Recombination in transformation of Bacillus subtilis

Buitenwerf, Johannes

SUMMARY

This thesis describes experiments aimed at advancing our knowledge concerning the process of transformation in *Bacillus subtilis*. To this purpose mutants, impaired in transformation, were isolated. Both in these mutants and in the highly-transformable wild-type synchronously transforming at low temperature, the fate of transforming DNA was studied.

After a general introduction in chapter 1, comprising a brief review of the salient features of the systems of transformation of *B. subtilis* and *D. pneumoniae*, in Chapter 2 a number of transformation-deficient mutants is described. In the well-transformable wild-type, double-stranded donor DNA is successively converted into double-stranded fragments, single-stranded segments and into donor-recipient complex. Mutant 7G-73 converts double-stranded fragments into single-stranded segments, but only a minor amount of donor-recipient complex is formed. The donor-recipient complex shows an aberrant buoyant density in CsCl-gradients as compared to donor-recipient complex formed in the wild-type. Mutant 7G-84 forms double-stranded fragments and single-stranded segments. Via an intermediate complex of donor and recipient DNA with aberrant density, a complex is formed, which is physico-chemically indistinguishable from donor-recipient complex formed in the wild-type. Mutant 7G-97 produces double-stranded fragments, but the donor DNA is not processed further.

Based on the conjecture that the aberrant buoyant density donor-recipient complex formed in the mutants 7G-73 and 7G-84 might represent an intermediate in normal recombination, the conversion of which is delayed in these mutants, Chapter 3 deals with experiments which are aimed to examine whether the aberrant buoyant density donor-recipient complex is also formed in wild-type *B. subtilis*. To this purpose a method was used that both synchronises and slows down the transformation process. Synchronous uptake of DNA was effected by the chelating agent ethylene-diamine-tetra-acetate (EDTA), which prevents entry, but not the binding of transforming DNA to competent cells. After the addition of excess divalent cations (Mg^{2+}) synchronous entry and conversion of the bound DNA into various molecular forms is achieved.
Addition of Mg^{2+} ions at $17^{o}C$ to cells, loaded with donor DNA in the presence of EDTA at $30^{o}C$, results in the formation of a donor-recipient complex that is endowed both with an aberrant buoyant density and little

biological activity. After shifting the transforming cells to 30°C, the aberrant donor-recipient complex is converted into a normal buoyant-density complex.

Chapter 4 deals with the further characterization of the intermediate recombination complex. Alkaline sucrose-gradient centrifugation showed that the donor DNA in the intermediate donor-recipient complex is covalently bonded to the recipient DNA. Treatment of the intermediate complex with the single-stranded DNA specific nuclease S1 resulted in the liberation of acid-soluble products. In addition, the biological activity of the complex is decreased following treatment with nuclease S1. Both on the basis of these results and the properties of the complex in CsCl-gradients, it is assumed that the intermediate donor-recipient complex is a structure in which a single-stranded segment of donor DNA is partially paired with the recipient DNA and is bound covalently to the recipient DNA at the paired extremity.