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Relationship Between Transformation in *Bacillus subtilis* and Infection by Bacteriophage SP02

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When bacteriophage SP02 infects a *Bacillus subtilis* culture during or shortly after transformation, the frequency of transformants among the resulting lysogens is greatly reduced relative to that in the uninfected culture. The effect can occur after the deoxyribonucleic acid has been taken up and covalently attached to recipient deoxyribonucleic acid.

The presence in *Bacillus subtilis* of a prophage of either SPO2 or ϕ105, is inhibitory to transformation (7, 10, 11). The mechanism of this inhibition has not been established. Here we extend these previous observations by showing that the frequency of transformation is decreased by SPO2, not only in cells in which a prophage is already established, but also in cells lysogenized during or after the transformation process. Using this facility, we show that the decrease in transformation frequency is achieved even when the phage are not present until after maximal uptake of donor deoxyribonucleic acid (DNA) has been achieved and after time has been allowed for covalent bonds to form between donor and recipient DNA.

Routine procedures were as described previously (8, 9). Transformations were performed at 37 C. In some experiments, 2×10^{-5} M $MnCl_2$, optimal for SPO2 infection, was added to all cultures of competent cells just before addition of DNA. *B. subtilis* strains used were: SB-133 (*phe-1*) and MB-210 (*trp-2*). SPO2 cultures were ultraviolet light induced from lysogenic cultures of MB-210 grown in VYS medium (0.5% yeast extract, 2.5% veal infusion, 2×10^{-5} M $MnCl_2$, 5×10^{-3} M $MgSO_4$). The DNA solution used was supplied by Julius Marmur.

In infection by SPO2, the proportion of cells surviving the infection ranged from less than 10% to nearly 100%. In some experiments, nearly all survivors had become lysogenic. In others, significant numbers of cells survived without becoming lysogenic. The basic results were the same in either case and, since the failure of some cells to be lysogenized would not affect any of our conclusions unless it could be shown that they were not infected at all, we have ignored this failure for the sake of simplicity of discussion.

When competent cells were infected with SPO2 at or after the time of DNA addition, the proportion of transformants among cells that survived the infection was much less than among cells of the uninfected culture. Table 1 shows one experiment in which the SPO2 decreased the transformation frequency by about 1,000-fold when added at the same time as the transforming DNA. Table 1 also shows that the effect is not on DNA uptake, as defined by the achievement of deoxyribonuclease (DNase) resistance, since SPO2 still had its effect at a time when the transformation had become insensitive to DNase.

Nester and Stocker (6) showed that the maximal level of linkage between donor and recipient markers is reached by 30 min after addition of DNA. Dubnau and Davidoff-Abelson (3) have also shown that most of the donor DNA that will become covalently bound to recipient DNA has done so within 20 min after addition of DNA. Thus, the SPO2 can apparently have its effect after the establishment of covalent linkage.

The following arguments lead to the conclusion that the important effect occurs in the transformation mixture, where all cells are equally exposed to phage, rather than after plating, where the assays for transformants are performed at a lower dilution (higher concentration of unadsorbed phage) than are the viable count assays. (i) Reconstruction experiments were performed in which transformed cultures were mixed with untransformed cultures just before plating. In each case, one culture had been infected with SPO2 at 0 or 30 min after addition of DNA; and the other had not. In the cases in which the transformed culture had been infected with SPO2, the observed transformation frequency was greatly reduced relative to

TABLE 1. *Effect of SPO2 and DNase on transformation frequencies*[a]

Time DNase added (min)	Time SPO2 added (min)	Transformation frequency
—[b]	—	3.5×10^{-3}
—	0	3.1×10^{-6}
—	30	1.5×10^{-4}
0	—	0
30	—	3.2×10^{-3}

[a] Competent MB-210 was transformed with SB-133 DNA (1.2 μg/ml). DNase (10 μg/ml) or SPO2 (multiplicity of infection ≥4) was added at indicated times. Time zero is time of addition of DNA; 90 min after addition of DNA, each tube was plated to assay for frequency of transformation to *trp*+.

[b] None added.

those in which the transformed culture had not been infected and had only been mixed with the SPO2-infected culture at the time of plating. (ii) The effect was observed even when unadsorbed phage were removed by centrifugation prior to plating. (iii) The effect of SPO2 on transformation frequency decreased as the time between treatment with DNA and SPO2 increased, although this should have no effect on what happens on the plates. (iv) The numbers of colonies were usually consistent from one dilution to another. (v) In some experiments all the surviving cells, including those diluted 1:10^5 before plating, were lysogenic, and therefore all were presumably immune to further infection. (vi) The extent of the effect of SPO2 was dependent on the concentration of Mn^{2+} and Mg^{2+} in the transformation mixture, but was independent of the extent to which the culture (and thus these ions) was diluted before plating.

Thus, we have established that infection by SPO2, during or after transformation, can decrease the frequency of transformation and that the effect is not at the level of uptake of DNA or establishment of the covalent bond.

There are two alternative possible general explanations for the effect of SPO2: either (i) the process of lysogenization (or the presence of the prophage) interferes with transformation, or (ii) SPO2 preferentially kills competent (or transformed) cells, e.g., in infection of a newly transformed (or competent) cell, the choice between lysogeny and lytic infection goes in the latter direction more often than it does in infection of non-competent cells.

At, first glance, possibility (i) seems unlikely because the effect can still be obtained at so late a time after addition of DNA. If SPO2 is inhibiting the transformation process, there must be a part of the process that occurs after the establishment of covalent linkage. However, this is not beyond the realm of possibility and, indeed, it has been suggested previously that successful replication of transformed DNA (and, thus, formation of the transformant colony) depends upon some step in the transformation process that is not essential for covalent integration (2) and does not take place until a long time after addition of DNA (1).

However, the second alternative seems more likely, particularly since it is well known that competent cells remain distinguishable from non-competent cells for some time after transformation (1, 4, 5, 6). If this alternative is true, it may mean that in SPO2 infection the choice between lysogeny and lytic growth is affected by one or more of the same factors that determine the level of competence for transformation.

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