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Mapping of New *Escherichia coli* K and 15 Restriction Sites on Specific Fragments of Bacteriophage φX174 DNA

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We have isolated several new φX174 mutants which contain sites sensitive to restriction by *Escherichia coli*. One contains an *E. coli* 15 restriction site and three are double mutants containing an *E. coli* K site as well as the *E. coli* 15 site. The replicative form (RF) DNA of one of the mutants containing a K site has been shown to be restricted in spheroplasts of a K-12 strain. The infectivity of this RF, but not wild-type RF, has also been shown to be inactivated by an *E. coli* K extract and by purified K restriction enzyme in vitro. The product of the RF treated with purified K restriction enzyme in vitro is a full length linear molecule. The mutant sites have also been localized to specific regions of the φX174 genome by a fragment mapping technique, making use of specific fragments of φX174 RF DNA obtained by digestion with a specific endonuclease.

None of the restriction enzymes of *Escherichia coli* (1) appear to attack the DNA of wild-type bacteriophage φX174 (3, 10, 23). These specific endonucleases recognize a limited number of specific sites in double-stranded DNA (19), and therefore such sites are presumed absent in the double-stranded replicative form (RF) DNA of φX174. However, Schnegg and Hofschneider (23) have isolated a mutant of φX174 sensitive to *E. coli* B restriction. This paper describes the isolation, characterization, and mapping of several new mutants of φX174 which confer sensitivity to restriction by *E. coli*. Since the mapping of restriction sites in φX is difficult by conventional means, we have used a physical mapping procedure to localize these restriction sites.

A genetic assay for small fragments of φX174 DNA has been described (8, 13, 20, 25) and used previously to determine which φX174 markers are contained in specific fragments (8, 20). The specific fragments were generated by cleavage with the restriction enzymes from *Hemophilus influenzae* (24) and *Hemophilus aegyptius* (20). The genetic assay orders the products of specific endonuclease cleavage since the order of the genetic markers is known from the recombination map of φX174 (2). Consequently, if a sequence such as a restriction site can be associated with a particular φX174 DNA fragment, it is then localized on the genetic map within the length of the fragment. We will describe the localization of three of the restriction sites by using these procedures.

MATERIALS AND METHODS

Phage and E. coli strains. All of the phage used in this paper were derived from φX174 *am3*, a mutant with an amber mutation in the lysis cistron (E) which allows production of large quantities of RF DNA and phage (9, 14, 15). Since all φX174 strains mentioned in this paper contain the *am3* mutation, this mutation will not always be referred to since it is neutral with respect to restriction and modification.

Table 1 describes the characteristics of the *E. coli* strains used. W6 and C600.5 were used respectively for $r_K m_K$ (K restriction and K modification) and $r^- m^-$ (mutant which neither restricts nor modifies) spheroplast assays of infective DNA. *E. coli* 1100 was used to prepare extracts for testing restriction in vitro. Host specificity (1) was determined by plating unmodified λ phage on the host to be tested. Plaques were then picked and replated on the host being tested and on an $r_0 m_0$ host (wild-type, absence of restriction and modification). Several cycles of this testing were carried out. The phage mutant, φX174 sB1·C sensitive to restriction by *E. coli* B, was obtained from P. Hofschneider. The mutant nomenclature is that proposed by Arber and Linn (1). The·C refers to the last host in which the phage was grown, in this case *E. coli* C.

Spheroplast assays for infective DNA. DNA infectivity was performed as described by Guthrie and Sinsheimer (11), except that after the spheroplasts had been prepared, protamine sulfate (Lilly

TABLE 1. *E. coli strains*

Strain and reference	φX174 sensitivity[a]	Suppressor	Host specificity[b]	Origin	Other characteristics
CR (7)	s	amber	r_0m_0	C/K-12 hybrid	thy⁻, Sm^r, F⁻
HF4714 (2)	s	amber	r_Km_K	C/K-12 hybrid	
W6 (13)	r	none	r_Km_K	K-12	
C600.5 (18)	r	none	$r_K^-m_K^-$	K-12	leu⁻, thr⁻, B_1⁻, lac⁻, T_1^r, T_5^r
WWU 1*am* (21, 22)	s	amber	$r_{15}m_{15}$	15	pro⁻, met⁻, thy⁻, ura⁻, try⁻
HF4704 (16)	s	none	r_0m_0	C	thy⁻, uvrA
1100 (7)	r	?	r_Km_K	K-12	endoI⁻
BC (23)	s	none	r_Bm_B	C/B hybrid	
C (14)	s	none	r_0m_0	C	

[a] s, Sensitivity to φX174; r, resistance to φX174.

[b] Host specificity was determined by plating unmodified λ as described in Materials and Methods using C as an r_0m_0 standard, W6 as an r_Km_K standard, and WWU 1*am* as an $r_{15}m_{15}$ standard.

Research Laboratories) was added to give a final concentration of 25 μg/ml (4). Infected spheroplasts were incubated at 30 C for 3 to 5 h. The spheroplasts were then treated with a few drops of chloroform and mixed on a vortex mixer to release progeny phage. The progeny were assayed for plaque-forming units on the appropriate host strains.

DNA preparations. Single-stranded viral DNA (+ strands) was prepared by phenol extraction of purified virus (10). RF was purified from infected cells in two ways. Larger batches (from 1 liter of cells) were purified by the method of Komano and Sinsheimer (15). The fraction of RFI in the preparation was determined by electron microscopy (8). Small batches (10 ml) of purified ^{3}H-thymidine-labeled RF were prepared as follows. HF4704 was grown in Difco minimal broth (Davis) with 10% glucose and Casamino Acids and 1 μg of thymidine per ml to 2×10^8 cells per ml at 37 C. The cells were then infected with φX174 at a multiplicity of infection of 5. To stop single-strand DNA synthesis, after 15 min of aeration chloramphenicol was added to a concentration of 30 μg/ml. Enough ^{3}H-thymidine was also added at this time to give about 5 μCi/ml. Aeration of the culture was continued for 90 min at 37 C. The cells were spun down, suspended in 3.7 ml of cold 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.1. Then 0.37 ml of egg white lysozyme (Sigma Chemical Co.) at 4 mg/ml in 0.25 M Tris buffer, pH 8.1, was added followed by 0.37 ml of 2% ethylenediaminetetraacetic acid (EDTA). The suspension was left for 20 min at 4 C. If considerable clearing had not occurred and there had been no increase in viscosity, the cells were put at 37 C for 3 to 5 min. When the suspension was quite clear and viscous, 0.44 ml of 1% Sarkosyl NL97 (Geigy Industrial Chemicals) was added. The solution became completely clear. It was then sheared three times through a 25-gauge needle to decrease the viscosity. Cesium chloride was then added to give a concentration of 1.57 g/cm^3. Propidium iodide (12) was added to a concentration of 400 μg/ml, and the solution was banded at 35,000 rpm in a Beckman SW50.1 rotor for 36 h at 20 C. Drops were collected into Dowex resin (0.5 ml of ~2.5% wt/vol suspension); the fraction of RFI in the tritium-labeled preparation was determined by nitrocellulose binding procedures as previously described (8).

Nitrosoguanidine treatment of infected cells. *E. coli* C (10 ml) was grown to 4×10^8 to 5×10^8 cells per ml in tryptone broth and was then infected with φX174 *am3* or φX174 *am3* s15-1 at a multiplicity of infection of 5. The cells were aerated for 7 min at 37 C and then *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (1 to 2 mg/ml, freshly made in tryptone broth) was added to give a concentration of 100 μg/ml. The culture was left without aeration for 60 min at 37 C (5). It was then spun down, resuspended in fresh tryptone broth, and left aerating for 2 to 3 h at 37 C. The culture was then spun down again and suspended in 2 ml of 0.05 M sodium tetraborate. EDTA was added to give 0.4% and then egg white lysozyme to give 0.5 mg/ml. After several hours at 4 C, the cell debris was spun down. The supernatant fluid containing the released phage (14) was stored at 4 C.

Mutant selection. Two methods were used for mutant selection. Both involved plating the treated phage stock on *E. coli* CR (r_0m_0) to obtain individual plaques. In the first procedure individual plaques were stabbed with straight pins which were then put in 1 ml of tryptone broth. After mixing, 40 to 50 μliters of each phage suspension was put in several wells of a disposable spot plate (Linbro Chemical Co.). One milliliter of top agar containing about 0.1 ml CR was put in the first well of the several wells for each plaque. Both mutant and nonmutant phage grow on CR, and therefore the well shows a large number of plaques or lysis after incubation at 37 C. Each of the other wells for each plaque are filled with 1 ml of top

agar containing 0.1 ml of one of the restricting bacterial strains. Since mutants undergoing restriction should plate on the proper restricting strain with an efficiency of about 10^{-2} to 10^{-3} with respect to CR, a mutant should show one well containing few if any plaques. This would indicate restriction by the bacterial strain in that well. Such a mutant should plate with an efficiency close to that of CR on any other restricting strain.

The second method, which is more convenient, involved the use of antiserum to ϕX174. In this method individual plaques were also stabbed with straight pins. The phage on the pins was then stabed onto two plates, one having a top agar layer containing CR and the second a top agar layer containing one of the restricting hosts and a quantity of anti-ϕX174 serum. The amount of antiserum used was an amount that, in a control experiment, prevented growth of ϕX174 sB1·C on *E. coli* BC, but not of ϕX174 *am3* on the same host. Thus, nonmutant phage for the restricting host being tested would grow on both plates, giving clear spots, whereas restriction mutants for that host would grow on only the plate without antiserum. The combination of antiserum and restriction inhibits growth of any mutant. Without antiserum even mutants grow, since enough phage is transferred on the pins to assure successful infection of at least several cells, eventually giving a clear spot.

Identification of mutants. Phage suspected of being mutant by the above selection procedures were tested by plating on CR and the appropriate restricting host. Plaques were picked from both infected strains and replated again on both strains to test that the mutants were truly restriction mutants and not host range mutants (1, 23). This testing was repeated through several cycles of phage growth. All plaques appearing on the plates with the restricting host were shown to be modified, and their progeny phage became susceptible to restriction again after passage through a nonrestricting host.

Preparation of K-12 extracts. Extracts of *E. coli* 1100 were prepared by the method of Linn and Arber (17).

K restriction enzyme. Purified K restriction enzyme (200 μg of protein per ml, >80% pure) was a gift from J. Heywood.

Digestion of RF by 1100 extract. Extracts of *E. coli* 1100 were prepared and used to treat ϕX174 RF by the procedures of Linn and Arber (17). Reaction mixtures (0.2 ml) contained about 0.002 optical density units (260 nm) RF. Treatment was at 37 C for 30 min. After treatment the mixture was assayed for infectivity.

Digestion of RF by K restriction enzyme. Reaction mixtures were 0.1 ml of 0.1 M TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer, pH 8, containing 0.02 mM EDTA, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.02 mM *S*-adenosyl methionine, and 0.2 mM adenosine 5′-triphosphate (ATP) plus about 0.005 optical density units (260 nm, 4×10^{10}: phage equivalents) RF and 2 μliters of K restriction enzyme (reference 19; J. Heywood, personal communication). Treatment was at 37 C for 2 h. After treatment the samples were assayed for infectivity, and the remainder was frozen at −20 C.

Electron microscopy. Electron microscopy was as described previously (8).

Fragment mapping. The assay procedure is outlined in Fig. 1. Gel fractions containing specific fragments of ϕ*Xam3* RF were obtained, and the DNA was eluted as described previously (8, 20) by using endonuclease Z, a specific endonuclease from *Hemophilus aegyptius* (20). Three percent acrylamide-0.5% agarose gels were used. Ten microliters of each eluted gel fraction was heated to melt the DNA and then mixed with 10 μliters

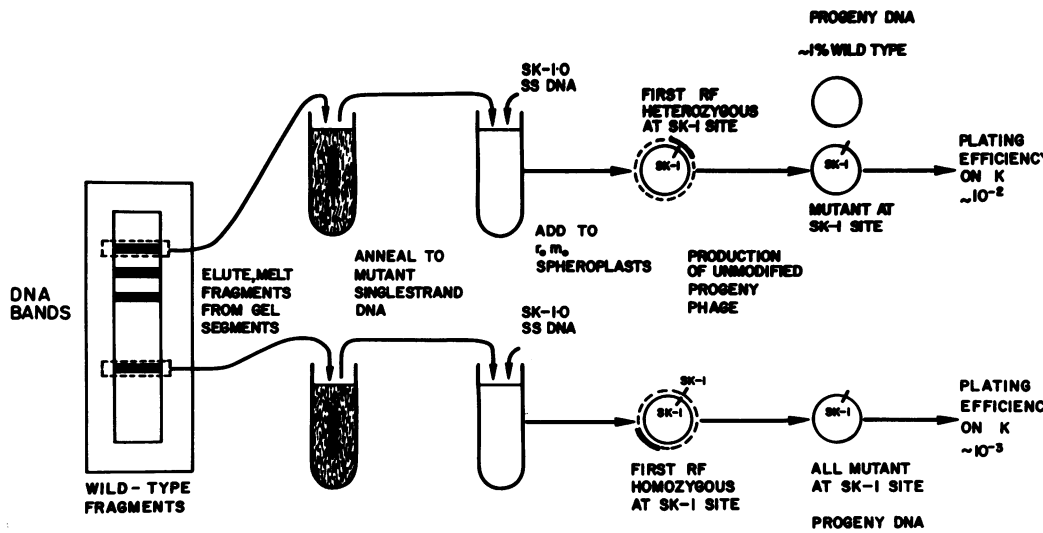

FIG. 1. *Mapping procedure for the restriction sites (see text for discussion of the procedure).*

of mutant viral strands. The mixture was then subjected to the standard annealing procedure (8). The total volume of each annealing mixture was transferred into 0.4 ml of 0.05 M Tris, pH 8.1, and used to infect spheroplasts of *E. coli* C600.5 (r^-m^-). The unmodified progeny phage were plated on HF4714, WWU 1*am*, and CR. The plating efficiencies on HF4714 (r_Km_K) and WWU 1*am* ($r_{15}m_{15}$) of the progeny from each fraction were determined by dividing the number of plaque-forming units on these two strains by the number on CR, the nonrestricting host. This procedure normalizes differences in spheroplast infectivity and DNA concentration among the samples.

RESULTS

Four mutants containing sites susceptible to *E. coli* restriction enzymes were isolated as shown in Table 2. φX*am*3 s15-1 was isolated as a mutant susceptible to restriction by *E. coli* 15 strains. It is not susceptible to restriction by other *E. coli* strains. The s15-1 mutant was mutagenized a second time with nitrosoguanidine, and three double mutants, also shown in Table 2, were isolated. The 15 and K sites are independent, since mutant phage grown on *E. coli* K strains are still restricted when plated on *E. coli* 15 strains. The uniqueness of the three K sites, as well as the 15 site, has been investigated by fragment mapping and will be discussed below.

These mutants were also grown on strains other than CR. Examples of the plating efficiencies of some of these phage stocks are also shown in Table 2. When modified, all the mutants plate with an efficiency of one on the corresponding restricting host. All the mutants were passed through several alternate cycles of growth on restricting and nonrestricting bacterial strains. All four mutant strains showed the regular characteristics of restriction mutants. All other phage functions appear normal.

Further characterizations of these mutants were carried out using the K site of φX*am*3 s15-1 sK-1. RF DNA was isolated from r_0m_0 cells infected with this mutant and from cells infected with φX*am*3. Both RF preparations were used to infect spheroplasts of either r^-m^- or r_Km_K type as shown in Table 3. The number of progeny phage produced for both RF preparations in both spheroplast strains increased linearly with the amount of added DNA in the concentration range used. Therefore, from these straight lines it was possible to determine DNA concentrations for the two RF types which gave an equal number of progeny phage following infection of r^-m^- spheroplasts. The number of progeny phage produced in r_Km_K spheroplasts after infection with the same RF DNA concentrations could then be determined in the same way. The ratio of sK-1 progeny to *am*3 progeny for these RF concentrations in the r_Km_K spheroplasts thus gives the efficiency of transfection of the restriction mutant in the restricting bacterial strain. The relative efficiency of transfection of r_Km_K spheroplasts by the sK-1 restriction mutant RF is about 20 times less than that of the *am*3 RF, indicating that the unmodified mutant DNA is inactivated in vivo by strains containing the K restriction enzyme.

The restriction mutant (sK-1) and *am*3 RF were also tested for restriction in vitro. An extract of *E. coli* 1100 (r_Km_K) was prepared by the

TABLE 2. *Plating efficiency of mutants of φX174 am3 on E. coli CR, HF4714 and WWU 1 am*

Mutant[a]	Host		
	CR (r_0m_0)	HF4714 (r_Km_K)	WWU 1am ($r_{15}m_{15}$)
φX174 *am*3 s15-1·CR	1	1	10^{-2}
φX174 *am*3 s15-1 sK-1·CR	1	2×10^{-3}	10^{-2}
φX174 *am*3 s15-1 sK-2·CR	1	10^{-3}	10^{-2}
φX174 *am*3 s15-1 sK-3·CR	1	3×10^{-3}	10^{-2}
φX174 *am*3 s15-1·K	1	1.5	10^{-2}
φX174 *am*3 s15-1·15	1	1.5	1.6
φX174 *am*3 s15-1 sK-1·K	1	1.5	10^{-2}
φX174 *am*3 s15-1 sK-2·K	1	1.5	10^{-2}

[a] Mutant sites are named according to the convention proposed by Arber and Linn (1).

TABLE 3. *Transfection of r^-m^- and r_Km_K E. coli strains with RF from φX174 am3 and φX174 am3 s15-1 sk-1*

RF	Plaque-forming units/ml	
	K-12-W6 (r_Km_K)	K-12-C600.5 (r^-m^-)
φX174 *am*3·C	2×10^6	7×10^5 [a]
φX174 *am*3 s15-1 sK-1·C	9×10^4	7×10^5
φX174 *am*3 s15-1 sK-1 ÷ φX174 *am*3	4.5×10^{-2}	1.0

[a] *Am*3·C RF required 1.3 ng of DNA whereas *am*3s15-1 sK-1·C RF required 0.8 ng of DNA to achieve 7×10^5 plaque-forming units. These values were determined from linear dose curves as explained in the results.

method of Linn and Arber (17). The two RF preparations were treated with two concentrations of extract, and the treated DNA was used to transfect spheroplasts of *E. coli* C600.5 (r^-m^-) as shown in Table 4. While there is some nonspecific inactivation of the *am*3 RF, the *am*3 s15-1 sK-1 RF is inactivated at a rate 20 times that of *am*3 RF, again indicating the presence of a specific restriction site in the mutant DNA.

Another indication of the presence of a restriction site is treatment of the mutant RF with purified restriction enzyme from *E. coli* K and subsequent observation of the treated RF in the electron microscope. Unmodified mutant and nonmutant RF were treated with the purified K restriction enzyme. The treated and untreated RF samples were assayed for infectivity in nonrestricting spheroplasts as shown in Table 5. Eighty-five percent of the infectivity of the mutant RF was inactivated by the enzyme. The treated and untreated mutant RF were observed in the electron microscope. The treated RF was found to be converted to linear molecules of the same length as the uncleaved open circle RFII molecules observed in the same picture. The untreated sample had no more linear DNA than was observed in a control grid of the bulk RF preparation. This microscope data indicates that the φX *am*3 s15-1 sK-1 mutant DNA is cleaved a single time by the *E. coli* K restriction enzyme. The infectivity data also indicates that no such cleavage occurs in wild-type DNA.

To find the relative positions of the mutant sites in the φX174 genome, a fragment mapping procedure was used. This procedure is essentially that described by Hutchison and Edgell (13) who used it to determine what genetic markers are contained in specific fragments of φX174 DNA (8, 20). The procedure is extended here to mapping mutant sites that are unusually difficult to map by standard procedures.

TABLE 4. *Restriction of φX174 RF in vitro by extracts of E. coli K-12-1100*

RF	Volume of extract (μliters)	PFU/ml[a]	Remaining infectivity (%)
φX174 *am*3	0	4.4×10^3	100
	70	2.6×10^3	59
	100	1.7×10^3	39
φX174 *am*3 s15-1 sK-1	0	8.6×10^4	100
	70	2.1×10^3	2.4
	100	1.3×10^3	1.5

[a] RF was treated with extracts of *E. coli* 1100 (r_Km_K) as described in Materials and Methods. After treatment, the RF was diluted 24 times into 0.05 M Tris buffer (pH 8.1) for spheroplast infection in C600.5 (r^-m^-). The values of PFU per milliliter are progeny phage per milliliter in the spheroplast tubes.

TABLE 5. *Restriction of unmodified φX174 RF in vitro by purified restriction enzyme from E. coli K-12*

RF	Restriction enzyme	PFU/ml[a]	Remaining spheroplast infectivity (%)
φX174 *am*3·C	−	4.3×10^4	100
	+	4.6×10^4	107
φX174 *am*3 s15-1 sK-1·C	−	3.5×10^4	100
	+	4.8×10^3	14

[a] Digestion of RF was as described in Materials and Methods. After treatment, the RF was diluted 10 times into 0.05 M Tris (pH 8.1) for spheroplast infection in C600.5 (r^-m^-). The values of PFU/ml are progeny phage per ml in the spheroplast tube.

Figure 1 shows a diagram of the procedure used to obtain phage recombinant for the restriction mutant sites. As previously described (8, 20), a mixture of ^{32}P-labeled and unlabeled *am*3 RF was digested with endonuclease Z, and the digest was subjected to electrophoresis. The gel was dried on filter paper and an autoradiogram was made showing the positions of DNA bands containing specific fragments of RF. The dried gel was cut into segments which were placed in test tubes (5 by 50 mm). Buffer was added to each tube, and the tubes were placed in a boiling water bath to both elute the DNA from the gel and to melt the double-stranded fragments eluted.

The fragments were then annealed to unmodified mutant single-stranded phage DNA and the annealed DNA was used to infect r_0m_0 spheroplasts. Presumably, those infecting single strands with a fragment annealed to them will have the complementary strand completed using the annealed fragment as primer (13). Thus a small fraction of the first RF molecules formed will contain a DNA fragment incorporated into the complementary strand. The top section of Fig. 1 shows the region complementary to that containing the mutant site. The first RF is heterozygous for the mutant site. Progeny RF will then be of two kinds, wild-type recombinants and mutants. Therefore, some of the progeny phage will be wild-type. The maximum fraction of wild-type, as determined previously (13), is on the order of 1%.

If the incorporated fragment does not contain the region complementary to that containing the

mutant site, as shown in the lower part of Fig. 1, then the original RF will be homozygous for the mutant site. All progeny RF, as well as progeny single-stranded molecules, will contain the mutant site; all progeny phage should be mutant.

Since unmodified mutant phage have a plating efficiency on $r_K m_K$ hosts of about 10^{-3} compared to $r_0 m_0$ hosts, a population of mutant phage containing 1% wild-type recombinants would have a plating efficiency of essentially 10^{-2} or 10 times that of a population containing no recombinants.

Figure 2 shows the results of fragment mapping experiments used to determine the positions of the sK-1 and sK-2 sites. The plating efficiencies of progeny phage from spheroplasts infected with single-stranded mutant DNA mixed and

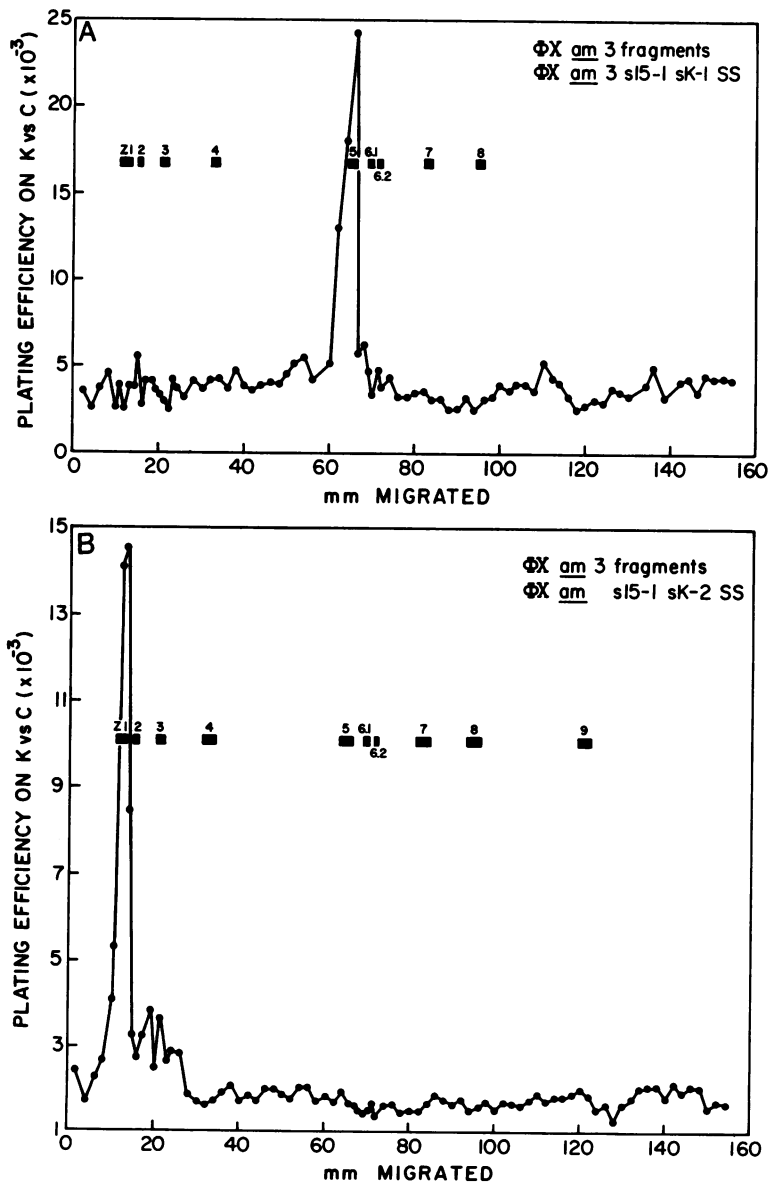

FIG. 2. *Fragment mapping of the sK-1 and sK-2 restriction sites. The plating efficiency of the progeny phage on a $r_K m_K$ host relative to that on a $r_0 m_0$ host is plotted for each fraction from the electrophoresis gel. The solid bars indicate the positions of the RF fragments as determined by an autoradiogram of the gel. A, Endonuclease Z digested wild-type fragments were annealed with sK-1 single-stranded DNA. B, Endonuclease Z digested wild-type fragments were annealed with sK-2 single-stranded DNA.*

annealed with gel segment eluates is plotted against the distance of the gel segment from the origin. The positions of the DNA bands as determined by autoradiography are also indicated.

It is clear that the sK-1 site is contained within the DNA fragment in band Z5 and the sK-2 site in band Z1. The sK-3 site is also located in band Z5. In each of these gel regions, the plating efficiency of the progeny phage is about eight times greater than that of the progeny phage from the rest of the gel. If, from $r_K m_K$ host plates, plaques from individual progeny of the samples in the peak are tested by passing through the $r_0 m_0$ hosts, then at least 85% of these progeny plaques are found to be recombinant, confirming the plating efficiency data.

The same annealing mixtures can also be used to determine where the s15-1 site is located. This site, however, is much harder to map, since the plating efficiency of the unmodified mutant on the $r_{15} m_{15}$ host is 10^{-2} compared to an $r_0 m_0$ host. Therefore, a sample containing 1% recombinants would have a plating efficiency of 10^{-2} because of recombinants and about 10^{-2} because of escape from restriction, or a total plating efficiency of about 2×10^{-2}. This gives a maximum increase in plating efficiency on the order of a factor of two. Such an increase is difficult to detect by these procedures. Preliminary data indicated such a small increase in plating efficiency for progeny of s15-1 DNA annealed to wild-type fragments from band Z3. When individual plaques in this peak appearing on $r_{15} m_{15}$ host plates are tested, about 50% are found to be recombinant. This number is quite close to the predicted number for 1% wild-type recombinants in the population.

DISCUSSION

The fragment mapping shows that the sK-1 site is on fragment z5, sites sK-2 and sK-3 are on fragment z1, and site s15-1 is on fragment z3. Therefore, sK-1 and sK-2 must represent different mutations. It is possible that the independent isolates of sK-2 and sK-3 are the same since they are associated with the same specific fragment of DNA. This fragment is 1,690 nucleotide pairs long and our unpublished data indicate that it spans the region from the "beginning" of cistron F into cistron G. The fragment bearing the s15-1 site is 1,025 nucleotide pairs long and extends from the middle of cistron A into cistron D. The fragment z5 has not yet been mapped.

These mapping experiments serve as an example of how specific DNA fragments produced by endonuclease treatment can be used generally to physically localize sequences on a genetic map. Essentially, any sequence or site for which one has an assay which can be applied to the fragmented DNA can be localized within the ordered set of fragments.

The new ϕX174 restriction-sensitive mutants described in this paper and the earlier mutant of Schnegg and Hofschneider (23) now make available in ϕX174 DNA sequences conferring sensitivity to restriction by three strains of *E. coli*. These mutants could be used to study the cleavage of DNA by these enzymes. They also allow the production of ϕX174 RF DNA fragments containing the sequences which confer sensitivity to restriction. Comparison of wild-type and mutant DNA sequences when such become available should prove interesting. The mapping of these sites should allow a more thorough investigation of them, both as to the recognition sequences involved in restriction and modification and for the site or sites of cleavage by the restriction enzyme.

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