Competence and competence development in Bacillus subtilis

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SUMMARY

This thesis includes five publications describing experimental work on the development and nature of the transient state of *Bacillus subtilis* in which this organism is genetically transformable (competent); it also comprises an introduction containing a survey of the relevant literature on these topics. The first two papers deal with the development of competence, the last three papers with the biochemical nature of the competent state.

In the first publication results of experiments are presented demonstrating that growing cultures of *B. subtilis* change their medium in such a way as to induce earlier initiation of competence development in a second culture grown in that medium. Evidence was obtained indicating that the competence-stimulating activity of culture fluids is due to (a) compound(s) excreted by the first culture.

The second paper deals with the isolation and characterization of a temperature-sensitive mutant impaired in the development of competence. This mutant shows normal competence development at the permissive temperature (37°C), but fails to become competent when grown at the restrictive temperature (42°C). Although the parental strain develops a reduced level of competence at 42°C, the effect of temperature is much more dramatic in the mutant. In addition, cultures of the mutant strain show an aberrant growth pattern at 42°C: their growth rate decreases abruptly in the late exponential phase of growth, while at the same time the cells become shorter as compared to wildtype cells. Genetic analysis strongly suggested that both the aberrant competence development and the changed growth behaviour of the mutant are due to the same genetic defect. Since cultures of the mutant strain immediately develop competence after a temperature shift from 42°C to 37°C, it was concluded that this strain is blocked in a relatively late step in the sequence of cellular changes leading to the competent state.

The investigations described in the papers III and IV aimed at the characterization of interactions occurring between exogenous DNA and the membrane of competent cells. These interactions were studied with the aid of structurally and functionally intact membrane vesicles, initially isolated from highly competent cultures which are mixed populations of

competent and noncompetent cells (Paper III). The membrane vesicles are capable of binding DNA. The binding is time, temperature and pH dependent. During exposure to membrane vesicles the DNA is attacked by Mg^{2+}-dependent nucleases. Both endo- and exonucleolytic activities are present. Since all of the bound DNA is accessible to added deoxyribonuclease I, it was concluded that the bound DNA does not penetrate the membrane but remains outside in a deoxyribonuclease I sensitive state.

Owing to the development of a suitable separation technique, competent and noncompetent cells could be separated in sufficiently large quantities to permit isolation of membrane vesicles from either type of cells (Paper IV). Vesicles were isolated from the separated cell populations and compared with respect to their interactions with DNA. Both types of membrane vesicles show similar endo- and exonuclease activities. Also, both types of vesicles bind DNA; however, especially at low DNA concentrations vesicles obtained from competent cells bind significantly more DNA than vesicles from noncompetent cells. This difference may reflect a property of the membrane in competent cells that is involved in the capacity of these cells to incorporate DNA.

In the last paper (V) experiments are described in which separated intact competent and noncompetent cells were compared with respect to their interactions with donor DNA. Competent cells were shown to possess a powerful cell envelope-associated exonucleolytic activity which is almost lacking in noncompetent cells. Ethylenediaminetetraacetate inhibits both this activity and native DNA-mediated transformation. The possible involvement of the competence-specific nucleolytic activity in genetic transformation of *B. subtilis* is discussed.