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# Cloning of Restriction Fragments of DNA from Staphylococcal Bacteriophage φ11

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*Eco*RI fragments of *Staphylococcus aureus* bacteriophage φ11 DNA were cloned in vector plasmid pSA2100 in *S. aureus*. The clones were analyzed in marker rescue experiments with suppressor- and temperature-sensitive mutants of φ11 to correlate the genetic and physical maps of the phage DNA. Several mutants could be identified on the physical map, and a clone containing fragment *Eco*RI-B of φ11 DNA expressed immunity to phage infection. In addition, it was found that recombinant plasmids containing φ11 DNA sequences can be transferred by high-frequency transduction after phage φ11 infection of the host cells.

Bacteriophage φ11 is a generalized transducing phage present as a prophage in *Staphylococcus aureus* 8325 (9). A preliminary characterization of the structure of the phage particle and the mature DNA has been reported (1). In the accompanying paper (7) we performed a detailed analysis of the mature DNA. Restriction enzyme maps of the mature, prophage, and replicative DNAs revealed that the mature DNA is circularly permuted. A genetic map of phage φ11 DNA was established by Kretschmer and Egan, who concluded that the map probably was circular (4).

The biology of phage φ11 has been studied from several different aspects. Novick (9) isolated a natural hybrid between phage φ11 and plasmid pI258, referred to as plasmid φ11*de*. It contains the *erm* and *mcr* genes of pI258 and late genes of phage φ11 (4, 9). Phage φ11 produces high-frequency transduction (HFT) lysates for the antibiotic resistance marker of the plasmid upon infection of a host harboring plasmid φ11*de*.

Sjöström and Philipson (11) studied the role of the φ11 genome in establishing and maintaining competence for transformation of *S. aureus*. There appears to be a requirement for lysogeny or infection with staphylococcal phages belonging to serological group B in order to establish competence. Cohen et al. (2) found that methicillin resistance (*mec*) was not transduced into *S. aureus* strain 8325-4 unless the host both was lysogenized with phage φ11 and harbored an appropriate penicillinase plasmid. They isolated four temperature-sensitive (*ts*) mutants of φ11 that did not mediate transduction for methicillin resistance at nonpermissive temperatures, although cells lysogenic with those *ts* mutants were competent for transformation of a tetracycline resistance plasmid, suggesting that the helper functions in transduction of *mec* and in transformation of *tet* resides in different genes of the phage.

This study attempts to correlate the genetic and physical maps of φ11 DNA. By cloning restriction fragments of φ11 DNA in *S. aureus* it was possible to locate some mutants on specific fragments. An explanation for the observed HFT of recombinant plasmids containing φ11 DNA sequences has also been advanced.

## MATERIALS AND METHODS

**Organisms and culture conditions.** The vector pSA2100 (earlier designation, pSC194) expressing chloramphenicol and streptomycin resistance has been described (3, 5, 6). Phage φ11 was propagated in *S. aureus* strain 8325-4 (10). Phages 83A and 55 were propagated in strains 8325-4 and PS55, respectively, by the same technique. All suppressor-sensitive (*sus*) and *ts* mutants of phage φ11 and the bacterial strains used in this study are listed in Table 1. Bacteria were grown in Trypticase soy broth (BBL Microbiology Systems Cockeysville, Md.).

**Preparation of DNA.** DNA was prepared from purified phage φ11 and plasmid-containing bacterial cells as described previously (5, 7), and DNA fragments were extracted from agarose gels as described by Löfdahl et al. (7).

**Digestion with restriction endonucleases and agarose gel electrophoresis.** DNAs of the vector plasmid, phage φ11, and isolated hybrids were digested with *Eco*RI, and the fragments were separated by agarose electrophoresis as described in the accompanying paper (7).

**Recombinant DNA.** *Eco*RI fragments of pSA2100 and phage φ11 DNA were ligated with T4 DNA ligase (Miles Laboratories, Inc., Elkhart, Ind.) as described earlier (6). When an *Eco*RI fragment purified from a preparative digest of φ11 DNA was cloned, donor and vector DNAs were used in equal amounts (0.1 to 0.3 μg), and ligation was carried out at 20°C for 1 h. Recombinant DNA experiments were always performed in a homologous system and did not require

TABLE 1. *Bacterial strains and phages*

| Designation | Relevant description | Source/reference |
|---|---|---|
| Bacteria | | |
| 8325-4 | 8325N, UV cured of φ11, φ12, and φ13 | R. P. Novick |
| 8325-4 *su*$^+$ | Suppressor mutant of 8325-4 | Kretschmer and Egan (4) |
| 8325-4(φ11) | 8325-4 lysogenized with phage φ11 | Our laboratory |
| 8325-4(83A) | 8325-4 lysogenized with phage 83A | Our laboratory |
| PS55 | Propagating strain for phage 55 | NBLS[a] |
| Phages | | |
| φ11 | Serological group B | R. P. Novick (9) |
| 83 | Serological group B | NBLS |
| 55 | Serological group B | NBLS |
| φ11 *sus* | Suppressor mutants: *A4*, *E64*, *H47*, *M28*, *O43*, *P68*, *Q54*, *U53*, *X27* | Kretschmer and Egan (4) |
| φ11 *ts*SP | Temperature-sensitive mutant: 31SP | Sjöström and Philipson (11) |
| φ11 *ts*N | Temperature-sensitive mutants: 57N, 73N | R. P. Novick |
| φ11 *ts*SC | Temperature-sensitive mutants: 4SC, 30SC, 46SC | S. Cohen et al. (2 |

[a] NBLS, National Bacteriological Laboratory, Solna, Sweden.

containment according to the National Institutes of Health and the Swedish guidelines.

**Transformation and transfection.** *S. aureus* was transformed with plasmid DNA as described previously (5). To transfer recombinant plasmids to strain 8325-4, phage 55 propagated on strain PS55 was used as the competence-inducing phage (13). Since phage 55 propagated on strain PS55 is restricted at infection of strain 8325-4, the transformants are not lysogenized, as determined by induction with UV light. The recombinant plasmids were designated pSL11NN, where SL indicates the initials of one of the investigators, 11 refers to φ11, and NN stands for the consecutive isolates of the plasmids.

**Marker rescue.** *sus* mutants of phage φ11 were propagated in Trypticase soy broth on strains 8325-4 su$^+$ and 8325-4 su$^-$ at 37°C (4). The *ts* mutants were propagated on strain 8325-4 at 30 and 42°C, respectively (11). Marker rescue of phage φ11 genes was determined by plating the *sus* mutants at 37°C on strain 8325-4 su$^-$ carrying wild-type phage φ11 *Eco*RI fragments in the pSA2100 vector. Marker rescue of *ts* mutants was carried out with the same strain at 42°C. Thus, plaque-forming units per milliliter were determined at nonpermissive conditions in each experiment.

**UV induction.** Cells grown overnight in Trypticase soy broth were washed and suspended in saline at a cell density of $10^8$. UV irradiation was carried out at 1,200 ergs/mm$^2$ per min (11). The cells were then incubated in an equal volume of 2× Trypticase soy broth at 37°C for 6 to 10 h or until lysis occurred. The media were then tested for released phages.

## RESULTS

**Cloning of the *Eco*RI fragments of phage φ11.** An attempt was first made to clone all *Eco*RI fragments of phage φ11 by a "shotgun" experiment. Phage φ11 DNA was digested with *Eco*RI, and the generated fragments were inserted into the *Eco*RI site of the pSA2100 vector. This vector carries genes for streptomycin and chloramphenicol resistance and contains one *Eco*RI site in the *str* gene (6). This site can therefore be used for negative selection of recombinant DNA. Recombinant DNA was transformed into strain 8325-4(83A) and phage 83A was used for superinfection to enhance competence (10). About 2,000 transformants were isolated by selection for chloramphenicol resistance (Cm$^r$); 1,000 of these were tested for resistance to streptomycin (Sm$^r$) by replica plating, and around 300 were identified as Sm$^s$ colonies. Plasmid DNA was prepared from several clones and the DNA was cleaved with *Eco*RI and analyzed by agarose gel electrophoresis. Figure 1 shows an example of such a gel, where the vector pSA2100 and phage φ11 DNA restricted with *Eco*RI were used as markers.

The following *Eco*RI fragments of phage φ11 were recovered in recombinant DNA: *Eco*RI-B, -D, -F, -I, -J, -K, -L, -M, -N, and -O. It was not possible from the migration on a 1% agarose to conclude whether fragment F or G had been cloned since they migrated close together. They could, however, be separated on a 0.7% agarose gel, and by DNA blotting on nitrocellulose (12) and hybridization to the $^{32}$P-labeled clone, it was verified that the F fragment had been cloned (data not shown). The clone containing fragment K also contains fragment D. In the search for clones containing the remaining *Eco*RI fragments several additional recombinants were analyzed. Among these, the D and F fragments were preferentially found, but the remaining fragments A, C, E, G, and H were never encountered. To isolate clones containing these fragments, purified *Eco*RI fragments from a preparative digest of phage φ11 DNA were isolated and inserted into the *Eco*RI site of pSA2100. The ligated DNA was used to transform strain 8325-

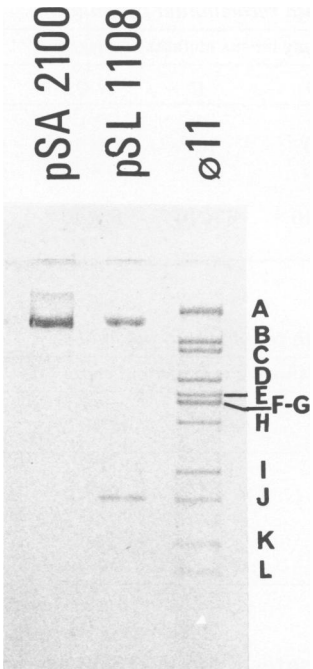


FIG. 1. *Electrophoretic analysis of one recombinant plasmid containing the EcoRI-J fragment of φ11 DNA. Vector pSA2100, recombinant plasmid pSL1108, and phage φ11 DNA were digested with EcoRI and analyzed on a 1% agarose gel.*

4(φ11), using superinfection with phage φ11 to enhance competence (10). Selection was first made for $Cm^r$ followed by negative selection for streptomycin. In this way clones with *Eco*RI fragments C and H were isolated and then transferred to strain 8325-4 by transformation. Phage 55 was then used to induce competence, which allowed characterization of the clones in a phage-free background. For the same reasons the first collection of φ11 clones had already been transferred to strain 8325-4 by transduction. The donor strain lysogenic for phage 83A was induced by UV irradiation, and the recipient strain 8325-4 was transduced at a low multiplicity of infection. The transductants did not release phage 83A, as revealed by UV induction. *Eco*RI fragments A, E, and G could not be recovered in recombinant DNA.

**Marker rescue.** The localization of phage φ11 genes on different cloned *Eco*RI fragments was determined by marker rescue of *sus* and *ts* mutants of phage φ11. Table 2 shows the rescue observed with different recombinant plasmids. Mutants *susM*28, *susO*43, *susP*68, and *susQ*54 (4) were rescued by plasmids carrying *Eco*RI fragment F of phage φ11 DNA. *susE*64 and *susU*53 were rescued by plasmid pSL1109, which contains both *Eco*RI fragments K and D. Finally, *susU*53 was rescued by recombinant plasmid pSL1104, which contains the *Eco*RI-D fragment.

In addition, an assessment was made of the presence of wild-type recombinants among the progeny. The φ11 mutants *susU*53 and *susE*64 were propagated both on strain 8325-4 pSL1109 and on strain 981 pSL1109. *susM*28, *susO*43, *susP*68, and *susQ*54 were propagated on strain 8325-4 pSL1105 and strain 981 pSL1105, respectively. One hundred plaques from each lysate were collected and plated on strain 8325-4. Each of the *sus* mutants in this experiment produced 100% wild-type phages on strain 8325-4 pSL1105 or 8325-4 pSL1109 after marker rescue.

Several *ts* mutants of phage φ11 were also analyzed for marker rescue by recombinant plasmids. The characteristics of these *ts* mutants have been described (2, 12; R. P. Novick, personal communication). Only those which could be rescued by recombinant DNA plasmids are listed in Table 3. Fragment *Eco*RI-J of φ11 rescues the temperature-sensitive functions in *ts*4SC, *ts*30SC, and *ts*46SC. *ts*57N is rescued by pSL1104 and pSL1109, both containing fragment *Eco*RI-D of φ11 DNA. *ts*73N, finally, is rescued by plasmid pSL1105 containing fragment *Eco*RI-F of φ11 DNA.

**Repressor of phage φ11.** Strains 8325-4, 8325-4(φ11), and 8325-4 carrying different recombinant plasmids were also tested for sensitivity to phage φ11 to identify the fragments containing the repressor gene. Strains 8325-4(φ11) and 8325-4 pSL1102 express complete immunity against phage φ11 (Table 4). The latter strain contains only fragment *Eco*RI-B of φ11, suggesting that the repressor gene is located in this fragment. An isolate of φ11*vir* (11) yielded the same titers of phage on strains 8325-4 and 8325-4 pSL1102, but since the characterization of this phage has not been completed, the results have not been included. To ascertain that the immunity for phage φ11 in strain 8325-4 pSL1102 was independent of a prophage, UV irradiation was attempted to release a presumptive prophage. Table 5 shows that no phages were released after UV induction of strain 8325-4 SL1102, and around $10^9$ PFU/ml were obtained in lysates from strains 8325-4(φ11) and 8325-4(φ11) pSA2100. These results support our suggestion that the repressor is encoded in the *Eco*RI-B fragment of phage φ11.

**Competence test by transfection.** It has been suggested (11) that an early gene of phage φ11 is involved in the development of competence in *S. aureus*. Strains of 8325-4 carrying various hybrid plasmids of φ11 DNA in pSA2100 were transfected with φ11*vir* DNA to study its

TABLE 2. *Marker rescue of phage φ11 sus mutants with recombinant plasmids*[a]

| Host strain | *Eco*RI fragment of φ11 DNA | Efficiency of plating for *sus* mutants of phage φ11 | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E* | *M* | *O* | *R* | *Q* | *U* |
| 8325-4 *su*$^+$ | | 1 | 1 | 1 | 1 | 1 | 1 |
| 8325-4 *su*$^-$ | | $<10^{-7}$ | $<5 \times 10^{-4}$ | $<10^{-7}$ | $10^{-5}$ | $7 \times 10^{-5}$ | $4 \times 10^{-7}$ |
| 8325-4 *su*$^-$ pSA2100 | | $<10^{-7}$ | $<5 \times 10^{-4}$ | $<10^{-7}$ | $10^{-5}$ | $7 \times 10^{-5}$ | $4 \times 10^{-7}$ |
| 8325-4 *su*$^-$ pSL1109 | D + K | $10^{-3}$ | | | | | $3 \times 10^{-3}$ |
| 8325-4 *su*$^-$ pSL1105 | F | | $3 \times 10^{-2}$ | $5 \times 10^{-1}$ | $6 \times 10^{-3}$ | $5 \times 10^{-2}$ | |
| 8325-4 *su*$^-$ pSL1104 | D | | | | | | $7 \times 10^{-4}$ |

[a] Only effective marker rescue is recorded for the plasmids.

TABLE 3. *Marker rescue of phage φ11 ts mutants with recombinant plasmids*[a]

| Host strain | *Eco*RI fragment of φ11 DNA | Temp (°C) | Efficiency of plating for *ts* mutants of phage φ11 | | | | |
|---|---|---|---|---|---|---|---|
| | | | 4SC | 30SC | 46SC | 57N | 73N |
| 8325-4 pSA2100 | | 30 | 1 | 1 | 1 | 1 | 1 |
| 8325-4 pSA2100 | | 42 | $2 \times 10^{-7}$ | $10^{-7}$ | $3 \times 10^{-7}$ | $10^{-7}$ | $2 \times 10^{-6}$ |
| 8325-4 pSL1104 | D | 42 | | | | $2 \times 10^{-3}$ | |
| 8325-4 pSL1105 | F | 42 | | | | | $10^{-1}$ |
| 8325-4 pSL1108 | J | 42 | $3 \times 10^{-3}$ | $2 \times 10^{-2}$ | $10^{-4}$ | | |

[a] Only effective marker rescue is recorded for the plasmids.

TABLE 4. *Identification of repressor function in EcoRI fragments of φ11 DNA*

| Strain | *Eco*RI fragment of φ11 DNA | Efficiency of plating of phage φ11 |
|---|---|---|
| 8325-4 | | 1 |
| 8325-4(φ11) | All | $<10^{-9}$ |
| 8325-4 pSL1102 | B | $<10^{-9}$ |
| 8325-4 pSL1103 | C | 0.5 |
| 8325-4 pSL1104 | D | 0.4 |
| 8325-4 pSL1105 | F | 0.3 |
| 8325-4 pSL1106 | H | 0.5 |
| 8325-4 pSL1107 | I | 0.5 |
| 8325-4 pSL1108 | J | 0.5 |
| 8325-4 pSL1109 | D + K | 0.5 |
| 8325-4 pSL1110 | L | 0.2 |

TABLE 5. *Release of phages after UV irradiation of S. aureus*

| Strain | Phage released (PFU/ml) |
|---|---|
| 8325-4 | Not detected |
| 8325-4(φ11) | $7 \times 10^8$ |
| 8325-4(φ11) pSA2100 | $10^9$ |
| 8325-4 pSL1102 | Not detected |

ability to express competence. None of the clones described here expressed competence (data not shown), but it should be borne in mind that the *Eco*RI-A fragment of φ11 DNA, which probably contains some early genes, could not be cloned. Furthermore, the *ts* mutant (*ts*31SP) which was temperature sensitive for competence (11) could not be rescued by the available recombinant clones.

**HFT lysates from bacteria harboring recombinant plasmids.** The natural hybrid plasmid φ11*de*, containing φ11 and pI258 DNA, was isolated by Novick (9). It is transduced at high frequency by phage φ11 propagated in bacterial strains harboring this plasmid. To investigate whether our recombinant plasmids containing fragments of φ11 DNA inserted in the vector pSA2100 also gave HFT lysates with phage φ11, strains 8325-4 pSA2100 and 8325-4 harboring various recombinant plasmids were infected by phage φ11, and the lysates were used for transduction into strain 8325-4. Table 6 shows that the transducing frequencies are 200- to 600-fold higher for the recombinant plasmids than for pSA2100 alone. It may therefore be concluded that several plasmids containing phage φ11 DNA sequences can be transduced at high frequency.

## DISCUSSION

**Recombinant DNA of phage φ11.** To correlate the genetic and physical maps of phage φ11 DNA, *Eco*RI fragments of the phage DNA were cloned by a shotgun experiment. *Eco*RI digests of the DNA gave identical fragments in independent recombinant plasmids, especially fragments *Eco*RI-F and -D. *Eco*RI fragments A, C, E, G, and H could not be recovered in the clones from the shotgun experiment. These fragments were therefore isolated and individually inserted into pSA2100, but only fragments *Eco*RI-C and -H could be isolated as recombinant plasmids after transformation. We have no

TABLE 6. *Transduction frequency of plasmid markers from recombinant clones containing phage φ11 DNA*

| Donor strain | *Eco*RI fragment of φ11 DNA | PFU of phage φ11 per ml | Transductants per ml[a] | Frequency |
|---|---|---|---|---|
| pSL1102 | B | <3 | <3 | |
| pSL1103 | C | $3.5 \times 10^9$ | $7.0 \times 10^7$ | $2.0 \times 10^{-2}$ |
| pSL1104 | D | $2.1 \times 10^9$ | $8.6 \times 10^7$ | $4.1 \times 10^{-2}$ |
| pSL1105 | F | $2.6 \times 10^9$ | $7.2 \times 10^7$ | $2.8 \times 10^{-2}$ |
| pSL1106 | H | $4.0 \times 10^8$ | $1.6 \times 10^7$ | $4.0 \times 10^{-2}$ |
| pSL1107 | I | $1.7 \times 10^9$ | $5.8 \times 10^7$ | $3.4 \times 10^{-2}$ |
| pSL1108 | J | $5.0 \times 10^9$ | $7.0 \times 10^7$ | $1.4 \times 10^{-2}$ |
| pSL1109 | D + K | $1.9 \times 10^9$ | $1.1 \times 10^8$ | $5.8 \times 10^{-2}$ |
| pSL1110 | L | $5.0 \times 10^9$ | $1.2 \times 10^8$ | $2.4 \times 10^{-2}$ |
| pSA2100 | | $3.4 \times 10^5$ | $3.2 \times 10^5$ | $0.9 \times 10^{-4}$ |

[a] Chloramphenicol resistance was scored after transduction into 8325-4 with the phage lysates.

explanation for our failure to clone fragments *Eco*RI-A, -E and -G. Failure to insert phage fragments in the homologous hosts has been reported in other systems, however (8, 14, 15).

**Comparison of genetic and restriction enzyme maps of phage φ11 DNA.** The results allow us to correlate the genetic and restriction maps of phage φ11 DNA. Figure 2 shows the genetic map of phage φ11 DNA (4) and the restriction enzyme map (7), in which the mapped genes and mutants have been included. The position of the attachment site (*att*) of the phage φ11 genome (7) has been included in the map of φ11 DNA. The region of the φ11 genome deleted in plasmid φ11*de* (9) is also indicated. This natural recombinant plasmid appears to involve a substitution between phage φ11 and pI258 DNA whereby restriction fragments *Eco*RI-K, -A, -B, and -E of φ11 DNA are lost and substituted by a region of pI258 containing the *erm* and *mcr* loci. Sequences from the φ11 DNA fragments *Eco*RI-E and -K may still be present in the hybrid plasmid since they are flanking the *Eco*RI-A and -B fragments, respectively. On the other hand, according to Kretschmer and Egan (4), *sus*E64 was not complemented by plasmid φ11*de*; this was verified by the results obtained with recombinant plasmid pSL1109, suggesting that gene E is localized in fragment *Eco*RI-K, part of which is absent in φ11*de*. Mutant *sus*E64 accumulates empty heads, suggesting an inability to package phage DNA (4). Alternatively, this mutant may be defective in replication or maturation of DNA.

That bacteria harboring fragment *Eco*RI-B are immune to φ11 infection made it difficult to map the φ11 mutants residing in this fragment by marker rescue. Since *Eco*RI fragments A and E could not be cloned, we could not map other φ11 mutations residing in the region deleted in plasmid φ11*de*. The clones containing *Eco*RI-B and -K are the only ones from this region, which contains a cluster of early genes flanked on both sides of short sequences of late genes (9). Gene *E*, residing on fragment *Eco*RI-K, is definitely a late gene. The clear-plaque mutant φ11-M15 (φ11*c*) maps close to gene *X* (4), and if it has a defective repressor, it should reside in the *Eco*RI-B fragment, but gene *X* has been defined as a late gene in the tail gene cluster (4). We therefore conclude that the late gene cluster ends within the *Eco*RI-B fragment. Sjöström and Philipson (11) showed that *ts*31SP showed temperature-sensitive expression of competence for transfection and transformation of *S. aureus*. This mutant may affect an early gene and it was mapped between the *A* and *X sus* mutants close to the former, which definitely is an early gene. These mutants as well as the competence induction mutant could not be rescued or induced with the φ11 recombinant clones.

Since the early gene cluster probably starts in *Eco*RI-B of φ1 DNA, the *ts*31SP mutants, like the *sus* mutant in gene *A*, are probably early mutants located in fragment *Eco*RI-A or -B. The nature of the competence factor is still not determined but since gene *X* is defined as a tail gene (4), with *ts*31SP in juxtaposition, the mutant may be part of a tail gene cluster. The competence factor may therefore correspond to a function of the tail. In accordance with this hypothesis, Thompson and Pattee (13) suggested that the competence factor for *S. aureus* was a structural protein of the phage. On the other hand, Sjöström and Philipson (11) showed that both RNA and protein syntheses are required for expression of competence after superinfection with phage φ11. Additional experiments are clearly required to determine the nature and gene location of the competence factor.

The results of marker rescue with the remaining *sus* mutants indicate that there is a gene cluster of late structural genes. The tail genes *Q* and *P* and the head genes *O* and *M* are localized on fragment *Eco*RI-F. Head gene *H* could not be mapped in the expected region between genes *E* and *M*. One possible explanation is that gene *H* is too close to the end of an *Eco*RI site to recombine with the *sus* mutant. The mutation affecting the formation of a tail base plate, *sus*U53, is obviously localized on the *Eco*RI-D fragment.

In addition to the *sus* mutants, some *ts* mutants could also be mapped on the genome (Fig. 2). There appears to be a good correlation between the genetic map of Kretschmer and Egan (4) and the restriction enzyme map reported in the accompanying paper (7), but the intergenic distances vary between the two maps.

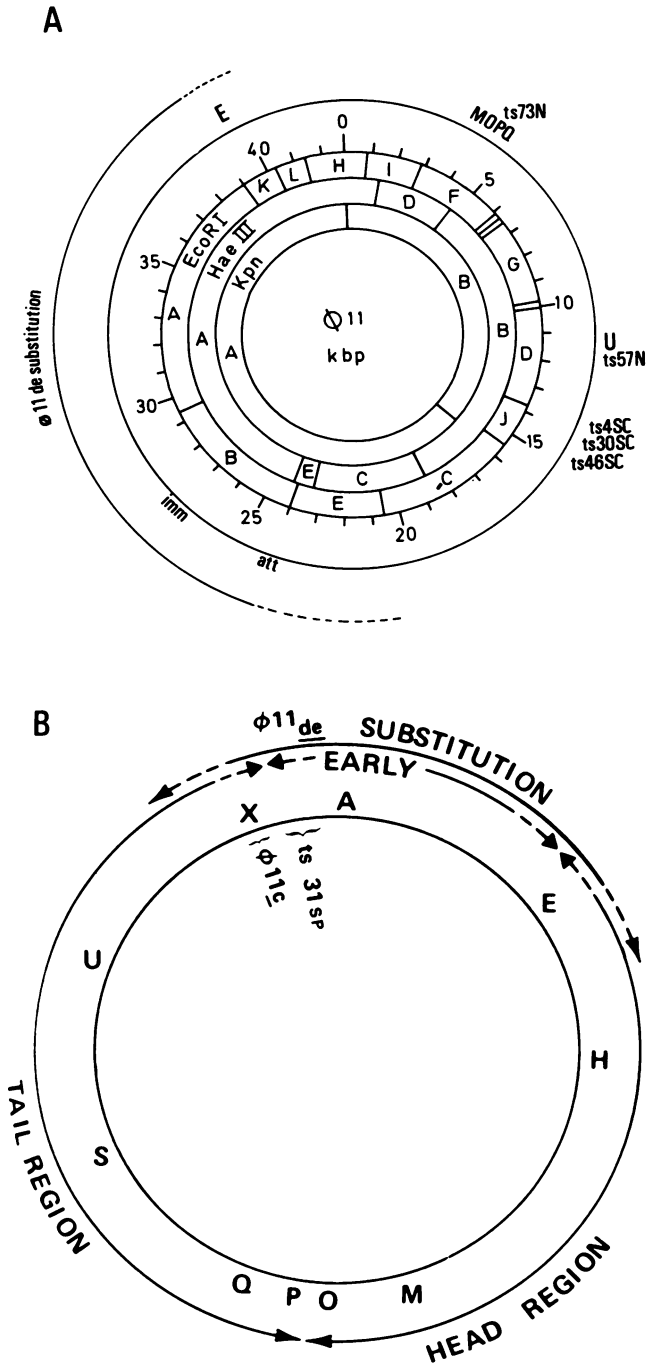


FIG. 2. *Correlation of genetic and physical maps of φ11 DNA. (A) Restriction enzyme map of φ11 DNA. The positions of the sus and ts mutants mapped by marker rescue, the immunity region expressed in pSL1102, and the attachment (att) site mapped in the accompanying report (7) are included. (B) Functional genetic map of φ11 published by Kretschmer and Egan (4). The φ11de substitution region is the deleted region of the φ11 genome. This region was identified by complementation and recombination (4) and by restriction enzyme analysis (not shown). The precise endpoints of this region in relation to the φ11 genome are unknown (indicated by broken lines).*

**HFT.** Plasmids containing phage φ11 sequences can be transferred by HFT after phage φ11 infection of the host cells (Table 6). Novick (9) demonstrated a similar phenomenon for the natural recombinant plasmid φ11*de*. The high transduction frequency of φ11*de* was explained by assuming that the plasmid control of replication was superseded by a phage control after infection by wild-type phage, thereby inducing the plasmid to multiply rapidly (9), resulting in a high copy number of the plasmid which could be packaged into φ11 phage particles.

Since several recombinant plasmids with φ11 DNA sequences can be transduced with high frequency, we propose an alternative interpretation. Since mature φ11 DNA is circularly permuted and terminally redundant (7), phage DNA is probably replicated as a rolling circle containing long concatamers. DNA molecules, larger than genome length, are cleaved and packaged into the phage heads at maturation. If a plasmid containing φ11 sequences recombines with the replicative φ11 DNA, the plasmid may be integrated in the concatameric DNA flanked by a duplication of the cloned φ11 sequences. At maturation the plasmid and its surrounding φ11 DNA will be included in the phage head as a full-length molecule. When this DNA molecule is introduced in the bacterial cell by transduction, a plasmid with the original φ11 sequences can be formed by intramolecular recombination. This model explains the HFT of all plasmids containing phage DNA, including φ11*de*, and also the observation that the peaks of transduction activity and plaque-forming units coincide when the phage preparations are banded at isodensity in cesium chloride (data not shown). If only the plasmid DNA is packaged in the phage heads, the virions containing plasmid DNA would have a lower density in cesium chloride since φ11*de* and the recombinant plasmids are smaller than mature φ11 DNA, but this was never observed. HFT of recombinant plasmid genes may therefore be dependent on a recombinational event prior to phage maturation.

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