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Role of the ϕ11 Phage Genome in Competence of *Staphylococcus aureus*

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Both phage ϕ11 and 83A, when present as prophage or when used as helper phage, induce competence for transfection and transformation to the same level in *Staphylococcus aureus*, strain 8325-4. Cells lysogenized with certain temperature-sensitive (*ts*) mutants of phage ϕ11 show competence at the nonpermissive temperature (41 C) without production of infectious phages. Phage ϕ11*ts* allele 31 can neither as a prophage nor as a helper phage develop competence under nonpermissive conditions. This mutant appears, therefore, to be mutated in the region of the phage genome controlling competence. The competence level for both transfection and transformation is increased by superinfecting strain 8325-4 (ϕ11) or 8325-4 (83A) at high multiplicities with phage ϕ11 with some of its mutants or with phage 83A. This superinfection enhancement appears to require protein synthesis but not deoxyribonucleic acid synthesis as judged from studies with inhibitors of macromolecular synthesis. Besides the phage particle, no extracellular or cell-bound factors so far detected can induce competence. The phage-induced product conferring competence is rapidly synthesized by strain 8325-4 ($ts\phi11_{31}$) after shift to permissive conditions, but requires deoxyribonucleic acid and protein synthesis to be expressed. Recombination between the *sus* mutants of phage ϕ11 of Kretschmer and Egan and $ts\phi11_{31}$ indicate that competence is controlled by an early gene in the lytic cycle which may be expressed also in lysogenic cells. The phage product inducing competence appears to have a half-life of 10 to 15 min in the conditional lethal mutant at shift to nonpermissive temperature. Ultraviolet inactivation of phage ϕ11 infectivity occurs more rapidly than inactivation of competence induction. In fact, the number of transformants is increased at low doses of irradiation. Competence induction is, however, decreased at high doses of ultraviolet irradiation.

The ability of bacteria to adsorb deoxyribonucleic acid (DNA) from related bacterial strains and become genetically transformed has been designated competence (26). Competence may also include the ability to assimilate phage DNA to achieve transfection since the same mechanisms are involved in both transfection and transformation in many systems (18, 20, 22). Both physiological and genetic properties may control competence, and the factors involved differ in different bacterial systems. Competence factors have been isolated in several species, and transformation competence appears to be strictly related to the activity of these mediators (3, 17, 27). In species such as *Escherichia coli* where competence factors have not yet been identified, calcium ions or helper phage are necessary for competence (4, 9, 12, 13, 16). Competence for both transfection (22) and transformation (20) in *Staphylococcus aureus* appears to be dependent on high concentrations of calcium ions as well as lysogeny or superinfection with phage ϕ11.

The present study delineates the role of phage ϕ11 and a related phage 83A in induction of competence, and with the aid of temperature-sensitive (*ts*) mutants we have established that an early gene of phage ϕ11 is responsible for induction both in the prophage state and at superinfection. Shift-up experiments suggest a half-life of 10 to 15 min for the intracellular factor inducing competence. No extracellular or cell-bound factor which could be transferred between competent and noncompetent cells could be detected.

MATERIALS AND METHODS

Staphylococcal strains. The *S. aureus* strains employed in this study are listed in Table 1. The standard host organism 8325-4, cured of the pro-

TABLE 1. *Source and characteristics of the strains of S. aureus*

Designation	Source	Derivation and description
8325-4	R. P. Novick	8325 N, UV-cured of φ11, φ12, and φ13.
8325-4 *str*^{R}	Our laboratory	Spontaneous mutant of 8325-4. Resistance level 75 μg of streptomycin/ml.
8325-4 *su*$^+$	P. Kretschmer	See reference 10.
8325-4 *thy*	Our laboratory	Thymine-requiring mutant of 8325-4. Spontaneous mutant isolated after enrichment in CHM medium in the presence of aminopterin (11).
8325-4 (φ11*de*)	R. P. Novick	See reference 15. Resistant to erythromycin. Resistance level 5 μg/ml.
P. S. 83A	The National Bacteriological Laboratory, Sweden	Propagating strain for phage 83A.

phages φ11, φ12, φ13, and strain 8325-4 (φ11*de*) were kindly provided by R. P. Novick, Department of Microbiology, The Public Health Research Institute of the City of New York. The suppressor host mutant, 8325-4 *Su*$^+$ was kindly supplied by P. J. Kretschmer and J. B. Egan, Department of Biochemistry, University of Adelaide, Adelaide, South Australia. The auxotrophic mutant requiring thymine (*thy*) (11) and the streptomycin-resistant derivative of strain 8325-4 were isolated in our laboratory. Stock cultures were kept on Trypticase soy agar (TSA) slants at 4 C.

Phages. The staphylococcal phages used are listed in Table 2. Most of the phages belong to the International Typing Set and were obtained from The National Bacteriological Laboratory, Sweden. Phage φ11, earlier designated P11 (15), and phage 80α were supplied by R. P. Novick, and the φ11 *sus* mutants were provided by P. J. Kretschmer. Table 2 also shows the serological grouping of the phages and their ability to induce competence as prophages in the host strain 8325-4 (see Results).

Media. Trypticase soy broth (TSB) was used for growth of bacteria and for propagation of most of the phages. TSA and TSB medium with 0.5% agar were used as bottom agar and soft agar, respectively, in assays for phages and transfectants. Transfection and transformation were performed in the competence buffer containing tris(hydroxymethyl)aminomethane-maleate buffer, 0.1 M at pH 7.0, with 0.1 M $CaCl_2$ (22). For ultraviolet (UV) inactivation, phage φ11 was suspended in a modified version of Novick buffer containing the following: β-glycerophosphate, 0.06 M; $MgSO_4$, 0.001 M; $CaCl_2$, 0.004 M; NaCl, 0.085 M; and 0.1% (wt/vol) glycerol, pH 8.0 (14). CHM medium used for isolation of pyrimidine-requiring mutants and as selective medium in transformation experiments was described earlier (11).

Purification of phages and preparation of transfecting and transforming DNA. Propagation and purification of phage 80α and phage φ11 has been described earlier (21, 22). The method used for phage φ11 was also used for phage 47, 75, and 83A. For preparation of a high-frequency transducing (HFT) lysate (15), strain 8325-4 (φ11*de*) was infected with phage φ11 or φ11*vir* at a multiplicity of infection (MOI) of about 5 in TSB medium, and incubated

TABLE 2. *Characteristics and origin of the staphylococcal phages*

Phage	Serological group[a]	Ability to induce competence as prophage	Source
29	B	–	NBLS[b]
52A	B	–	NBLS
52	B	–	NBLS
79	B	–	NBLS
53	B	–	NBLS
83A	B	+	NBLS
φ11	B	+	R. P. Novick (15)
80α	B	–	R. P. Novick (14)
47	A	–	NBLS
6	A	–	NBLS
42E	A	–	NBLS
54	A	–	NBLS
75	A	–	NBLS
81	A	–	NBLS
77	F	–	NBLS

[a] Serological groups refer to the serological classification of *S. aureus* phages (1, 15).

[b] NBLS, National Bacteriological Laboratory, Sweden.

with shaking at 37 C until the cells lysed. The lysate contained phage φ11 or φ11*vir*, depending on the phage used, and in addition phage φ11*de*. The phage φ11*de* is a hybrid between φ11 and the plasmid $P1_{258}$ which contains a gene(s) for erythromycin resistance (15). The phages were concentrated and purified as previously described for phage φ11. DNA for transfection and transformation was prepared from the purified phages containing a mixture of φ11*vir* and φ11*de* by phenol extraction (22), and the concentration of the DNA was determined as the optical density at 260 nm (OD_{260}) (22).

Transfection and transformation procedure. Transfection and transformation with phage φ11*vir*-φ11*de* DNA was carried out as previously described for transfection (22) with cells in early logarithmic

phase of growth where competence is optimal. Samples (0.1 ml) of the incubation mixture, containing cells at 10^9 colony-forming units (CFU)/ml and DNA at 10 μg/ml in competence buffer, were plated after washing and dilution in TSB medium on TSA plates. Transformants were scored after phenotypic expression for 2 h at 37 C in erythromycin agar (5 μg/ml). Transformation of the chromosomal thymine marker was performed as earlier described (20).

Isolation of ts mutants of phage ϕ11. An overnight culture of strain 8325-4 was washed twice in saline and diluted to 10 ml in TSB medium to an OD_{524} = 0.05 corresponding to about 2 × 10^7 CFU/ml. The culture was incubated at 37 C with shaking to an OD_{524} = 0.200 (10^8 CFU/ml) and then transferred to 30 C, and purified phage ϕ11 was added together with Ca^{2+} (4 mM) at a MOI of 5. After 15 min of incubation, membrane-filtered *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was added to a final concentration of 200 μg/ml. After 30 min of mutagenization the culture was diluted 1:25 in TSB medium and incubated until the cells lysed. The lysate was centrifuged and the supernatant was filtered through a 0.45-μm filter (Millipore Corp.). After dilution in TSB medium the phages were assayed with soft agar on strain 8325-4. The plates were incubated at 30 C overnight. Single plaques were then picked with sterile toothpicks to TSB medium and replicated onto two TSA plates seeded with strain 8325-4. One plate was incubated at the nonpermissive temperature 41 C and the other was incubated at the permissive temperature 30 C. Phages which failed to grow at 41 C were isolated by cutting out the lysis zones on plates incubated at 30 C. The *ts* character was confirmed and the mutants were numbered consecutively. Phage stocks of the *ts* mutants were prepared by the soft agar layer method described by Swanström and Adams (24) or by broth propagation. Yields of about 10^9 plaque-forming units (PFU)/ml were obtained.

UV inactivation of phage ϕ11. Phage ϕ11 was suspended in Novick buffer (14) containing 0.1% glycerol to a final concentration of 10^{10} PFU/ml in a 5-cm diameter petri dish and irradiated for different times. The UV irradiation was carried out with a Philips TUV 15-W GE germicidal lamp at a distance of 50 cm corresponding to a dose of 1,200 ergs/mm^2/min.

Complementation, reversion, and recombination studies with phage ϕ11 mutants. Temperature-sensitive mutants of phage ϕ11 were assayed on strain 8325-4 (ϕ11*de*) and on strain 8325-4 at both permissive and nonpermissive temperature in order to determine complementation with the deleted phage ϕ11*de* genome and the reversion of the *ts* mutants, respectively. Complementation between the *ts* mutants was determined by mixed infection of strain 8325-4 with sets of two *ts* mutants at nonpermissive temperature at a MOI of 2 to 4 for each and assayed for infectious centers immediately after dilution. Phage *ts*$\phi 11_{66}$ require adsorption at permissive temperature for complementation.

Recombination between *ts* mutants and the suppressor-sensitive mutants of phage ϕ11 was performed by mixed infection of strain 8325-4 Su^+ (2 × 10^7 CFU/ml) at a MOI of about 5 for each at permissive temperature (30 C). After 10 min of phage adsorption in TSB medium with 4 mM KCN to prevent replication (7), the culture was diluted 10^4-fold and incubated until lysis. The phage yield was determined on strain 8325-4 Su^+ at 30 C, and phage recombinants were scored on strain 8325-4 at 41 C.

Chemicals and enzymes. TSA and TSB were obtained from BBL (Cockeysville, Md). NTG was from EGA-Chemicals (Germany). 6(*p*-Hydroxyphenylazo)-uracil (HPUra) was a gift from Neal C. Brown, Department of Biochemistry, University of Massachusetts Medical School, Worcester, Mass. Rifampin was provided by LePetit, Milan-Rome (Italy). Lysostaphin was obtained from Schwarz/Mann (Orangeburg, N.Y.). Electrophoretically purified pancreatic deoxyribonuclease was from Worthington Biochemical Corp. (Freehold, N.J.).

RESULTS

Phages determining competence. It was reported earlier (22) that the phage ϕ11 genome was responsible for transfection competence in strain 8325-4 irrespective of whether it was present in a prophage or vegetative form. In the latter case it could both enhance competence at superinfection of the lysogenic host cell and induce competence as a helper phage introduced together with the DNA in the nonlysogenic host. To establish whether other prophages had the same effect, strain 8325-4 was made lysogenic for different phages which could multiply in strain 8325-4. Competence was measured by transfection with phage 80α DNA. Table 2 shows that among 15 phages tested only phage 83A besides phage ϕ11 could determine competence.

Cross-immunity between phages 83A and ϕ11. As shown in Table 2 the two phage ϕ11 and 83A belong to the same serological group. The two phages were also tested for similarities in other characters than competence and antigenicity. Phage ϕ11 and phage 83A were assayed on different indicator strains of *S. aureus* at 37 C to determine efficiency of plating (EOP). Table 3 shows that strain 8325-4 (83A) exhibits complete immunity for phage ϕ11. Strain 8325-4 (ϕ11) shows in the reverse test a 10^{-4}-fold reduction in EOP for phage 83A and the plaques observed are small. Phage 80α and phage ϕ11*vir* show moderate reduction in EOP (10^{-2}-fold) on strain 8325-4 (83A). Phage 83A does not give HFT lysates upon infection of strain 8325-4 (ϕ11*de*), indicating that this phage cannot control replication or assembly of the plasmid into particles in the same way as phage ϕ11 (15).

Competence of the strains 8325-4 (ϕ11) and 8325-4 (83A). The frequencies of transfection and transformation for the two strains 8325-4 (ϕ11)) and 8325-4 (83A) were also compared. Competence was tested by the standard procedure (see Materials and Methods) by adding DNA from phage ϕ11*vir*-ϕ11*de* to the cells. This phage carries genes for erythromycin resistance since it is a hybrid between a phage and a plasmid (15). After 10 min at 30 C in competence buffer, both the transfectants, as ϕ11*vir* plaques, and the transformants as ero^R colonies, were scored from the same incubation mixture. Spontaneous acquisition of erythromycin resistance was not observed. Table 4 shows that the transfection frequency with strain 8325-4 (ϕ11) is about 10-fold higher than that for strain 8325-4 (83A). The transformation frequencies for the two strains are about the same. The enhancement of competence by superinfection at a MOI of 30 with phage ϕ11 described earlier (22) was also observed with phage 83A. When strain 8325-4 (83A) was superinfected with phage ϕ11, no increase in transfectants was observed and the enhancement of transformation was lower than with phage 83A as superinfecting phage. The double lysogen, strain 8325-4 (83A, ϕ11) developed a lower competence level than strain 8325-4 (ϕ11). Thus phage ϕ11 is less effective in its ability to induce competence in strain 8325-4 (83A), and the reduction in transfection may depend on the relatively poorer EOP of ϕ11*vir* on this strain (see Table 3) since the transformation frequency is the same as for strain 8325-4 (ϕ11).

TABLE 3. *Efficiency of plating for different phages on different host strains*

Strain	Phage	PFU/ml	EOP
8325-4	ϕ11	5.0×10^{11}	1
8325-4	83A	5.0×10^{11}	1
8325-4	80α	2.0×10^{10}	1
8325-4	ϕ11*vir*	4.0×10^{10}	1
8325-4 (ϕ11)	ϕ11	5.2×10^{2}	10^{-9}
8325-4 (ϕ11)	83A	4.0×10^{7}	10^{-4}
8325-4 (ϕ11)	80α	2.0×10^{10}	1
8325-4 (ϕ11)	ϕ11*vir*	4.0×10^{10}	1
8325-4 (83A)	ϕ11	<3	$<5.0 \times 10^{-11}$
8325-4 (83A)	83A	<3	$<5.0 \times 10^{-11}$
8325-4 (83A)	80α	2.0×10^{8}	10^{-2}
8325-4 (83A)	ϕ11*vir*	3.0×10^{8}	10^{-2}

Effect of UV inactivation on competence induction of helper phage in nonlysogenic bacteria. It was reported earlier that strain 8325-4 (ϕ11*de*) does not show competence. This strain harbors a plasmid which is a hybrid between a penicillinase plasmid (Pl_{258}) and a deleted phage ϕ11 genome (15). The deletion of the phage genome appears to comprise a third of the genome mainly covering early regions (R. P. Novick and P. J. Kretschmer, personal communications). It was therefore of interest to determine whether competence induction only required part of the genome also when it was introduced as helper phage. UV irradiation of the helper phage was studied to assess the requirement for infectivity and lysogeny in induction of competence.

Phage ϕ11 was UV-irradiated for different times and assayed for infectivity and ability to induce competence for transfection and transformation on strain 8325-4 as described in the legend to Fig. 1. In spite of a rapid decrease in phage infectivity after irradiation, competence for transfection is slightly increased at low levels of UV irradiation. A significant increase in transformants was observed with increased

TABLE 4. *Competence of strain 8325-4 (ϕ11) and 8325-4 (83A) with and without superinfection*

Strain	Phage at a MOI of 30	CFU/ml	ϕ11*vir* transfectants (PFU)/ml[a]	Transfection frequency	ero^R transformants/ml[a,b]	Transformation frequency
8325-4 (ϕ11)		1.1×10^{9}	5.2×10^{4}	5.0×10^{-5}	3.0×10^{2}	3.0×10^{-7}
8325-4 (ϕ11)	ϕ11	1.1×10^{9}	1.2×10^{6}	1.0×10^{-3}	1.0×10^{4}	1.0×10^{-5}
8325-4 (ϕ11)	83A	1.1×10^{9}	2.3×10^{6}[c]	2.0×10^{-3}	2.2×10^{4}	2.0×10^{-5}
8325-4 (83A)		1.5×10^{9}	4.0×10^{3}	2.7×10^{-6}	1.9×10^{2}	1.2×10^{-7}
8325-4 (83A)	ϕ11	1.5×10^{9}	3.7×10^{3}	2.5×10^{-6}	5.1×10^{3}	3.4×10^{-6}
8325-4 (83A)	83A	1.5×10^{9}	2.8×10^{5}	1.9×10^{-4}	5.0×10^{4}	3.3×10^{-5}

[a] ϕ11*vir*-ϕ11*de* DNA at 10 μg/ml was added for 10 min at the time of superinfection.
[b] ero^R, Erythromycin resistance.
[c] Indicator strain 8325-4 (ϕ11, 83A).

UV doses. All transformants obtained with untreated helper phage were lysogenized (20), but nine of ten transformants obtained after infection with phages UV-irradiated for 10 min lysed after prolonged incubation. The delayed lysis may suggest that the cells were not lysogenized with the irradiated phage.

Characteristics of ts mutants of phage ϕ11. Since only part of the viral genome appears to determine competence, we isolated mutants of phage ϕ11. Four of the *ts* mutants obtained after NTG treatment have been further characterized. Table 5 shows the designation of the isolated *ts* mutants and their reversion frequency. Results from complementation tests among the mutants and with strain 8325-4 (ϕ11*de*) are also reported. Four complementation groups were obtained. Phage *ts*$\phi 11_{31}$ and *ts*$\phi 11_{65}$ did not complement with phage ϕ11*de*. Phage *ts*$\phi 11_{65}$ requires permissive temperature for adsorption.

Recombination between ts and sus mutants of phage ϕ11. Kretschmer and Egan (manuscript in preparation) have established a circular genetic map of phage ϕ11 involving the 10 *sus* genes earlier described (10). The *ts* mutants were mapped by recombination with the suppressor-sensitive mutants. Table 6 shows the recombination frequencies obtained in crosses *sus* × *ts* (see Material and Methods). Phage *ts*$\phi 11_{28}$ mapped both close to genes M and Q. Phage *ts*$\phi 11_{91}$ appears in a region between U and X, and phage *ts*$\phi 11_{31}$ appears close to gene A. Phage *ts*$\phi 11_{65}$ recombined in low frequencies with all amber mutants and was not mapped further.

The position of the *ts* mutants on the ϕ11 map presented by Kretschmer and Egan (manuscript in preparation) is shown in Fig. 2. They proposed that ϕ11*de* probably is deleted in the region of the map from between U and X to between E and H. Our complementation results with phage ϕ11*de* (Table 5) agree with this proposal.

Competence of strain 8325-4 lysogenic for ts mutants of ϕ11. The four newly isolated *ts* mutants of phage ϕ11 were used to lysogenize strain 8325-4, and the lysogens were tested for transformation and transfection at permissive and nonpermissive temperatures. Inocula from TSA plates incubated overnight at 41 C were grown in TSB medium at 30 and 41 C. When the cultures reached OD_{524} = 0.100, competence for transfection and transformation was tested at a cell density of 4 × 10^8 CFU/ml with ϕ11*vir*-ϕ11*de* DNA. DNA uptake was terminated after 10 min of incubation at 30 and 41 C,

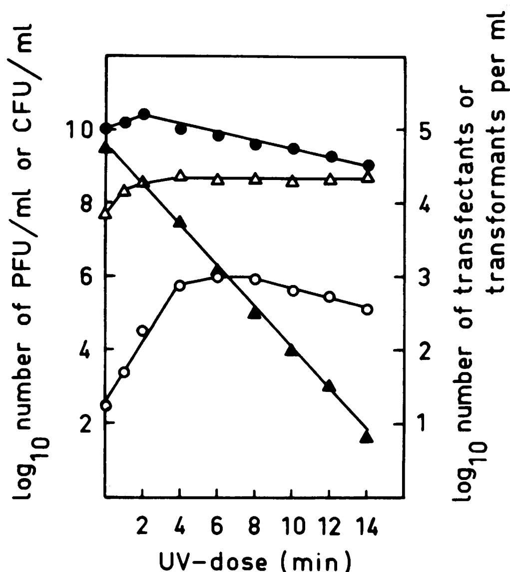

FIG. 1. *Effect of UV inactivation of the helper phage ϕ11 on competence induction. Phage ϕ11 was UV-irradiated in Novick phage buffer containing 0.1% glycerol for different times. A 0.2-ml amount of irradiated phage suspension was added to 1.8 ml of S. aureus strain 8325-4 (7 × 10^8 CFU/ml) in competence buffer. The mixture was immediately divided into two parts, A and B. To part A phage ϕ11vir DNA (10 µg/ml) was added, and to part B phage ϕ11-ϕ11de DNA was added. After 10 min of incubation at 30 C and DNase treatment (150 µg/ml) for 5 min, samples from A were assayed for transfectants and from B plated for ero^R transformants/ml. Symbols: ●, transfectants/ml (ϕ11vir PFU/ml); ○, transformants/ml (ero^R/ml); ▲, ϕ11 PFU/ml; △, viable count (CFU/ml) after 10 min of contact between bacteria and phage ϕ11.*

respectively, by addition of deoxyribonuclease (DNase) (150 µg/ml).

Spontaneous release of infective phage particles by the lysogens was tested at the different temperatures concurrently. Table 7 shows that phage *ts*$\phi 11_{31}$ can neither induce competence nor synthesize phages at the nonpermissive temperature. The other *ts* mutants *ts*$\phi 11_{28}$, *ts*$\phi 11_{65}$, *ts*$\phi 11_{91}$ can induce competence at 41 C, but no phages are released. However, when growth medium from strain 8325-4 (*ts*$\phi 11_{28}$) incubated at 41 C was assayed on strain 8325-4 at 30 C, plaques were obtained which may suggest that *ts*$\phi 11_{28}$ has at least one renaturable defect in a structural protein. The competence level reached with strain 8325-4 lysogenic with the *ts* mutants of phage ϕ11 are about 6 to 10 times lower than for the wild type at permissive temperature (30 C). The number of transfected and transformed cells are always lower at 41 C

TABLE 5. *Characteristics of the ts mutants of phage φ11*

Phage	Reversion frequency[a]	Complementation with phage φ11*de* at 41 C	Complementation groups	Tentative characters
ts $\phi 11_{28}$	$\approx 2 \times 10^{-6}$	+	I	Late
ts $\phi 11_{31}$	$<2 \times 10^{-9}$	−	II	Early
ts $\phi 11_{65}$	$\approx 10^{-7}$	−	III[b]	Small plaques, late[c]
ts $\phi 11_{91}$	$\approx 4 \times 10^{-8}$	+	IV	Late

[a] Reversion frequency was determined on strain 8325-4 by assaying phage stocks obtained from single plaque isolates at 30 and 41 C.
[b] Adsorption of *ts* $\phi 11_{65}$ at 30 C.
[c] Strain 8325-4 (*ts* $\phi 11_{65}$) lyses upon UV induction at 41 C (data not published).

TABLE 6. *Recombination between suppressor-sensitive φ11 mutants and the ts mutants of phage φ11*

Phage cross *sus* × *ts*	Recombination (%)[a]		
sus	ts_{28}	ts_{31}	ts_{91}
A 4	6.0	0.016	2.6
E 64	44.0	20.0	8.0
H 47	15.4	6.0	6.0
M 28	1.2	4.0	8.0
O 43	1.8	2.0	6.0
P 68	4.0	8.0	4.0
Q 54	1.0	8.8	10.0
U 53	13.2	3.0	0.8
X 27	7.0	0.14	1.4

[a] Percent recombination was calculated as 200 × [(PFU/ml on strain 8325-4 Su^- at 41 C)/(PFU/ml on strain 8325-4 Su^+ at 30 C)].

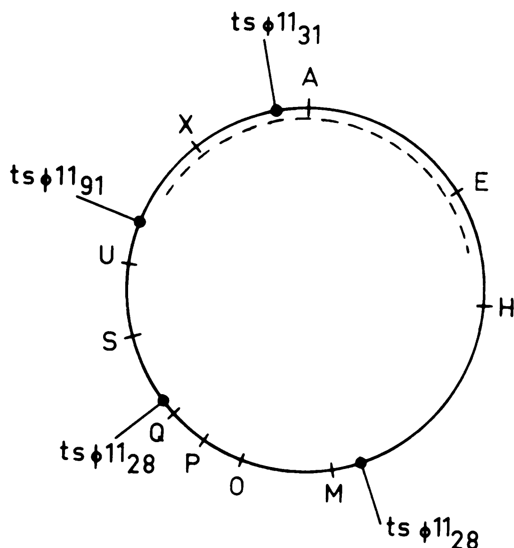

FIG. 2. *Map of phage φ11 showing the 10 sus genes and the tentative positions of the ts mutants of phage φ11. The dashed line shows the region deleted in the phage φ11de genome according to Novick and Kretschmer (personal communications).*

because of the temperature effect on DNA uptake (22).

Temperature sensitivity for competence and spontaneous phage release of the ts mutants of phage φ11. The temperature profile for competence and phage multiplication was determined for the *ts* mutants by growing strain 8325-4 lysogenic for these mutants in TSB medium with shaking at different temperatures. Inocula were taken from a TSA plate incubated at 41 C overnight. When the cultures reached OD_{524} = 0.100, competence was tested by adding φ11*vir* DNA. After incubation and DNase treatment, samples were assayed for transfectants. Spontaneously released phages were determined at the same time in a separate sample. Figure 3 shows the profiles obtained for strain 8325-4 lysogenic for $ts\phi 11_{31}$ (A), for φ11 wild type (B), and for $ts\phi 11_{65}$ (C). Three different profiles for competence and phage multiplication were observed: (i) neither competence nor spontaneous phage release at 41 C and a rapid decline in both functions at temperatures above 35 C; (ii) temperature independence of competence and phage release; (iii) temperature independence of competence coupled with temperature sensitivity of phage release. These experiments infer that at least one phage gene product is necessary for competence, whereas infective particles do not appear to be required.

Effect of superinfection on competence. Superinfection with phage φ11 has earlier been shown to enhance competence for transfection (22). Table 4 shows that phage 83A also can increase competence by superinfection. This phenomenon was examined further since the increase in competence by superinfection may suggest a helper phage mechanism involving penetration of DNA. Temperature-sensitive

TABLE 7. *Competence and phage production of strain 8325-4 lysogenic for ts mutants at permissive and nonpermissive temperatures*

Strain	Temp (C)	Free phages in culture medium (ϕ11 PFU/ml)	ϕ11 *vir* transfectants (PFU)/ml	Transfection frequency	*ero*^{R} [a] transformants/ml	Transformation frequency
8325-4 (ϕ11)	30	2.5×10^5	1.4×10^5	4.7×10^{-4}	3.2×10^3	1.0×10^{-5}
8325-4 (ϕ11)	41	1.2×10^5	8.0×10^4	2.7×10^{-4}	1.2×10^3	4.0×10^{-6}
8325-4 ($ts\phi11_{28}$)	30	2.2×10^5	2.8×10^4	4.0×10^{-5}	9.0×10^2	1.3×10^{-6}
8325-4 ($ts\phi11_{28}$)	41	<3[b]	1.0×10^4	2.0×10^{-5}	4.0×10^2	6.4×10^{-7}
8325-4 ($ts\phi11_{31}$)	30	4.2×10^4	3.4×10^4	1.1×10^{-4}	9.5×10^2	3.2×10^{-6}
8325-4 ($ts\phi11_{31}$)	41	<3	<3	$<3.0 \times 10^{-8}$	<3	$<3.0 \times 10^{-8}$
8325-4 ($ts\phi11_{65}$)	30	9.0×10^4	3.7×10^4	8.8×10^{-5}	1.2×10^3	2.8×10^{-6}
8325-4 ($ts\phi11_{65}$)	41	<3	1.2×10^4	2.6×10^{-5}	7.0×10^2	2.3×10^{-6}
8325-4 ($ts\phi11_{91}$)	30	8.4×10^4	1.2×10^4	6.0×10^{-5}	3.0×10^2	1.0×10^{-6}
8325-4 ($ts\phi11_{91}$)	41	<3	9.0×10^3	2.8×10^{-5}	1.0×10^2	3.3×10^{-7}

[a] *ero*^{R}, Erythromycin resistance.
[b] 10^4 PFU/ml was obtained at assay performed at 30 C.

mutants of phage ϕ11 were therefore compared with the wild type with regard to their effect on competence when superinfecting at permissive and nonpermissive temperatures. The results are shown in Table 8 where the number of transfected (A) and transformed (B) cells obtained at 41 C without superinfection are given the value of one and the results under other conditions are normalized to this value. Phages ϕ11 and $ts\phi11_{28}$ induce a 30- to 100-fold increase in competence at superinfection at both temperatures. Phage $ts\phi11_{31}$, however, failed to induce competence upon superinfection regardless of temperature. The lack of enhancement at permissive temperature is unexpected and is inconsistent with results obtained when the phage $ts\phi11_{31}$ is used as helper phage at permissive temperature (see below), but may possibly be due to partial inhibition of expression of the competence gene(s) at superinfection in lysogenic host cells. Two unrelated phages (phages 47 and 75) which cannot induce competence in *S. aureus* were also tested for superinfection enhancement. Strain 8325-4 (ϕ11) lysogenized with these two phages was tested for competence after superinfection, but these two phages which lack ability to generate competence as prophage also failed to induce competence upon superinfection. Thus competence induction by ϕ11 and 83A is a specific mechanism.

Effect of inhibitors on phage multiplication. To define the role of the phage genome during expression of competence, we evaluated the effect of inhibitors of macromolecular synthesis. These inhibitors were first

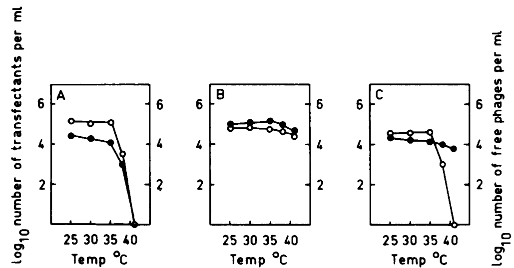

FIG. 3. *Temperature profiles for competence induction and phage multiplication of different S. aureus strains, (A) 8325-4 ($ts\phi11_{31}$); (B) 8325-4 (ϕ11); (C) 8325-4 ($ts\phi11_{91}$). Cultures were grown in TSB medium at different temperatures to a cell density of about 10^8 CFU/ml. Competence tests were then performed by adding phage ϕ11vir DNA. After 10 min of incubation at respective temperatures and DNase treatment (150 µg/ml) for 5 min, samples were assayed for transfectants. Free ϕ11 phages in culture were determined on strain 8325-4. Symbols: O, ϕ11 PFU/ml; ●, transfectants/ml determined as PFU/ml of ϕ11vir.*

studied on the lytic cycle of phage ϕ11. Brown (2) has studied the effect of HPUra on DNA synthesis of the host strain 8325-4 and of phage ϕ11-M15, a clear-plaque mutant of phage ϕ11. It was first established that HPUra, chloramphenicol, and rifampin (28) completely inhibit phage multiplication of phage ϕ11 in strain 8325-4 (Fig. 4). Almost 100% of the cells were infected after 10 min of incubation, but no progeny phages were released in the presence of the inhibitors. After the cells were washed free of the drug and further incubated in TSB

TABLE 8. *Comparison of competence at superinfection of strain 8325-4 (φ11) with phage φ11 and ts mutants of phage φ11 at permissive and nonpermissive temperatures*

Strain	Phage	MOI	Temp (C)	Ratio A_s/A_o [a]	Ratio B_s/B_o [b]	Frequency A	Frequency B
8325-4 (φ11)			30	3	3	7.0×10^{-5}	1.0×10^{-6}
8325-4 (φ11)			41	1	1	1.3×10^{-5}	3.3×10^{-7}
8325-4 (φ11)	φ11	25	30	350	120	5.0×10^{-3}	4.0×10^{-5}
8325-4 (φ11)	φ11	25	41	140	50	1.8×10^{-3}	1.7×10^{-5}
8325-4 (φ11)	$ts\phi 11_{28}$	10	30	72	28	1.0×10^{-3}	9.0×10^{-6}
8325-4 (φ11)	$ts\phi 11_{28}$	10	41	31	10	4.0×10^{-4}	3.3×10^{-6}
8325-4 (φ11)	$ts\phi 11_{31}$	25	30	3	2	7.0×10^{-5}	1.0×10^{-6}
8325-4 (φ11)	$ts\phi 11_{31}$	25	41	1	1	1.4×10^{-5}	7.0×10^{-7}

[a] A_o = transfectants per milliliter without superinfection at 41 C; A_s = transfectants per milliliter under test conditions.
[b] B_o = transformants per milliliter without superinfection at 41 C; B_s = transformants per milliliter under test conditions.

medium in each case, the cells lysed and the phage yields were the same as for the control culture without drug. Viable counts of uninfected cells were not affected after reversion of treatment with the inhibitors except with rifampin which reduced viable counts by a factor of 2 to 3 (not shown). Thus, HPUra, rifampin, and chloramphenicol could be used as inhibitors of DNA, ribonucleic acid (RNA), and protein synthesis, respectively, and they were all reversible with regard to phage replication.

Superinfection in presence of inhibitors. The inhibitors were used in superinfection experiments to determine whether DNA, RNA, or protein synthesis are required for enhancement of competence. Strain 8325-4 (φ11) (2×10^8 CFU/ml) was superinfected at the competence maximum at a MOI of 25 with phage φ11 with chloramphenicol, rifampin, and HPUra present at the concentrations 75 μg/ml, 0.1 μg/ml, and 100 μM, respectively. φ11*vir* DNA was added, and, after incubation and DNase treatment, the infected cells were washed three times and assayed for transfected cells. Table 9 shows that chloramphenicol and rifampin prevent the increase in competence usually seen upon superinfection. Indeed, the inhibitors depressed the level of transfection even in the nonsuperinfected controls. HPUra gave a 10-fold increase of the competence level compared with 50-fold for the control without inhibitor. Thus protein and possibly RNA synthesis, but not DNA synthesis, appears to be required for expression of the phage gene(s) involved in enhancement of competence after superinfection.

Competence in nonlysogenic bacteria with ts helper phages. Competence in *S. aureus* can be transferred by growth medium from a

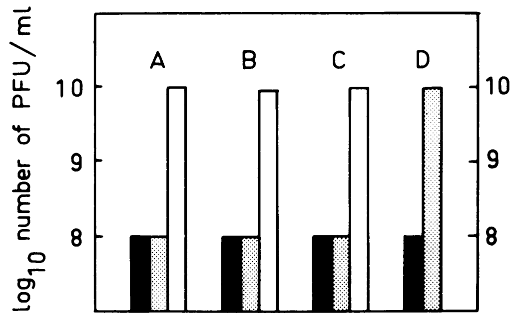

FIG. 4. *Effect of inhibitors on the synthesis of phage φ11 by S. aureus strain 8325-4. At zero time, drug and phage φ11 was added, the latter at a MOI of 5 to 2 × 10⁸ CFU/ml in the presence of 4 × 10⁻³ M CaCl₂. After 10 min of adsorption at 30 C the infected cells were separated from unadsorbed phages by centrifugation and resuspended in the same volume of TSB medium with the inhibitor present. The number of infected cells was determined on strain 8325-4. Incubation was continued at 37 C, and after 120 min the total number of PFU/ml was determined. Treated bacteria were washed three times and incubated to lysis in TSB medium. Symbols: A, rifampin (0.1 μg/ml); B, chloramphenicol (75 μg/ml); C, 6(p-hydroxyphenylazo)-uracil (100 μM); D, control; ■, number of infected cells (PFU/ml) after 10 min; ▨, total number of PFU/ml after 120 min; □, total number of PFU/ml after reversion.*

competent lysogenic strain, 8325-4 (φ11), to a noncompetent strain, 8325-4. This transfer was accomplished by the phage φ11 (22). Since *ts* mutants were available, induction of competence in nonlysogenic cells was investigated in detail. Strain 8325-4 (8×10^8 CFU/ml), equilibrated at permissive and nonpermissive temperature, was infected with phage φ11 and phage $ts\phi 11_{31}$ at a MOI of 0.08 and DNA

prepared from phage ϕ11*vir* was added concurrently. After incubation and DNase treatment at the respective temperatures, samples were assayed for transfectants on strain 8325-4 (ϕ11). Table 10 shows that the wild type of phage ϕ11 can act as helper with about equal efficiency at both temperatures. The small difference observed may depend on temperature-dependent DNA uptake (22). Phage *ts*$\phi 11_{31}$ cannot act as helper at nonpermissive temperature (41 C), but can induce competence at 30 C albeit at a fivefold-lower level than the wild type.

Transfer of competence between cells in mixed cultures. Competence factors of protein character have been identified and purified in bacterial systems (3, 17, 27). Competence can be transferred to noncompetent strains.by treating the cells with these factors before addition of DNA. Transfection and transformation in *S. aureus* are mediated by phage ϕ11, which acts as a competence factor (20, 21). *S. aureus* appears to be the only system where a phage-coded product induces competence. Mixed cultures of competent and noncompetent cells were used to establish whether products other than the phage or cell-to-cell contact could mediate competence. Two streptomycin-resistant strains 8325-4 str^R and 8325-4 (*ts*$\phi 11_{31}$)str^R were isolated to permit identification of donor and recipient in mixed cultures. Donor and recipient strains at the same cell density were incubated at nonpermissive temperature (41 C). When the mixed cultures reached an OD_{524} = 0.100 (5 × 10^7 CFU/ml), competence for transformation was assayed with ϕ11-ϕ11*de* DNA. Erythromycin transformants were scored with and without streptomycin in the medium. Spontaneously released phages were assayed on strain 8325-4 at both temperatures. Table 11 shows that only strains lysogenized with genomes allowing phage release induce competence in strain 8325-4 and 8325-4 (ts$\phi 11_{31}$). Thus only strain 8325-4 (ϕ11) and 8325-4 (*ts*$\phi 11_{28}$) can transfer competence to the noncompetent strains at 41 C, resulting in colonies resistant to both erythromycin and streptomycin. Cell-to-cell contact or extracellular products besides phage ϕ11 cannot therefore transfer competence between competent and noncompetent cells.

Competence at temperature-shift experiments with strain 8325-4 (ts$\phi 11_{31}$). The time required for development of competence was determined by temperature-shift experiments from nonpermissive to permissive conditions with strain 8325-4 (*ts*$\phi 11_{31}$). Figure 5 shows that competence is immediately expressed at the shift and the maximum level is reached after 45 min of incubation at the permissive temperature. No transfectants or transformants were obtained in the control kept at 41 C. Figure 6 shows the results obtained at a shift-up experiment with strain 8325-4 (ϕ11) and strain 8325-4 (*ts*$\phi 11_{31}$). The number of competent cells are reduced about 40 times during 1 h and 45 min for strain 8325-4 (*ts*$\phi 11_{31}$) after the shift. Only a twofold reduction was observed for the control

TABLE 9. *Superinfection of strain 8325-4 (ϕ11) with phage ϕ11 in presence of inhibitors of macromolecular synthesis*

Strain	MOI	Inhibitor[a]	ϕ11 *vir* transfectants (PFU)/ml	Transfection frequency	A_s/A_o[b]
8325-4 (ϕ11)			1.5×10^3	1.5×10^{-5}	1
8325-4 (ϕ11)	25		8.0×10^4	8.0×10^{-4}	53
8325-4 (ϕ11)	25	CM	8.0×10^2	8.0×10^{-6}	0.5
8325-4 (ϕ11)	25	Rif	9.0×10^2	1.8×10^{-5}	0.6
8325-4 (ϕ11)	25	HPUra	1.6×10^4	1.6×10^{-4}	11

[a] CM, Chloramphenicol at 75 μg/μliter; Rif, rifampin at 0.1 μg/ml; HPUra, 6(*p*-hydroxyphenylazo)-uracil at 100 μM.

[b] A_o = transfectants per milliliter without superinfection; A_s = transfectants per milliliter with superinfection.

TABLE 10. *Transfection of strain 8325-4 with helper phage at permissive and nonpermissive temperatures*

Strain	Helper phage	MOI	CFU/ml	Temp (C)	ϕ11 *vir* transfectants (PFU)/ml
8325-4	ϕ11	0.08	8.0×10^8	30	1.0×10^3
8325-4	ϕ11	0.08	8.0×10^8	41	3.0×10^2
8325-4	*ts*$\phi 11_{31}$	0.08	8.0×10^8	30	2.2×10^2
8325-4	*ts*$\phi 11_{31}$	0.08	8.0×10^8	41	<3

TABLE 11. *Transfer of competence in mixed cultures at nonpermissive temperature*

Strains	ero^R [a] transformants/ml		Free phages (φ11 PFU/ml) in culture medium assayed at	
	Plated on TSA with erythromycin	Plated on TSA with erythromycin and streptomycin	30 C	41 C
8325-4 (φ11)	8.4×10^2	<3	2.1×10^5	2.5×10^5
8325-4 ($ts\phi11_{91}$)	2.6×10^2	<3	2.0×10^1	<3
8325-4 ($ts\phi11_{65}$)	1.0×10^2	<3	<3	<3
8325-4 ($ts\phi11_{28}$)	6.5×10^2	<3	1.0×10^4	<3
8325-4 ($ts\phi11_{31}$) str^R [b]	<3	<3	<3	<3
8325-4 str^R	<3	<3	<3	<3
8325-4 (φ11) + 8325-4 str^R	1.1×10^3	7.0×10^2	2.1×10^4	2.4×10^4
8325-4 (φ11) + 8325-4 ($ts\phi11_{31}$) str^R	9.2×10^2	4.4×10^2	1.2×10^4	1.0×10^4
8325-4 ($ts\phi11_{91}$) + 8325-4 str^R	1.7×10^2	<3	2.0×10^1	<3
8325-4 ($ts\phi11_{91}$) + 8325-4 ($ts\phi11_{31}$) str^R	1.2×10^2	<3	<3	<3
8325-4 ($ts\phi11_{65}$) + 8325-4 str^R	1.0×10^2	<3	<3	<3
8325-4 ($ts\phi11_{65}$) + 8325-4 ($ts\phi11_{31}$) str^R	1.1×10^2	<3	<3	<3
8325-4 ($ts\phi11_{28}$) + 8325-4 str^R	3.6×10^2	1.0×10^2	1.7×10^3	<3
8325-4 ($ts\phi11_{28}$) + 8325-4 ($ts\phi11_{31}$) str^R	9.0×10^2	6.0×10^2	1.2×10^3	<3

[a] Erythromycin resistance at 5 μg/ml.
[b] Streptomycin resistance at 75 μg/ml.

strain, 8325-4 (φ11). The half-life of the competence factor is calculated to about 10 to 15 min from this experiment.

Effect of inhibitors on competence induction of the prophage. The pattern of macromolecular synthesis required for competence induction was established with inhibitors at temperature shifts from nonpermissive to permissive conditions with strain 8325-4 ($ts\phi11_{31}$). Figure 7 shows that synthesis of the phage-directed product starts immediately after the shift to permissive conditions in the control (see also Fig. 5). No competence was developed when inhibitors of RNA and protein synthesis were present, which agree with the results obtained at superinfection of strain 8325-4 (φ11) with phage φ11 where both RNA and protein synthesis were required for induction of competence (Table 9). In contrast to the results with superinfection, it appears that the prophage requires DNA synthesis before it can induce competence, and in separate experiments it has been ascertained that HPUra does not inhibit competence induced by superinfection under identical conditions (not shown).

DISCUSSION

The requirement for lysogeny with φ11 for expression of transfection and transformation competence in *S. aureus* has previously been established (20, 22). The present study attempts to delineate the role of the phage genome in competence. Phage φ11 and also phage 83A can induce competence regardless of whether the phage is present as prophage or in a vegetative form. In the lytic cycle the phage could be introduced either as a helper phage in nonlysogenic bacteria or as a superinfecting phage in immune bacteria and in both cases confer competence.

It was of importance to establish that helper or superinfecting phage did not act by simply facilitating the uptake and assimilation of transfecting DNA. The fact that the *ts* mutant ($ts\phi11_{31}$) (Table 10) did not function as a helper phage at nonpermissive temperature and that the serologically unrelated phages 47 and 75 failed to induce competence at superinfection appears to rule out a direct helper effect at the cell envelope. In addition, the requirement for both RNA and protein synthesis (Table 9) prior to expression of competence at superinfection suggests that the phage genome must be expressed before competence is established. It appears safe to conclude, therefore, that the phage φ11 genome must contain the genes responsible for induction of competence in *S. aureus*. It has recently been suggested that the

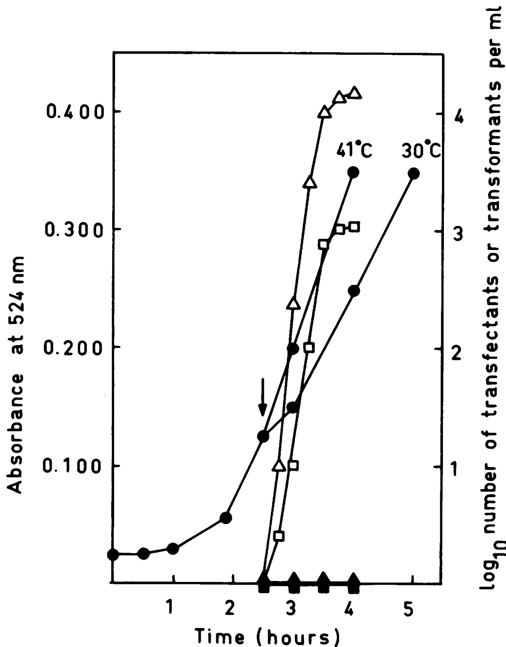

FIG. 5. *Competence induction in S. aureus strain 8325-4 (tsφ11₃₁) after shift to permissive temperature. Strain 8325-4 (tsφ11₃₁) was incubated in TSB medium at nonpermissive conditions to OD_{524} = 0.125. Half the culture was moved to 30 C; the other half was maintained at 41 C. Competence tests were performed at zero time and at intervals of 15 min after the shift indicated by the arrow. Cell density was adjusted and phage φ11vir-φ11de DNA (10 μg/ml) was added to the bacteria in competence buffer. After 10 min of incubation and DNase treatment (150 μg/ml) for 5 min at respective temperatures, samples were assayed for transfectants and scored for transformants. Symbols: Δ, transfectants/ml (φ11vir PFU/ml) at 30 C; □, transformants/ml (ero^R/ml) at 30 C; ●, optical density at 524 nm; ▲, transfectants/ml (φ11vir PFU/ml) at 41 C; ■, transformants/ml (ero^R/ml) at 41 C.*

phage φ11 genome is required also for methicillin transduction in *S. aureus* (5).

The phage genome could possibly increase competence by a marker rescue mechanism since phage φ11*vir* DNA was used to score transfection. The prophage, the helper or the superinfecting phage would then rescue genes introduced with the transfecting DNA. However, since both transformation of erythromycin resistance and transfection increase similarly at superinfection, it is unlikely that marker rescue is involved and the enhancement of competence by superinfection could probably be regarded as a gene dose effect. The enhancement of competence induction at superinfection was previously not observed when plasmid characters were transformed at higher frequency than observed here (20). This may be due to the level of competence in the recipient before infection, since superinfection can only enhance transformation competence up to a maximal frequency of 10^{-5} (data not shown). Recently it has also been established that the thymine marker is transformed at increased frequency after superinfection (data not shown), which also favors a gene dose effect.

The strain 8325-4 (φ11*de*) harboring a plasmid which is a hybrid between a compatibility group I plasmid and the phage φ11, and which appears to lack both the early (Novick, personal

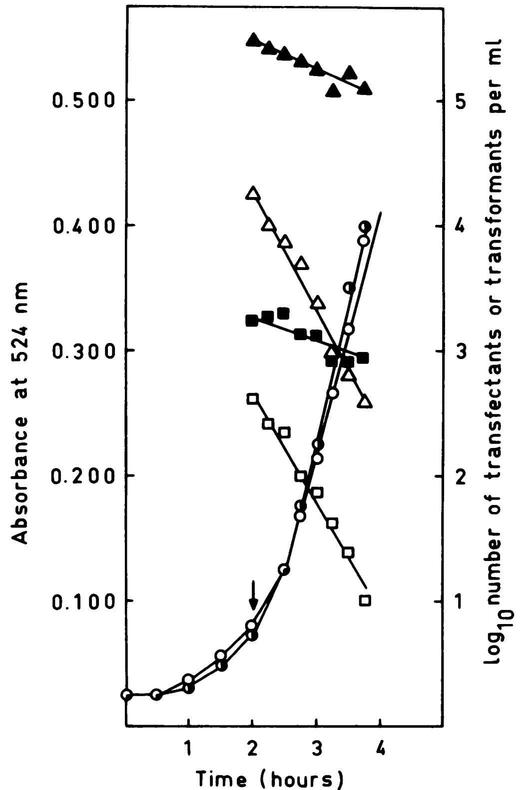

FIG. 6. *Decrease in competence after shift to nonpermissive temperatures of S. aureus strain 8325-4 (φ11) and 8325-4 (tsφ11₃₁). The strains were grown in TSB medium at 30 C. At OD_{524} = 0.075 the cultures were moved to nonpermissive conditions (41 C) and cell samples, adjusted to the same density, were taken at zero time and 15-min intervals after the shift indicated by the arrow. The competence level was measured by adding phage φ11vir-φ11de DNA. DNA incubation was performed at 41 C for all samples and after DNase treatment the number of transfectants and transformants was determined. Symbols: ▲, transfectants/ml (φ11vir PFU/ml); ■, transformants/ml (ero^R/ml); O, optical density at 524 nm for strain 8325-4 (φ11); Δ, transfectants/ml (φ11vir PFU/ml); □, transformants/ml (ero^R/ml); ◑, optical density at 524 nm for strain 8325-4 (tsφ11₃₁).*

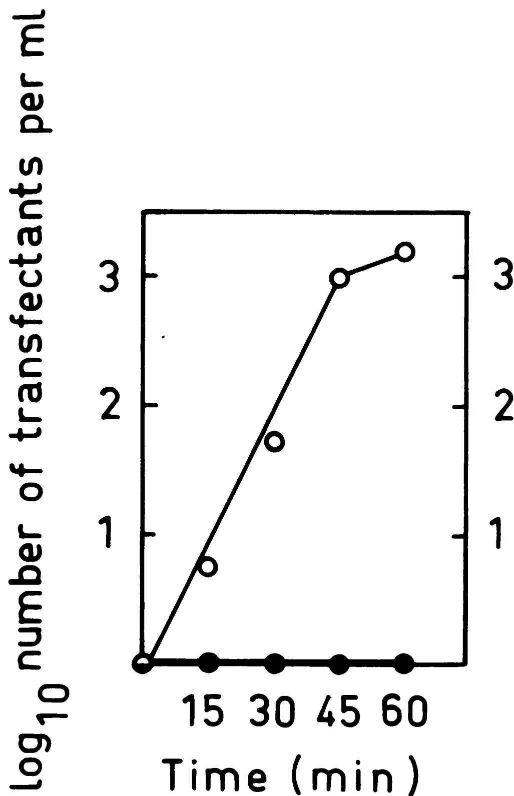

FIG. 7. *Effect of inhibitors on competence induction after shift to permissive temperatures of S. aureus strain 8325-4 (tsφ11₃₁). At zero time the culture was transferred from nonpermissive to permissive temperature (30 C) and the competence was immediately measured by adding phage φ11vir DNA (10 μg/ml). After 10 min of incubation and DNase treatment (150 μg/ml) for 5 min at respective temperatures, samples were assayed for transfectants. At the shift the culture was divided into four portions containing 0.1 μg of rifampin per ml, 75 μg of chloramphenicol per ml, 100 μM HPUra, and no addition, respectively. Samples were taken for test of competence at intervals of 15 min by adding phage φ11vir DNA (10 μg/ml) to the bacteria in competence buffer with drug still present. The number of CFU/ml was adjusted to 5 × 10^7 CFU/ml in all samples before DNA addition. After incubation and DNase treatment, the bacteria were washed three times in saline before reversion in TSB medium. Samples were then assayed for transfectants. Symbols: ○, transfectants/ml (φ11vir PFU/ml) in the control sample, ●, transfectants/ml (φ11vir PFU/ml) in samples with inhibitors.*

communication) and some late (Kretschmer and Egan, manuscript in preparation) genes of the phage genome, cannot express competence (22). Therefore, only a part of the phage φ11 genome appears to be required to establish competence. The UV inactivation of the helper phage (Fig. 1) gave an enhancement of transformation competence at a dose where phage infectivity was drastically reduced. Recombination experiments between the *ts* mutants and the phage φ11 *sus* mutants of Kretschmer and Egan (10) suggest that the phage *ts*φ11₃₁ which is temperature sensitive for competence is an early mutant of the phage. This early region is probably absent in phage φ11*de* since *ts*φ11₃₁ could not complement with phage φ11*de*. The position of the *ts*φ11₃₁ gene on the phage φ11 map (Fig. 2) close to the early *sus* mutant A also suggests that an early gene is involved. It may therefore be concluded that competence induction resides in the phage φ11 genome and probably in an early gene which may not be under repressor control at lysogeny because of the maximal 100-fold enhancement observed at superinfection.

The expression of the phage gene(s) controlling competence shows several interesting features. In the prophage state it appears that both DNA and protein synthesis are required before competence is expressed in temperature-shift experiments to the permissive temperature of the phage *ts*φ11₃₁ (Fig. 7). This may suggest that the competence factor itself is not a *ts* protein but that the synthetic mechanism is temperature dependent. An alternative and more likely explanation would be that the prophage of phage φ11 must replicate in order to be transcribed and expressed, which might only occur once during each cell cycle. It was previously reported that the competence pattern during growth of 8325-N *nuc* was complex with new competence peaks at intervals in the growth cycle (22). Assuming that the growth was comparatively synchronous in these experiments, the competence peak may reflect the increase in competence at replication of the prophage. It has been proposed that DNA replication is required for uptake and integration of transforming DNA in *Bacillus subtilis* (8), but more recent experiments with HPUra have established that DNA replication is not mandatory (6). A requirement for DNA synthesis has been established for the expression of genes in eukaryotes, where synthesis of histones is confined to a distinct period (the S-phase) of the cell cycle (19). In prokaryotes no conclusive evidence is available to indicate requirement for DNA replication for gene expression although it has not been ruled out in production of deletions in the chromosome of *E. coli* (23).

Provided that the gene(s) responsible for competence in the prophage is only expressed

once per cell cycle, it is easier to understand the effect of superinfection which may provide a template for transcription in phage ϕ11 lysogens where the DNA is not available for transcription. This will lead to an increased gene dose enhancing the competence. In fact, the maximal frequency for transfection after superinfection is around 10^{-3} and 10^{-5} for transformation. Competence is enhanced 30- to 100-fold by superinfection. Neither the superinfecting phage nor the helper phage requires DNA synthesis to induce competence, but protein synthesis is necessary. The competence is rapidly established as revealed by temperature shift-down experiments, and the induction by helper phage in the nonlysogens requires less than 2 min (not shown).

The character of the competence factor induced by the phage gene has not yet been established, but experiments to identify the product are in progress. The temperature shift-up experiments suggest a half-life for competence of about 10 to 15 min, but it is not clear whether this is due to the short half-life of the competence factor itself or whether it reflects the half-life of a temperature-sensitive event in the synthetic pathway leading to competence.

In conclusion, the results suggest that the phage ϕ11 genome contains an early gene which can induce competence for transfection and transformation in *S. aureus* strain 8325-4. This gene can be expressed by the prophage, helper phage, or after superinfection of immune bacteria, suggesting that the gene is not under repressor control. Expression of competence appears to require DNA and protein synthesis by the prophage, but only protein synthesis by helper or superinfecting phage, suggesting that the prophage gene(s) can only be expressed during DNA replication.

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