Relationship Between Competence for Transfection and for Transformation

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Deoxyribonucleic acid (DNA) extracted from phage SPP1 is highly infectious on *Bacillus subtilis* competent cells; the efficiency of infection is 5×10^3 to 6×10^3 phage equivalents per plaque-forming unit. This DNA was used to study the relationship between competence for transfection and for transformation. The experiments were concerned with the frequency of infection and transformation in mutants exhibiting different levels of competence, the effect of periodate on competence for infection and for transformation, the competition between phage and bacterial DNA, the transformation of cells preinfected with phage DNA, and the infection of cells pre-treated with bacterial DNA. The data show that *B. subtilis* cells competent for transformation are also competent for transfection and vice versa; transfection with phage DNA represents, therefore, a simple way to measure the total number of competent cells in a culture. The fraction of competent cells, determined by SPP1 DNA infection, varied from 10^{-2} to 7×10^{-2} .

Transfection is defined as "infection of cells by nucleic acid extracted from a virus resulting in the production of complete virus" (16). Kaiser and Hogness (9) first succeeded in infecting *Escherichia coli* cells with λ deoxyribonucleic acid (DNA) and a related "helper" phage. Romig (14) first obtained infection of cells of *Bacillus subtilis*, competent for genetic transformation, by DNA extracted from a *B. subtilis* phage. Further studies on transfection in *B. subtilis* have been reported by several authors and reviewed by Spizzen et al. (16). Recently, a study of competence in *B. subtilis* by means of transfection with ϕ 29 phage DNA has been reported (3). These studies indicate the existence of a certain relationship between bacterial transformation and transfection, concerning the irreversible binding of DNA to the cell.

In the present paper, more data are reported on the relationships between transformation and transfection. The DNA used was from a newly isolated *B. subtilis* phage called SPP1, which has a number of interesting physicochemical properties (13), and exhibits the highest efficiency of transfection so far described in *B. subtilis*. Experiments will be described concerning the effect on the two processes of a number of treatments and of states affecting competence. The data indicate that competence for infection is determined by

the same physiological events by which the bacterial cells develop competence for transformation, and that infection by phage DNA can therefore be used, under certain conditions, to estimate directly the total fraction of competent cells in a culture.

MATERIALS AND METHODS

Strains. The strains used are listed in Table 1.

Media. The media used were the minimal medium of Davis and Mingioli (4) for selection of transformants, PS medium (13) for the production of phage lysates, Spizzen medium (15) for preparing competent cells, and Bennett's medium (17) for the counting of infectious centers.

DNA preparation. Phage DNA was prepared as described by Riva et al. (13); bacterial DNA was extracted according to the method of Polsinelli and Barlati (10). Radioactive phage was prepared as follows: a 500-ml flask containing 100 ml of minimal medium, supplemented with 5 μ g/ml of adenine, 25 μ g/ml of tryptophan, 0.4 mg/ml of Casamino Acids (Difco), and 1 mc of ³H-adenine (Radiochemical Centre, Amersham, England), was inoculated with 10^6 cells per ml of *B. subtilis* strain SB 58, requiring adenine and tryptophan. After 9 hr of growth with shaking at 37 C, the cells were centrifuged and resuspended at a concentration of 5×10^8 cells/ml in 200 ml of PS medium, prewarmed at 37 C and supplemented with 0.5 mc of ³H-thymidine (Radiochemical Centre). The cells were then multiply infected (4:1) with a phage suspension; lysis occurred after 2 hr. The DNA was extracted from purified phage as already described (13). The specific activity of the DNA was

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TABLE 1. List of *Bacillus subtilis* strains used

Strains	Genotype ^a	Origin
SB 25	<i>trp⁻ his⁻</i>	J. Lederberg
SB 58	<i>trp⁻ ad⁻</i>	J. Lederberg
PB 3015	Prototroph	Derivatives of SB 25
PB 424	<i>trp⁻ his⁻ met⁻</i>	
PB 3177	<i>trp⁻ his⁻ leu⁻</i>	
PB 551	<i>trp⁻ his⁻ act^r</i>	
PB 553	<i>trp⁻ his⁻ act^r</i>	
PB 555	<i>trp⁻ his⁻ act^r</i>	

^a Symbols: *trp⁻*, *his⁻*, *ad⁻*, *met⁻*, *leu⁻*, requirement for tryptophan, histidine, adenine, methionine, and leucine, respectively; *act^r*, resistance to actinomycin.

about 12,000 counts per min per μ g as measured with a Packard Tri-Carb scintillation spectrometer. The DNA titer was determined by the method of Dische (5).

Phage DNA fractionation. Radioactive phage DNA was fractionated in a 5-ml sucrose gradient (5 to 20%, w/v). A 0.1-ml amount of solution, containing 4 μ g of DNA, was layered on the top of the gradient and run at $110,000 \times g$ for 5 hr in a Spinco L 50 ultracentrifuge, rotor SW39. Fractions were collected by puncturing the bottom of the tube and were analyzed for radioactivity and infectivity.

Infection and transformation procedures. Competent cells were prepared according to the method of Young and Spizizen (18). A 1-ml amount of competent cells was incubated with phage DNA or bacterial DNA at 37 C with shaking. DNA uptake was interrupted by adding deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J.) to a final concentration of 10 μ g/ml. The infective centers were counted by plating samples of cells according to the standard phage assay (1). SB 25 cells grown to stationary phase were used as indicator. Plaques were counted after 20 hr of incubation at 37 C. Unless otherwise stated, transformation was performed for tryptophan independence.

RESULTS

Properties of the transfection process. Competent cells of *B. subtilis* SB 25, when incubated with phage DNA at 37 C for 50 min and plated as described in Materials and Methods, gave phage progeny. The infectivity was destroyed by deoxyribonuclease treatment (10 μ g/ml, 10 min at 37 C) and by thermal denaturation of DNA, but not by ribonuclease. Figure 1 shows the relation between the concentration of phage DNA in the culture of competent cells and the number of infective centers produced. The efficiency of infection, that is, the number of phage DNA equivalents per plaque-forming unit (PFU), is 5×10^3 to 6×10^3 . At saturating DNA concentration (>10 μ g/ml), the fraction of cells that could be infected was about 3%. In

other experiments, the fraction of infected cells varied from 1 to 7%.

To check whether the efficiency of infection could be enhanced by the use of DNA enriched in high molecular weight material, radioactive phage DNA was fractionated in a sucrose gradient as described in Materials and Methods. The fractions were assayed for radioactivity and for infectivity (Fig. 2). The coincidence of the infection curve with that of the radioactive DNA indicates that the DNA preparation had a homogeneous molecular weight composition. The highest efficiency of infection obtained in this experiment was 7×10^3 DNA molecules per PFU, which is not significantly different from that obtained with unfractionated DNA.

Kinetics of infection and transformation. Competent cells (3×10^8 /ml) of SB 25 were incubated with phage DNA (20 μ g/ml) and, in parallel, with bacterial DNA (2 μ g/ml). At various times after the addition of phage or bacterial DNA, samples of the cultures were removed and transferred to tubes containing deoxyribonuclease. After 10 more min, samples of the cells were assayed for infection or for transformation. Figure 3 shows that the number of transformants and the number of infected cells increased with the time of exposure to DNA. The transformation plateau was reached after about 15 min, whereas the infection plateau was

FIG. 1. Dependence of infectious center formation on phage DNA concentration. Competent cells (3×10^8 /ml) were incubated with different concentrations of DNA at 37 C with shaking. After 50 min, deoxyribonuclease was added (10 μ g/ml), and incubation was continued for 10 more min. Samples of the suspension were plated, and plaques were counted after 20 hr at 37 C.

FIG. 2. Sucrose gradient fractionation of ^3H -phage DNA. With *Escherichia coli* ribosomes as marker, an $S_{20w}^{0.01}$ of $32 \pm 1S$ for the DNA band was calculated which is in agreement with the value determined elsewhere (13) and corresponds to a molecular weight of 2.5×10^7 daltons. Nineteen fractions were collected; 0.03 ml of each fraction was counted in a Packard Tri-Carb scintillation spectrometer, and 0.05 ml was assayed for infectivity as described in the text.

FIG. 3. Kinetics of transformation or infection.

not reached before 50 min. This difference is probably due to the different size of the fragment of DNA that must enter the cell in order to express the biological activity: a whole phage equivalent for transfection, i.e., 2.5×10^7 daltons, and the minimal size taken up for transformation, i.e., about 10^6 daltons (2). In this experiment, the

maximal frequency of transformed cells was 2×10^{-3} , and the maximal frequency of infected cells was about 1.5×10^{-2} .

Treatment of competent cells with periodate. Treatment of competent cells with sodium periodate strongly decreases bacterial transformation by reducing DNA uptake (10). Competent cells were treated with different concentrations of sodium periodate and assayed with saturating levels of one of the DNA preparations. The residual fractions of infected or transformed cells as a function of NaIO_4 concentration are shown in Fig. 4. The two curves are essentially coincident, suggesting that sodium periodate indeed acts on structures common to the two processes, infection and transformation.

Infection of strains with different degrees of competence. It has been reported that mutants of *B. subtilis* resistant to actinomycin show a reduced transformability (6, 11), probably because of a diminished permeability of the cell wall. Three actinomycin-resistant strains and a sensitive one, processed for competence, were in-

FIG. 4. Inhibition of infection and transformation by treatment of competent cells with sodium periodate. Cells were exposed to different concentrations of sodium periodate for 10 min at 37 C according to the procedure described by Polsinelli and Barlati (10).

fected and transformed in parallel experiments with saturating DNA concentrations. The data in Table 2 show that a decrease of transformation is parallel to a decrease of infection.

The data confirm those obtained with periodate (see above), indicating that the DNA receptors are common to the two processes.

Competition experiments. If there is a common attachment site for bacterial and phage DNA, then one would expect a mutual interference of the two processes by either DNA; that is, an excess of phage DNA should compete with bacterial DNA and inhibit transformation, and vice versa.

Competent cells of SB 25 were incubated with phage and bacterial DNA premixed in different proportions. Infection and transformation were assayed after 50 and 15 min of incubation, respectively. The shorter incubation time used for transformation had the purpose of reducing to a minimum the loss of transformed cells due to the cellular lysis caused by phage production; in fact, as shown in Fig. 3, at 15 min, only a small fraction of cells was able to undergo the infectious process. The data in Table 3 show that there was reciprocal competition between the two DNA preparations. The results confirm, therefore, the existence on the cell of common receptors for the two DNA preparations.

Furthermore, taken at face value, the results would indicate that the class of cells competent for infection is exactly coincident with the class of cells competent for transformation; this can be deduced by the essentially total inhibition of either process by the presence of an excess of the other DNA. On the other hand, it is conceivable that the observed competition is a fairly general

TABLE 2. *Correlation between actinomycin resistance and transformability or transfectability in Bacillus subtilis*

Strains	Relative transformation percentage ^a	Relative infection percentage ^a
SB 25, sensitive to actinomycin	100	100
PB 551, resistant to 1 µg/ml of actinomycin	2	2.2
PB 553, resistant to 4 µg/ml of actinomycin	1.2	1.2
PB 555, resistant to 6 µg/ml of actinomycin	1.2	0.9

^a The cells (2×10^8 to 3×10^8 /ml) were incubated with saturating doses of bacterial and phage DNA, i.e., 2 and 20 µg/ml, respectively. They were assayed after 50 min of exposure.

TABLE 3. *Competition between phage and bacterial DNA in transformation and infection experiments^a*

Bacterial DNA	Phage DNA	Residual infection	Phage DNA	Bacterial DNA	Residual transformation
µg/ml	µg/ml	%	µg/ml	µg/ml	%
0	0.5	100	0	0.1	100
0.01	0.5	75	0.1	0.1	40
1	0.5	15	0.5	0.1	20
10	0.5	0.5	10	0.1	1

^a The DNA concentrations indicated in the second and fifth columns lie in the linear range of the dose-response curves for infection and transformation (see Fig. 1). Recipient and donor strains were SB 25 *his*⁻ *trp*⁻ and SB 3015 prototroph, respectively. Transformation was performed for tryptophan independence.

phenomenon and that any receptor specific for DNA will also bind any DNA. If such were the case, the observed competition would then reflect simply an interference at the level of a relatively aspecific binding site rather than the sharing of the incorporation machinery by the two processes. We thought, then, that a more direct approach to the problem of whether the cells competent for transformation were also competent for transfection, and vice versa, would be provided by experiments in which either process was studied in the same cells but in a "temporal sequence"; thus, the two DNAs would never be present at the same time and could not compete with each other.

Effect of pretreatment with phage or bacterial DNA on transformation and infection. Competent cells of strain PB 424 *his*⁻ *trp*⁻ *met*⁻ were incubated with a saturating level of bacterial DNA (10 µg/ml) for 20 min at 37 C with shaking; the cells were then treated with deoxyribonuclease to destroy the residual bacterial DNA, whether free in the culture or bound to the bacterial wall. Deoxyribonuclease was removed by centrifugation and subsequent washing of the cells with Spizizen medium on a membrane filter (Millipore Corp., Bedford, Mass.). The cells were then incubated with 50 µg/ml of phage DNA for 50 min at 37 C and plated to assay separately for transformed or infected cells. As shown in Table 4, transformed cells which survived the subsequent infection by phage DNA (experiment 2) were only 5% of the control (experiment 1); that is, about 95% of transformable cells were later infected and killed. In the reciprocal experiment, the cells were first incubated with shaking for 50 min at 37 C with 50 µg/ml of phage DNA and, after treatment

TABLE 4. *Effect of pretreatment with phage or bacterial DNA on transformation and infection^a*

Expt	DNA used for first incubation	Deoxyribonuclease treatment	DNA used for second incubation	Frequency of <i>met</i> ⁺ colonies	Frequency of <i>trp</i> ⁺ colonies	Frequency of infected cells
1	Bacterial	No	None	2.0×10^{-3}		
2	Bacterial	Yes	Phage	1.0×10^{-4}		5.6×10^{-3}
3	None	Yes	Phage			8.4×10^{-3}
4	None	No	Phage			9.4×10^{-3}
5	Bacterial	Yes	Bacterial	1.4×10^{-3}	3.4×10^{-4}	
6	None	Yes	Bacterial		5.5×10^{-4}	
7	Phage	No	None			3.9×10^{-2}
8	Phage	Yes	Bacterial		8.0×10^{-6}	3.1×10^{-2}
9	None	Yes	Bacterial		4.8×10^{-4}	

^a The recipient strain was PB 424 *his*⁻ *trp*⁻ *met*⁻. Strain SB 25 was the *met*⁺ donor DNA for the first incubation. Strain PB 3015 prototroph was the *trp*⁺ donor DNA for the second incubation. The concentration of bacterial and phage DNA were saturating, i.e., 10 and 50 μ g/ml, respectively. Incubation of cells alone or cells and bacterial DNA was for 20 min. When cells were incubated with phage DNA, the time was 50 min. The concentration of the cells was 2×10^8 to 3×10^8 /ml. Deoxyribonuclease was washed by centrifugation and resuspension in Spizizen medium and by filtration on a Millipore filter with a great volume of the same medium. Finally, the cells were resuspended at approximately the initial concentration.

with deoxyribonuclease and washing as above, were incubated with bacterial DNA (10 μ g/ml) for 20 more min. Following this treatment, transformed cells (experiment 8) were less than 2% of those found in the control without phage DNA (experiment 9). This reduction cannot be due to the effect of residual deoxyribonuclease. As a control, bacteria not exposed to bacterial DNA were exposed to phage DNA and assayed for infection; whether or not the cells had been exposed to deoxyribonuclease before the exposure to phage DNA, the infectivity was unchanged (experiments 3 and 4), showing that residual deoxyribonuclease cannot be responsible for the decrease of transformation in the basic experiments. Other control experiments showed that the absence of transformed cells when phage DNA was added to the same cells before bacterial DNA must be due to a killing of the infected cells and not to an inability of the cells that have taken up some DNA to accept more DNA. Experiment 5 shows that, after exposure to *met*⁺ transforming DNA and subsequent removal by deoxyribonuclease of the nonincorporated molecules, the competent cells were still able to take up and integrate another kind of transforming DNA (*trp*⁺ DNA), and the efficiency of transformation for the latter was essentially identical to that of cells which had not been first exposed to the *met*⁺ DNA (experiment 6). Conversely, the disappearance of transformed cells when phage DNA was added after bacterial DNA must also be due to the lethal effect of the infection and not to any damaging action of the later incorporation of DNA on the integration of the DNA previously taken up; in fact, the

frequency of transformation for *met*⁺ in experiment 5 where the cells were later exposed to *trp*⁺ DNA was the same as for cells exposed to *met*⁺ DNA and not subsequently exposed to any further treatment (experiment 1). Also, the ability to take up infectious DNA and develop phage was not affected by either previous or subsequent treatment with bacterial DNA; the number of cells infected when they had been exposed to bacterial DNA before exposure to phage DNA (experiment 2) was not significantly different from the number of infected cells when there had not been previous exposure to bacterial DNA (experiment 3). Furthermore, the number of cells infected after exposure to phage DNA (experiment 7) was not significantly higher than the number when phage DNA incubation was followed by transforming DNA treatment (experiment 8). In other words, all these controls show that after taking up one kind of DNA, if the residual DNA is removed by deoxyribonuclease and washing, the same cells are quite capable of taking up either kind of DNA; that they are the *same* cells is shown by the disappearance of transformants when one of the DNA preparations used is the phage DNA, whether added before or after the bacterial DNA.

In conclusion, these experiments show that all of the cells competent for transformation are also competent for infection; it does not show the converse, since it is possible that a number of cells are competent for infection but not for transformation. Such a difference could occur because the two processes enable the incorporation of DNA into the cells, whereas only the

transformation process requires also the integration into the host genome.

Estimation of the fraction of competent cells. The infection by phage DNA could in any case be a useful tool to estimate the total fraction of competent cells. In transformation experiments, only a fraction of cells that incorporate in the genome donor DNA fragments are scored as transformants. In fact, the recipient strain generally carries one or few markers for which transformation can be performed, whereas the bacterial chromosome breaks into many fragments (20 to 100) during the DNA extraction. Even if an extra step were needed for transformation with respect to transfection, namely, the integration step, the total number of cells able to bind irreversibly high molecular weight DNA would be an acceptable measure of competence, and this is obtainable by determining the fraction of cells infected by phage DNA. Experiments will be described showing that the estimation of competence obtained by directly infecting the cells with phage DNA is in agreement with the indirect estimation reported by Goodgal and Herriott (8). These authors calculated the fraction of competent cells from the data of transformation for two unlinked markers. For example, if the markers are A and B, the frequency of competent cells would be $n_A \times n_B / n_{AB}$, where n_A is the frequency of transformants for the marker A, n_B the frequency of transformants for the marker B, and n_{AB} the frequency of cells transformed for A and B. Competence so determined is a measure of the fraction of cells that are both able to incorporate DNA and to integrate it. If competence so determined were to be identical to the competence

measured with phage DNA, then one could conclude that the two processes take place exactly and only in the same cells, and that a cell competent for one process is also competent for the other one.

Experiments of infection and transformation were performed with the recipient strain PB 3177 *his⁻ trp⁻ leu⁻* (the leucine marker is not linked to the other two markers). Samples of the culture processed for competence were either transformed with a *trp⁺ leu⁺* DNA or infected with phage DNA. The single transformants for *trp⁺* or *leu⁺* and the double transformants for *trp⁺ leu⁺* were selected, and the above ratio was calculated at various DNA concentrations. The ratios obtained did not depend on the DNA concentrations, as expected for two unlinked markers, and were in agreement with the values for frequency of competent cells (2 to 3%) obtained by infecting the same batch of cells with saturating doses of phage DNA (see Table 5).

DISCUSSION

In the infection experiments reported above, 2×10^6 to 5×10^6 infectious centers per μg of DNA were obtained. The molecular weight of the DNA being 2.5×10^7 daltons, an efficiency of infection of 5×10^3 to 6×10^3 phage equivalents per infectious center can be calculated (Fig. 1). This efficiency is the highest so far reported in *B. subtilis*.

The existence of a relationship between transformation and transfection in *B. subtilis* has been suggested by other authors (3, 7, 12, 14). In our experiments, the total fraction of infected cells was, in different experiments, 10 to 20 times higher than the fraction of cells transformed for

TABLE 5. Estimation of competence by infection and by transformation

Bacterial DNA concn (A)	Frequencies of transformants ^a			Product of single frequencies (B) $\times$ (C)	Frequency of competent cells [(B) $\times$ (C)]/(D)
	<i>trp⁺</i> (B)	<i>leu⁺</i> (C)	<i>trp⁺ leu⁺</i> (D)		
$\mu\text{g/ml}$					
20	3.74×10^{-3}	4.8×10^{-3}	5.3×10^{-4}	1.79×10^{-5}	3.39×10^{-2}
2	2.26×10^{-3}	3.0×10^{-3}	2.1×10^{-4}	6.8×10^{-4}	3.23×10^{-2}
0.2	1.25×10^{-3}	1.27×10^{-3}	2.5×10^{-6}	1.59×10^{-6}	6.35×10^{-2}
0.02	3.3×10^{-4}	2.75×10^{-4}	2.3×10^{-6}	9.1×10^{-8}	3.95×10^{-2}
Phage DNA concn			Frequency of infected cells		
$\mu\text{g/ml}$					
50			3.5×10^{-2}		
100			2.0×10^{-2}		

^a The recipient strain was PB 3177 *his⁻ trp⁻ leu⁻*. Strain PB 3015 prototroph was the donor DNA. The concentration of cells was about $3 \times 10^8/\text{ml}$.

a single marker; the fraction of transformants for tryptophan independence was 2×10^{-3} to 3×10^{-3} , whereas the fraction of competent cells determined with transfection was 3×10^{-2} to 7×10^{-2} . This estimate was in good agreement with that obtained according to the method devised by Goodgal and Herriott (8).

In the present investigation, data on infection of strains with different competence (Table 2), on the effect of sodium periodate treatment on infection (Fig. 4), and those obtained from an experiment concerning DNA competition (Table 3) strongly suggest the existence of a common mechanism for transformation and transfection, at least in the early stages.

Experiments on transformation of cells preincubated with phage DNA and on infection of cells preincubated with bacterial DNA indicate that all the cells that can be transformed can also be infected by SPP1 phage. The near identity of the fraction of infectable cells with that of competent cells measured by the method of Goodgal and Herriott (8) shows that the converse is also true, and that the classes of cells competent for transfection and for transformation are exactly coincident. Therefore, the fraction of competent cells of a culture can be measured directly by determining the fraction of infected bacteria. It follows that the DNA binding and entrance steps, which are common to the two processes, are limiting for the transformation process, and that once the DNA is incorporated, the probability of integration is close to one.

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