence in *Bacillus subtilis* represents a prokaryotic specialization process which has attracted much attention for several reasons. First, because *B. subtilis* is easily amenable to genetic analysis and is, therefore, the best-studied Gram positive prokaryote. In addition, *B. subtilis* is a non-pathogenic representative of an industrially important genus commonly used for fermentation processes. Furthermore, competence development is an example of a relatively simple cell-differentiation system and improved understanding of this process may facilitate further insight into the very complicated regulatory schemes underlying eukaryotic developmental programmes. Finally, from a scientific point of view the uptake of DNA is an intriguing transport process which is distinct from other transport systems since it requires unique adaptations to allow passage of the DNA macromolecule through the cell-envelope.

In recent years much has been learnt about the regulatory aspects of competence. Competence develops as a post-exponential response to nutrient deprivation and is under the control of a signal transduction system which monitors environmental and cytoplasmic changes. It has become apparent that the competence signal transduction pathway is embedded into a larger regulatory network controlling all known post-exponential adaptation responses.

Chapter I of this thesis reviews identified components of the competence signal transduction pathway, the signals they are responding to, their mutual interactions, and the means by which they effect synthesis of proteins involved in DNA binding, DNA uptake, and recombination.

Chapters II, III, and IV describe the cloning, sequence analysis and characterization of *srfA*, a large operon required for two different post-exponential processes, i.e. the non-ribosomal production of the peptide-antibiotic surfactin and competence development. *srfA* encodes a large multi-enzyme complex consisting of seven amino acid activation domains responsible for the activation and sequential addition of the seven amino acids which compose the surfactin molecule. It is shown that only the portion of *srfA* encoding the fourth, valine-activating domain plays a regulatory role in competence and functions as an assembly link between other regulatory components of the competence signal transduction pathway.

In chapter V it is demonstrated that the genetic determinant of the competence-required *srfA* region comprises a small open reading frame, *comS*, located within but translated in a frame different from that of *srfA*.

Chapter VI describes the cloning and characterization of *comK*, which, as reported in chapter VII, functions as
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chapter VII, functions as the final autoregulatory control switch before a cell becomes competent. All regulatory branches of the competence signal transduction pathway converge at the point of *comK* expression, both at the transcriptional as well as the post-translational level. In chapter VIII the gene product of *comK* is identified as the competence transcription factor (CTF), which is directly responsible for the transcriptional activation of the genes encoding components of the DNA uptake apparatus. Transcriptional activation of the latter genes as well as *comK* itself is shown to be dependent on the binding of ComK (CTF) to promoter-upstream regions of its target genes.

Chapters IX and X describe the finding that the *addAB* operon, *recA*, and *dinR*, genes which are involved in (the regulation of) recombination and DNA repair, are also transcriptionally activated during the development of competence. This transcriptional induction is shown to be dependent on CTF which binds to the (promoter-)upstream regions of these genes. The biological significance of the simultaneous synthesis of the DNA-entry apparatus and the induced expression of genes involved in recombination is evident: in this way it is guaranteed that the internalized single-stranded DNA will be used for the production of heteroduplex molecules.

Chapter XI describes the differential expression of two deoxyribonuclease(DNase)-encoding genes. The expression of *nucA* is under competence control and dependent on *comK*, although the DNase does not appear to play a role in competence. The *nucB* gene specifies an extracellular sporulation-specific DNase which seems to be expressed in a cell type-specific manner.

Chapter XII is included as an addendum and describes the sequence analysis and characterization of *tlpC*, a gene encoding a protein similar to methyl-accepting chemotaxis proteins. This gene was identified as a byproduct of the sequence determination of the intergenic region between *srfA* and *nucA*.

It has become evident that competence development is dependent on a multi-sensory signal transduction pathway as part of a regulatory network responsive to a wide variety of stimuli and controlling all known post-exponential adaptation processes. During recent years many components of this network have been identified and considerable progress has been made to elucidate the regulatory machinery involved. However, little is known about the nature of the environmental signals, their targets and the actual sensory mechanism involved in signal recognition. Active cooperation and communication between laboratories working on post-exponential regulation in *B. subtilis* will (continue to) be necessary to unravel the molecular basis underlying competence regulation and other post-exponential phenomena.