The competence transcription factor of Bacillus subtilis

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CHAPTER 10

SUMMARY AND CONCLUSIONS

Development of genetically transformable cells is in *Bacillus subtilis* an accurately regulated cellular differentiation process, which enables cells to take up DNA from the medium. Over the years a dozen regulatory proteins have been identified, which form a complex signal transduction network, involved in the development of genetic competence. One of these proteins, ComK, appears to be pivotal in this regulatory network. In this thesis the regulation and function of ComK in the development of competence has been further investigated.

Competence develops when nutrients in the growth medium become limiting and exponential growth ceases. Chapter 1 discusses the specific conditions required for competence to develop optimally, and briefly describes the components comprising the DNA binding, uptake and recombination systems, that are activated during competence. In addition to competence, in *B. subtilis* other postexponential phase processes are activated, such as sporulation, motility and the production of enzymes and antibiotics. The regulation of these processes is intimately intertwined, and Chapter 1 deals with the regulation of competence in relation to these other postexponential phase processes.

One of the operons essential for competence and activation of ComK, is *srfA*. This operon specifies a large protein complex, the surfactin synthetase, which is responsible for the synthesis of the lipopeptide antibiotic, surfactin. A comprehensive deletion and mutagenesis study described in Chapter 2, shows that not the surfactin synthetase is involved in competence development but a small gene, *comS*, located in the *srfA* operon.

ComK is essential for competence, and genetic analyses positioned *comK* at the end of the regulatory cascade, which is responsible for the expression of the structural competence genes, specifying the DNA binding, uptake and recombination systems. Chapter 3 demonstrates, by means of gel retardation experiments, that ComK specifically binds to the promoters of all of these competence genes. This, and genetic data, suggested that *comK* encodes the competence transcription factor. This inference appeared to be correct as shown in Chapter 5.

ComK activates also the transcription of its own gene, and this autostimulatory expression is an important step in the development of competence. Autostimulation of ComK is kept in control by the negative regulators MecA and ClpC. In Chapter 4 it is demonstrated that these proteins together with ComK form a stable ternary protein complex, preventing ComK from binding to its own promoter. Addition of purified ComS destabilizes this complex, enabling ComK to bind to its own promoter, which explains the importance of ComS in the development of competence.

Chapter 5 describes a comprehensive biochemical analysis to ascertain the DNA sequence recognized by ComK. ComK appears to bind as a tetramer comprised of two dimers, each of which recognizes an AT-rich palindromic sequence, the so called AT-box. Surprisingly, the distance between the two AT-boxes varies in a gene-specific manner, with one, two and even three helical DNA turns. Finally, this Chapter presents a number of experiments which suggest that ComK recognizes the AT-box sequence via the minor groove of the DNA.

Of several regulatory proteins involved in competence, it is unknown by what mechanism they regulate *comK* expression. Expression of *comK* requires the presence of the response regulator DegU. Probably, DegU functions as a transcription factor, however no promoters were known which are directly regulated by DegU. In Chapter 6 such a promoter is described for the first time: the *comK* promoter. Moreover, binding of DegU appears to stimulate binding of ComK to the *comK* promoter, suggesting that DegU is essential to enhance the affinity of ComK for its own promoter, thus adding to the efficiency of the autostimulatory transcription of *comK*.

AbrB is another protein which regulatory mechanism in the development of competence is unknown. The function of AbrB is complex as its role in competence development is positive as well as negative. AbrB is involved in various postexponential phase processes, and acts in these processes as transcriptional inhibitor by binding to the transcription initiation signals of certain promoters. In Chapter 7 it is made plausible that the negative function of AbrB in the development of competence is based on the same mechanism, as purified AbrB also binds to the *comK* promoter, and as such masks the transcriptional initiation signals of this gene.

During transformation of competent *B. subtilis* cells, homologous DNA taken up from the medium, is integrated into the genome according to a RecA-catalysed homologous recombination process. The expression of *recA* is stimulated in cells developing competence, and Chapter 8 demonstrates that this stimulation is *comK*-dependent, and that ComK binds to the *recA* promoter. During normal growth the expression of *recA* is inhibited by binding of the negative regulator LexA (DinR) to the *recA* promoter. In this way LexA prevents binding of the RNA polymerase, which is essential for gene transcription. Competence-dependent *recA* expression occurs since ComK is able to oppose LexA-imposed repression. The mechanism as to how ComK relieves this repression is unknown. However, Chapter 9 shows that ComK does not replace LexA from the *recA* promoter. Still, the presence of ComK is sufficient to partially overcome LexA repression, and in this Chapter it is postulated that ComK interacts with the RNA polymerase, which enables the latter to compete efficiently with LexA for binding to the *recA* promoter.

In conclusion, this thesis shows that ComK is directly responsible for the expression of all known structural competence genes. The way in which ComK regulates these genes and its own gene, is diverse. First, there are three classes of ComK-regulated promoters, based on differences in the spacing of the ComK recognition sequence. Second, ComK uses an

additional transcription regulator (DegU) for the regulation of the *comK* promoter, and third, in case of *recA* expression, ComK opposes the effect of a repressor (LexA).

Finally, this thesis gives evidence of a surprisingly complex transcriptional regulation in case of *comK* expression. At present, all together four different transcriptional regulators are known, of which three are described in this thesis, that bind specifically to the *comK* promoter, a situation not previously documented for prokaryotes.