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Transformation and Transfection in Bacillus subtilis

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SUMMARY

This thesis describes a number of aspects of transformation and transfection in *Bacillus subtilis*, with emphasis on the fate of transforming and transfecting DNA following uptake by competent cells. Paper I deals with transforming DNA and shows that the eclipse phase, observed in *B. subtilis* transformation (Venema *et al.*, 1965) is due to double-stranded fragmentation, probably occurring concomitantly with uptake of donor DNA into the competent cell. Depending upon the method of treatment of lysates obtained from transforming cells, a variable part of the irreversibly absorbed donor DNA exhibits aberrant buoyant behaviour in CsCl gradients.As a function of time of incubation of the transforming cells the buoyant density of donor DNA shifts to that characteristic for DNA of *B. subtilis*, suggesting the presence of a transient complex with a cellular component.

Sucrose gradient analysis of re-extracted DNA obtained at various times after irreversible fixation by competent cultures shows that initially donor DNA sediments more slowly than resident DNA and that concomitantly with recovery of donor marker activity from the eclipse, donor DNA is co-sedimenting with the resident DNA, indicating that recovery from the eclipse is due to genetic integration. Initially, the donor marker activity is more resistant to shear than the resident marker activity. When integration of donor DNA is completed, the shear resistance of donor marker activity has become equal to that of the resident marker activity. These data, and the observation that the ratio of donor marker to resident marker activity, being initially dependent of the concentration of DNA, becomes independent from the concentration of DNA when donor marker activity has recovered its biological activity, support the conclusion that eclipse of donor marker activity is due to double-stranded fragmentation of the donor DNA and that recovery from the eclipse is caused by integration.

The molecular weight of eclipse DNA was determined from sucrose gradient centrifugation as well as from the effect of different shear intensities on the ratio of donor marker to resident marker activity. The two values obtained agree reasonably well (9.3×10^6 and 8.0×10^6, respectively).
The relative donor marker frequencies in reisolated DNA mixtures, sheared to MW $\simeq 8.0 \times 10^6$ or less, thus eliminating size dependent differences in transforming activity of donor and resident DNA, decrease as a function of time of incubation of the transforming cells to a final value of 0.25 of the initial value. Since, on the basis of single-strand integration, a decrease by a factor of 2 is to be expected, it is assumed that only about 50% of the eclipse phase DNA molecules participate in genetic integration.

Paper II describes experiments in which the effect of a number of enzymes on recombinant type and donor marker activity in DNA obtained from transforming cells as a function of time of incubation after irreversible binding of transforming DNA was analysed. Both recombinant type and donor marker activity increase upon incubation with DNA ligase, indicating that recombinational intermediate molecules, lacking phosphodiester bonds between the donor and resident moieties of DNA are present during transformation. If the re-extracted DNA is additionally incubated with DNA polymerase I and exonuclease III jointly, no further increase in either of the transforming activities is observed. This observation lends support to the idea that recombination in transformation is effected by a mechanism similar to that proposed for recombination in bacteriophage λ by Cassuto *et al.* (1971).

Paper III deals with transfection of *B. subtilis* with DNA obtained from a newly isolated virulent *B. subtilis* phage, H_1. Bacteriophage H_1 DNA contains HMU instead of thymine and has a molecular weight of 83×10^6. Transfection of *B. subtilis* with H_1 DNA shows a 4-5 powered concentration dependence, indicating that 4-5 phage DNA molecules are required for the formation of an infective center. H_1 transfection of *B. subtilis* uvr^+ increases about 100 fold (1) by UV irradiation of the competent culture or (2) by incubation of the competent culture with UV irradiated homologous or heterologous DNA, prior to the addition of transfecting DNA.

The fate of transfecting H_1 DNA in normal conditions and in enhancing conditions was followed using re-extraction experiments. Like transforming DNA, transfecting H_1 DNA suffers double-strand breakage concomitantly with uptake by the competent cells. Endonucleolytic degradation of transfecting H_1 DNA is equal in normal and enhancing conditions. However, cellular exonucleolytic breakdown of transfecting DNA is inhibited in conditions enhancing transfection. The increased transfection efficiency in enhancing conditions can quantitatively be explained by the observed inhibition of exonuclease activity. The involvement of the uvr genotype of the host bacterium on transfection enhancement by UV was studied using two UV sensitive strains: strain 1G23, a normally transformable and transfectable hcr^- strain, and strain 8G5 recA1, a hcr^+ recombination deficient strain, which is normally transfectable with H_1 DNA. Contrary to uvr^+ strains and strain 8G5 recA1, in which transfection is enhanced by either method of enhancement, transfection enhancement in strain 1G23 is observed only upon incubation of competent cultures with UV irradiated DNA. The absence of transfection enhancement upon UV irradiation of competent 1G23 cultures is accompanied by the lack of inhibition of cellular exonuclease activity. Alkaline sucrose gradient analysis

revealed that strain 1G23 is deficient in the incision step of the excision-repair process of elimination of UV damage. From these results a model for the mechanism of transfection enhancement by UV is proposed, which is based on the assumption that DNA carrying UV damage traps cellular exonucleases.

Paper IV, finally, concerns a probable case of heterologous transfection, involving bacteriophage GA-1 DNA and *B. subtilis*. Results are presented showing that the host of bacteriophage GA-1 is probably not *B. subtilis* and that GA-1 phage particles are not infective for *B. subtilis* although GA-1 phage particles are produced when competent *B. subtilis* cells are exposed to GA-1 DNA. The transfecting activity of GA-1 DNA is sensitive to proteolytic enzymes, indicating that a protein which is required for transfection is bound to phenol-extracted purified GA-1 DNA. The buoyant density of protease treated GA-1 DNA in CsCl density gradients is 0.004 g/cm^3 higher than that of untreated DNA. Since the competitive inhibition of transformation by protease treated GA-1 DNA is equal to that of untreated DNA, possible explanations for the effect of proteases on the infectivity of GA-1 DNA are that the protein associated with GA-1 DNA is essential for the intracellular development of GA-1 phage particles, or for protection of the GA-1 chromosome from being degraded by cellular nucleolytic activity. The shape of entry curves of GA-1 DNA favors the possibility that the protein is bound to the end(s) of the GA-1 DNA molecule.