Function and regulation of the bacillus subtilis addAB genes in homologous recombination

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Summary and general discussion

Homologous recombination is a biological process essential to all organisms, prokaryotes and eukaryotes, because it is involved in the generation of genetic diversity, the maintenance of genomic integrity and the proper segregation of chromosomes. The first part of chapter 1 gives a brief overview of our knowledge of the molecular mechanisms of homologous recombination derived from studies in the prokaryote *Escherichia coli*. This process has only been studied to some degree in the species *Bacillis subtilis*, as described in the second part of chapter 1. To date, in this Gram-positive, spore forming soil bacterium, 13 genes, including *addA* and *addB*, which are involved in the complex process of homologous recombination, have been identified. The products of these genes are involved in many cellular processes such as natural transformation, DNA repair, bacteriophage-mediated transduction and DNA repair.

The work, described in chapter 2 to 6, is mainly focused on the *B. subtilis addAB* genes and their gene products. The *B. subtilis addA* and *addB* genes encode the two subunits of the ATP-dependent deoxyribonuclease enzyme complex, which is involved homologous recombination and DNA repair. The heterodimeric enzyme is an ATP-dependent helicase and exonuclease.

Chapter 2 shows that the *B. subtilis* AddAB enzyme is the functional equivalent of the *E. coli* RecBCD enzyme, although no extended amino acid sequence similarity exists between the proteins. The introduction of the *addAB* genes into an *E. coli recBCD*$^-$ strain nearly completely restored the viability, the ability to repair U.V. damaged DNA and recombination in conjugation. However, the *B. subtilis* AddAB enzyme did not show Chi-activity in phage lambda recombination. The individual *B. subtilis* Add proteins neither complemented *recB* nor *recC* mutations.

Chapter 3 and 4 communicate the characterization of AddAB enzymes, mutated in the ATP binding site of subunit A and B, respectively. Chapter 3 shows that the lysine to glycine substitution in the ATP binding site of AddA led to significantly reduced transformation with chromosomal DNA, cell viability, the capacity to repair DNA damage inflicted by U.V irradiation or mitomycin C treatment, and bacteriophage PBS-1 mediated transduction. These effects could be related to significantly reduced rates of ATP-dependent exonuclease- and helicase activities,

Chapter IX

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Chapter 5 describes th
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AddA shows a high degree of
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Chapter 6 deals with the
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The transcription of *re*
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suggesting that at least one of these activities is involved in homologous recombination. A similar mutation in the ATP-binding site of subunit B had only marginal effects on these processes. Chapter 4 describes the purification and characterization of the wild type AddAB enzyme and the two mutant enzymes. It was demonstrated that the lysine of the ATP binding site of subunit AddA is essential for the helicase- and exonuclease activities of the enzyme, because it is required for the hydrolysis of ATP and not fot the binding of ATP. No function could be attributed to the corresponding lysine of subunit B.

Chapter 5 describes the examination of the C-terminal region of subunit AddA. In this chapter it is shown that this region is required for the exonuclease activity but not for the helicase activity of the enzyme. Despite the lack of the exonuclease activity, an AddAB enzyme, containing a C-terminal truncated subunit A, could almost fully complement an *E. coli recBCD* and a *B. subtilis recBCD* mutant with respect to homologous recombination and DNA repair. These results, together with those obtained in chapter 3, suggest that the helicase-, but not the exonuclease- activity, is essential for these processess. The C-terminal region of AddA shows a high degree of homology with the C-terminal region of subunit RecB of the *E. coli* RecBCD enzyme, the functional equivalent of AddAB, suggesting a similar function in exonuclease activity for this region in RecB.

Chapter 6 deals with the transcriptional regulation of *addAB*. The *addA* and *addB* genes are located in an operon structure as was suggested by the limited overlap between the two open reading frames. The genes are transcribed from a σ^A type promoter with a low, constitutive level of expression. However, during competence development, a *comK*-dependent induction of *addAB* transcription was observed. A direct interaction between ComK, the competence transcription factor, and the promoter region of *addAB* was demonstrated by means of gel retardation assays. These findings indicate that a direct regulatory link exists between the establishment of the competent state and the synthesis of AddAB, required for recombination.

The transcription of *recA* is regulated in a similar way as that of *addAB*: chapter 7 demonstrates that ComK also stimulates the expression of *recA* by binding to its promoter upstream region. This binding was studied in more detail in chapter 8, in which it was demonstrated that at least 4 ComK molecules bind to the *recA* promoter region, forcing the region to bend. DNAseI and hydroxyl radical protection analyses demonstrated that ComK covers an extended region of the *recA* promoter

region, spanning almost 100 bp. Chapter 7 shows that the expression of *recA* is also regulated by DinR, the transcriptional repressor of the SOS DNA repair system. It was demonstrated that ComK and DinR can bind simultaneously to the *recA* promoter region, which suggest that the ComK-dependent transcriptional activation of *recA* during competence development is not caused by competition for binding between ComK and DinR.

In conclusion, the present results indicate that AddAB plays an important role in homologous recombination and DNA repair for which the helicase activity, but not the exonuclease activity, seems essential. Possibly, the enzyme plays an important role in the initiation stage of recombination: the unwinding of dsDNA molecules to generate ssDNA tails, which are essential substrates for further processing by the recombination machinery. However, the exact role of AddAB, in, for instance, the transformation process with chromosomal DNA, still remains to be established. This process can be examined by studying the fate of internalized DNA in various AddAB mutants.

Samenvatting en algemene

Homologe recombinat
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schade, transformatie, trans

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