HOMOLOGOUS RECOMBINATION IN *ESCHERICHIA COLI*: DEPENDENCE ON SUBSTRATE LENGTH AND HOMOLOGY

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ABSTRACT

We studied the *in vivo* recombination between homologous DNA sequences cloned in phage lambda and a pBR322-derived plasmid by assaying for the formation of phage-plasmid cointegrates by a single (or an odd number of) reciprocal exchange. (1) Recombination proceeds by the RecBC pathway in wild-type cells and by low levels of a RecF-dependent pathway in *recBC*$^-$ cells. The RecE pathway appears not to generate phage-plasmid cointegrates. (2) Recombination is linearly dependent on the length of the homologous sequences. In both RecBC and RecF-dependent pathways there is a minimal length, called the *minimal efficient processing segment* (MEPS), below which recombination becomes inefficient. The length of MEPS is between 23–27 base pairs (bp) and between 44–90 bp for the RecBC- and RecF-dependent pathways, respectively. A model, based on overlapping MEPS, of the correlation of genetic length with physical length is presented. The bases for the different MEPS length of the two pathways are discussed in relationship to the enzymes specific to each pathway. (3) The RecBC and the RecF-dependent pathways are each very sensitive to substrate homology. In wild-type *E. coli*, reduction of homology from 100% to 90% decreases recombinant frequency over 40-fold. The homology dependence of the RecBC and RecF-dependent pathways are similar. This suggests that a component common to both, probably *recA*, is responsible for the recognition of homology.

THE enzymatic machinery of generalized recombination requires homologous DNA substrates. One mechanism for testing if two sequences are homologous is for the recombination enzymes to form transient heteroduplexes of the sequences. If the heteroduplex is stable, recombination may proceed. If not, the transient, unstable complex cannot support the subsequent steps of recombination (THOMAS 1966; STAHL 1979; GONDA and RADDING 1983; WATT *et al.* 1985).

Recombination between a pair of homologous sequences can occur at any of a number of homologous positions. The farther apart a pair of markers are, the more probable that they are segregated by recombination events occurring between them (MORGAN 1911). This gives rise to the phenomenon of genetic distance or length.

The global phenomena of genetic homology and genetic lengths are prob-

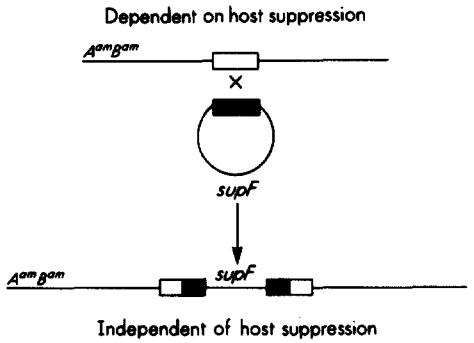


FIGURE 1.—Homologous recombination between an amber-marked phage, that requires host amber-suppression, and a *supF*-plasmid can result in a phage-plasmid cointegrate that is independent of host amber-suppression.

ably the cumulative effects of local events occurring in small physical intervals. Therefore, the questions of how homology is recognized and how genetic lengths are related to physical lengths may be addressed by looking at small intervals. What properties of small physical intervals are relevant to homology recognition, and what are the genes and their products responsible for homology recognition? What is the correlation between genetic and physical lengths in small physical intervals, and what genes and their products are responsible for the correlation? Given a minimal substrate length required for efficient recombination (BAUTZ and BAUTZ 1967; SINGER *et al.* 1982; GONDA and RADDING 1983; WATT *et al.* 1985), what is the molecular basis for the minimal length requirement?

E. coli provides a model system to address these questions. Three pathways for homologous recombination have been identified: the RecBC, RecF and RecE pathways. Each is characterized by its genetic requirements. The RecBC pathway requires *recA* and *recB recC* (CLARK 1974). The RecF pathway requires *recA*, *recF*, *recJ* (HORII and CLARK 1973; LOVETT and CLARK 1985), *ruv* and *recN* (PICKSLEY, LLOYD and BUCKMAN 1984). The RecE pathway is activated by mutation of *sbcA* (GILLEN, WILLIS and CLARK 1981; FOUTS *et al.* 1983) in the cryptic prophage called *rac* (KAISER and MURRAY 1979) and requires *recE*, as well as *recA* and some of the genes of the RecF pathway, for recombination during conjugation (GILLEN, WILLIS and CLARK 1981). The requirement for *recA* seems dispensable when the RecE pathway operates upon plasmid-plasmid (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982) and lambda-lambda recombination (CLARK 1974).

We studied the recombination between homologous sequences carried by lambda phage and by plasmid vectors to form phage-plasmid cointegrates (Figure 1; SEED 1983). We used this system to compare the different pathways for their requirements for substrate length and substrate homology. Since the different pathways require a common gene (*recA*), as well as genes specific to each pathway (*recBC*, *recF* and *recE*), we can begin to deduce which genes are involved in the recognition of genetic length and homology.

MATERIALS AND METHODS

Bacterial strains: All bacterial strains are *E. coli* K12 derivatives, the relevant genotypes are listed in Table 1. Cells were routinely grown in L broth (10 g Bacto-tryptone, 5 g Bacto yeast extract and 10 g NaCl per liter, pH adjusted to 7.5 with NaOH) or in NZMg [10 g NZamine (Sheffield Products), 5 g NaCl per liter and 10 mM $MgCl_2$]. Agar plates contain 15 g agar per liter of L or NZMg broth. NZMg top agar contains 7 g agar per liter of NZMg broth. Cell and phage dilutions were made in SM (0.1 M NaCl, 50 mM Tris(hydroxymethyl) aminomethane HCl, pH 8.0, 10 mM $MgCl_2$ and 0.05% gelatin). For *lac* assays, 0.01 ml of a 100 mM stock of isopropylthio-β-galactoside (IPTG, Sigma) and 0.05 ml of a 20 mg/ml stock of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal, Bachem, Inc.) in dimethylformamide were added to 5 ml of NZMg top agar per 90 mm plate. The UV-sensitivity and proficiency for conjugational recombination phenotype of each host strain were routinely tested using a GE 63OT8 UV lamp for UV-sensitivity, and using JC158 (Hfr thr^+ leu^+ str^s) as donor for conjugational recombination proficiency (WILLETS, CLARK and LOW 1969). Control for conjugational proficiency used JC7221 (F′thr^+leu^+/*pyrB66*) as donor. Selection was for thr^+leu^+ exconjugants on M9 minimal agar plus streptomycin, histidine, arginine, thiamine and proline. Cells were transformed by the procedure of KUSHNER (1978).

Plasmid and phage construction: The procedures in MANIATIS, FRITSCH and SAMBROOK (1982) were followed, with minor modifications. Enzymes were obtained from Bethesda Research Labs, Boehringer-Mannheim and New England Biolabs and were used according to the conditions in MANIATIS, FRITSCH and SAMBROOK (1982).

Phage: All phage except for λlinkH were isolated from a Charon 4A phage library of BALB/c mouse DNA (CREWS *et al.* 1981; S. CREWS and H. V. HUANG, unpublished results). λV3 contains the V3 sequence; λV9 and λV11 contain V11 sequence; λV2, λV13 and λV18 contain the V13 sequence; λV10, λV19 and λV3207 contain the V1 sequence, and λV14 contains the V14A and V14B sequence (Figure 2). The V3, V11, V13 and V1 sequences are mouse immunoglobulin V_H gene segments of the T15 family (CREWS *et al.* 1981). The V14A and V14B sequences are also V_H gene-segments, but of the levan family (S. CREWS and H. V. HUANG, unpublished results).

λlinkH is a hybrid of λgtWES-λB, EMBL3A and Charon 4A (LEDER, TIEMEIER and ENQUIST 1977; FRISCHAUF *et al.* 1983; BLATTNER *et al.* 1977). The left arm and λB fragment are from λgtWES-λB; thus, λlinkH carries amber mutations in gene *W* and *E*. Our isolate of λgtWES-λB has a 6.7 kb tandem duplication of *H* to *lom*, from position *ca.* 13060 to position *ca.* 19730 of lambda (DANIELS *et al.* 1983), and λlinkH retains this duplication. The λB fragment is in the inverted orientation relative to other lambda sequences, and the *Sac*I 25881-*Xba*I 24508 region is replaced by a polylinker bordered by *Sac*I and *Xba*I recognition sites:

GAGCTCGAATTCCCGGGGATCCGTCGACCTGCAGATCTCTAGA.

The right arm of λlinkH is derived from an *in vivo* recombinant between EMBL3A and Charon 4A; it consists of EMBL3A sequences from *N* into the *OP* interval (including the *KH54* deletion) and of Charon 4A sequences from the *OP* interval into *QSR*80 and the right end of φ80. The size of λlinkH is 41.9 kb (86% lambda).

Plasmids: BP221 (Figure 3) consists of the 405-bp *Bgl*II to *Pst*I fragment, called the V3 sequence (Figure 2), from λV3 cloned into the *Bgl*II and *Pst*I sites in the polylinker of πMT1. πMT1 is a plasmid vector that consists of the *Tha*I 2522 to *Eco*RI, *rep bla* region of pBR322 (SUTCLIFFE 1978), with a synthetic *supF* (SEED 1983) cloned into the *Eco*RI site. Between *supF* and *rep* is a polylinker sequence containing *Eco*RI, *Sma*I, *Bam*HI, *Sal*I, *Pst*I, *Bgl*II, *Xba*I and *Hin*dIII restriction endonuclease recognition sites. Plasmids BP2212, BP2213 and BP2214 (Figure 4) are derived from BP221 by deletion of sequences between the *Bam*HI site in the polylinker and the *Rsa*I (92), *Ava*II (165) and *Rsa*I (241) sites, respectively. BP2211 is derived from BP221 by deleting the *Pst*I fragment (position 45 to 416, Figure 2). The plasmids BP2221, BP2222 and BP2223 are derived from BP221 by deletion of sequences between the *Bgl*II site (position 1,

TABLE 1

Bacterial strains (*E. coli* K12 derivatives) and their relevant genotypes

	Relevant genotype	Reference/source
Strains		
AB1157[a]	Rec$^+$	HOWARD-FLANDERS and THERIOT (1966)
JC5519	*recB21 recC22*	WILLETS and CLARK (1969)
JC3881	*recB21 recC22 recF143*	KATO, ROTHMAN and CLARK (1977)
RK1064	*recA56 recB21 recC22*	FISHEL, JAMES and KOLODNER (1981)
JC8679	*recB21 recC22 sbcA23*	GILLEN, WILLIS and CLARK (1981)
LE392	*supE supF*	MANIATIS, FRITSCH and SAMBROOK (1983)
MC1061	sup°	CASADABAN and COHEN (1980)
LG75	sup° *lacZ*^{am}	B. SEED
JC7221	F'*thr*$^+$ *leu*$^+$/*pyrB66*	GILLEN, WILLIS and CLARK (1981)
JC158	HfrH PO1 *serA6*	CLARK (1963)
Lambda phage		
λV3	Charon 4A clone containing V3 sequence	CREWS *et al.* (1981)
λV9	Charon 4A clone containing V11 sequence	S. CREWS and H. V. HUANG (unpublished results)
λV11	Charon 4A clone containing V11 sequence	CREWS *et al.* (1981)
λV2	Charon 4A clone containing V13 sequence	S. CREWS and H. V. HUANG (unpublished results)
λV13	Charon 4A clone containing V13 sequence	CREWS *et al.* (1981)
λV18	Charon 4A clone containing V13 sequence	S. CREWS and H. V. HUANG (unpublished results)
λV10	Charon 4A clone containing V1 sequence	CREWS *et al.* (1981)
λV19	Charon 4A clone containing V1 sequence	CREWS *et al.* (1981)
λV3207	Charon 4A clone containing V1 sequence	CREWS *et al.* (1981)
λV14	Charon 4A clone containing V14A, V14B sequences	S. CREWS and H. V. HUANG (unpublished results)
λlinkH	Charon 4A clone containing 38-bp poly-linker	This study
Plasmids		
BP221	Entire V3 sequence (405 bp) in πMT1	This study
BP2221	BP221 with truncated V3 sequence (315 bp)	This study
BP2222	BP221 with truncated V3 sequence (242 bp)	This study
BP2214	BP221 with truncated V3 sequence (239 bp)	This study
BP2213	BP221 with truncated V3 sequence (166 bp)	This study
BP2223	BP221 with truncated V3 sequence (166 bp)	This study
BP2212	BP221 with truncated V3 sequence (90 bp)	This study
BP2211	BP221 with truncated V3 sequence (44 bp)	This study

TABLE 1—Continued

	Relevant genotype	Reference/source
pV1305	168 bp of V13 sequence	This study
πMT1	*bla* and *rep* from pBR322, *supF* from πVX (SEED 1983)	This study
πMT638	entire polylinker (38 bp)	This study
πMT631	πMT638 with truncated polylinker (31 bp)	This study
πMT627	πMT638 with truncated polylinker (27 bp)	This study
πMT623	πMT638 with truncated polylinker (23 bp)	This study
πMT621	πMT638 with truncated polylinker (21 bp)	This study
πMT619	πMT638 with truncated polylinker (19 bp)	This study
πMT600	πMT638 with polylinker deleted	This study

[a] The complete genotype is F⁻ *thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 argE3 rpsL31 tsx-33 supE44 his-4.* (Strains JC5519, JC3881 (*his*⁺), RK1064 and JC8679 (*his-60*) are isogenic to AB1157).

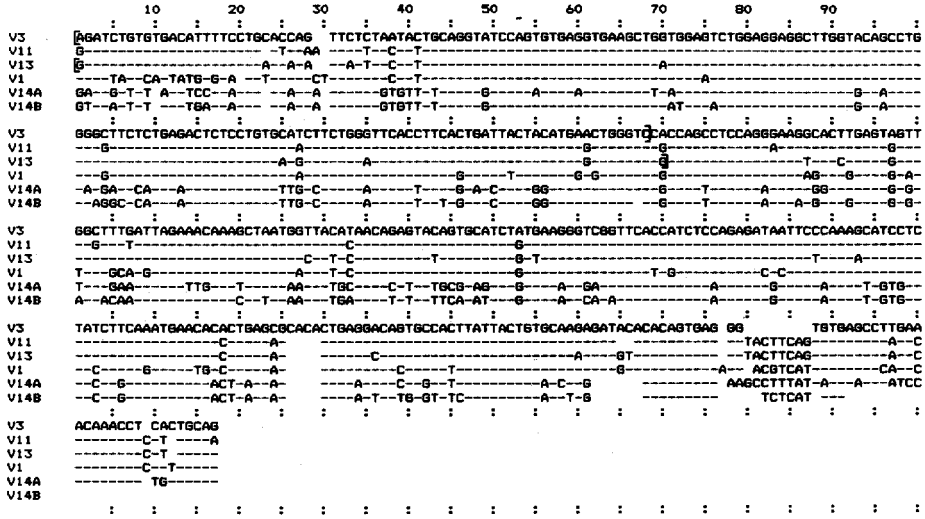


FIGURE 2.—Comparison between the V3 and the V11, V13, V1 and V14A, V14B sequences. Horizontal dashes indicate identity to V3. Differences are shown by the indicated base changes. Gaps are shown as blanks and are introduced to obtain best alignment. The extent of V3 and V13 sequences in BP2213 and pV1305, respectively (Table 4), are indicated by square brackets

Figure 2) and the *Rsa*I (92), *Ava*II (165) and *Rsa*I (241) sites of the V3 sequence, respectively. pV1305 is a 168-bp *Sau*3AI-*Ava*II fragment of the V13 sequence (position 1-170, Figure 2) from λV13, cloned in πMT1. πMT638 (Figure 5) is *supF* and has the same *rep bla* region as πMT1. The polylinker sequence of πMT638 is the only region of homology between it and λlinkH. πMT631, πMT627, πMT623, πMT621, πMT619, πMY600 (Figure 5) are derived from πMT638 by deletion of various lengths of the polylinker. The length of homology between each of the plasmids and λlinkH is shown in Figure 5.

Phage-plasmid crosses: Each cross was performed at least three times. Ten-milliliter aliquots of L broth, 50 μg/ml ampicillin, were inoculated from single colonies of plasmid-containing cells of each strain. The cultures were grown overnight at 37° with shaking. One hundred microliters of the overnight culture were inoculated into 10 ml of L broth, 50 μg/ml ampicillin, and were grown to early log phase (*ca.* 4×10^7 cells/

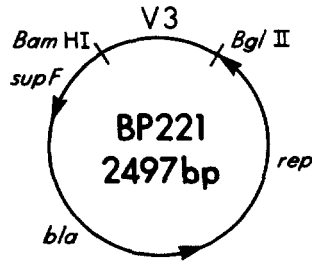


FIGURE 3.—The structure of the plasmid BP221. *bla* and *rep* are the beta-lactamase gene and the replication region, respectively, derived from pBR322. Restriction endonuclease sites relevant to the construction of BP221-derivatives are shown.

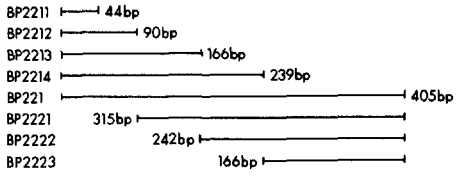
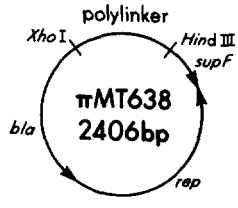


FIGURE 4.—Deletion derivatives of BP221 (see METHODS AND MATERIALS for the construction schemes). The extent and length of V3 sequence remaining in each derivative is indicated. The plasmids are otherwise identical to BP221 (Figure 3).



		Homology To λLinkH
λLinkH	GCTTCGAATGAGCTCGAATTCCCGGGGATCCGTCGACCTGCAGATCTCTAGAATATGATGAAAGAT	
πMT638	TG—ACCCCCTCGAG—GC-T—CGCTA—	38bp
	bla ←	→ *supF*
πMT631	TG—ACCCCCTCGAG— AGC-T—CGCTA—	31bp
πMT627	TG—ACCCCCTCGAG— AGC-T—CGCTA—	27bp
πMT623	TG—ACCCCCTCGAG— AGC-T—CGCTA—	23bp
πMT621	TG—ACCCCCTCGAG— AGC-T—CGCTA—	21bp
πMT619	TG—ACCCCCTCGAG— AGC-T—CGCTA—	19bp

FIGURE 5.—The structure of the plasmid πMT638. The *bla* and *rep* are the beta-lactamase gene and the replication region, respectively, derived from pBR322. The location of the polylinker sequence is shown. The sequence of the polylinker in λlinkH and πMT638 and the extent and length of the polylinker remaining in each plasmid derivative of πMT638 are shown.

ml). Cells were concentrated by pelleting at 4°, for 10 min at 1000 × *g*, and were resuspended with 100 μl of ice-cold SM. Appropriate dilutions were spread on L plates with or without 50 μg/ml ampicillin to measure the frequency of plasmid-containing cells. Appropriate dilutions of the cells were also tested for conjugational recombination and for UV-sensitivity to confirm the phenotype expected of each strain (see References in Table 1). One hundred-microliter aliquots of 10^8 cells were mixed with 100 μl, containing 10^7 plaque-forming unit (input multiplicity of infection = 0.1), of the appropriately diluted phage stocks and were incubated for adsorption at 37° with no shaking. After 10 min, 10 ml of ice-cold SM was added, and the cells were pelleted. The cells were resuspended with 10 ml of ice-cold SM and were pelleted again to remove un-

adsorbed phage. The cell pellets were resuspended with 100 μl of ice-cold SM. Appropriate dilutions were added to 100-μl aliquots of a fresh LE392 (*supE supF*) overnight culture and 5 ml of NZMg top agar and were plated on 90-mm NZMg plates to measure the total number of infected cells. Dilutions of the cells were mixed with 0.5 ml of MC1061 (*sup°*, 3×10^9 cells; a fresh overnight culture was pelleted and resuspended in 1/6 volume of ice-cold SM) and 15 ml of NZMg top agar and were plated on 150 mm NZMg plates to enumerate cells that produce recombinant phage. The recombinant frequency is defined as the total number of recombinant infective centers divided by the total number of infective centers. The large excess of sup° cells was used to minimize secondary recombination events when a large number of infected cells (10^3–10^6) are deposited per 150-mm plate. Recombinant plaques were very small under these conditions. Random recombinant plaques were stabbed onto lawns of LG75 (*sup° lacZ*^{am}) in the presence of IPTG and Xgal to assay for *supF* in the recombinant cointegrate. Representative recombinant phage were grown, phage DNA were isolated and were characterized by restriction mapping and DNA sequencing (HUANG, CREWS and HOOD 1981; MANIATIS, FRITSCH and SAMBROOK 1982).

RESULTS

Homologous recombination between phage and plasmid: We performed crosses between the plasmid BP221 and the lambda phage λV3. The plasmid BP221 (Figure 3) consists of the 405-bp V3 sequence from λV3, cloned in πMT1 [a plasmid vector that includes the *bla* and *rep* regions of pBR322 and a synthetic *supF* (SUTCLIFFE 1978; SEED 1983)]. λV3 is a Charon 4A clone of 16-kb of BALB/c mouse DNA, including the V3 sequence and flanking mouse DNA (METHODS AND MATERIALS; CREWS *et al.* 1981; HUANG, CREWS and HOOD 1981). λV3 has amber mutations in genes *A* and *B* and requires the product of *supF*, for example, to complete its infection cycle. It is not able to form plaques on sup° cells. If recombination occurs between the V3 sequences of the phage λV3 and the plasmid BP221, a phage-plasmid cointegrate can be formed (Figure 1). The cointegrate carries the *supF* gene, can be packaged and can form plaques on sup° cells.

AB1157 cells containing BP221 were infected with λV3 and were plated for infective centers in the presence of excess MC1061, sup° cells. Twelve percent of the infected cells gave rise to plaques. Four lines of evidence show that the host suppressor-independent phage giving rise to these plaques are λV3-BP221 cointegrates, resulting from homologous recombination between the V3 sequences. First, the frequency of suppressor-independent plaques of λV3 after infection of plasmid-free sup° cells was $<10^{-8}$, in contrast to 1.2×10^{-1} found after infection of sup° cells containing BP221. Second, the frequency of host suppressor-independent plaques was 3.5×10^{-7} when λV3 infected AB1157 containing πMT1, the plasmid vector without the V3 sequence. Thus, phage-plasmid homology is essential for a high frequency of suppressor-independent plaques. Third, host suppressor-independent phage give blue plaques on LG75 (sup° *lacZ*^{am}) in the presence of IPTG and Xgal and, thus, carry *supF*. Fourth, restriction mapping and DNA sequencing (results not shown) of host suppressor-independent phage demonstrate that the plasmid is indeed integrated into the phage in an arrangement expected of cointegrates formed by homologous recombination (see Figure 1).

TABLE 2

Genetics of phage-plasmid recombination

Strain	Relevant genotype	Recombinant frequency[a]	Deficiency index[b]
MC1061	+	1.2×10^{-1}	
AB1157	+	1.2×10^{-1}	≡1
JC5519	*recB21 recC22*	9.5×10^{-3}	15
JC3881	*recB21 recC22 recF143*	3.9×10^{-4}	359
RK1064	*recA56 recB21 recC22*	2.0×10^{-5}	7000
JC8679	*recB21 recC22 sbcA23*	7.1×10^{-3}	20

[a] Cells containing the BP221 plasmid are infected with the λV3 at a multiplicity of 0.1, excess phage is removed by washes and dilutions are plated with an excess (more than tenfold) of MC1061 (sup°) or LE392 (*supE supF*). The recombinant frequency is defined as the number of infective centers on MC1061 divided by the number of infective centers on LE392.

[b] Deficiency index is defined as the recombinant frequency of AB1157 divided by the recombinant frequency of each recombination deficient strain.

Genetic requirements for phage-plasmid recombination: No known recombination functions are encoded by plasmid BP221, and λV3 is deleted for all known phage recombination genes (*int red gam*) (ECHOLS and GUARNEROS 1983; SMITH 1983). Thus, recombination is expected to be dependent on host functions. Two different wild-type strains, AB1157 and MC1061, harboring the plasmid BP221 give equal frequencies of recombinants with λV3 (Table 2). We measured the frequencies of recombinants between λV3 and BP221, using strains with mutations in recombination genes but otherwise isogenic to AB1157 (Table 2). The frequency of recombinants is decreased in both *recBC*$^-$ and *recA*$^-$ *recBC*$^-$ strains. Thus, homologous recombination between phage and plasmid is dependent on *recA* and *recB recC*. The recombinants in the *recA*$^-$ *recBC*$^-$ strain might be due to nonhomologous recombination events (MARVO, KING and JASKUNAS 1983).

The *recA*$^-$ *recBC*$^-$ and the *recBC*$^-$ *recF*$^-$ strains give recombinant frequencies lower than the *recBC*$^-$ strain. Thus, the low level of recombination in the *recBC*$^-$ strain is *recA*- and *recF*-dependent. We have not been able to measure the frequency of recombinants in a *recBC*$^-$ *sbcB*$^-$ strain, which gives wild-type levels of conjugational recombination, because pBR322-derived plasmids are unstable in the strain (results not shown; VAPNEK *et al.* 1976; REAM, CRISONA and CLARK 1978).

The results show that phage-plasmid recombination, like conjugational recombination, is mediated by the RecBC and RecF-dependent pathways. The *recBC*$^-$ *sbcA*$^-$ strain, JC8679, is UV-resistant and gives wild-type levels of recombination in conjugation (results not shown; GILLEN, WILLIS and CLARK 1981), but the same cultures are as deficient as the *recBC*$^-$ strain for phage-plasmid recombination.

Length dependence of phage-plasmid recombination: The recombinant frequencies measured using the wild-type strain AB1157 are linearly dependent on substrate length in the range 27–405 bp (Table 3; Figure 6a). A least-

TABLE 3

Effects of substrate length

Substrate length (bp)	Recombinant frequency		
	AB1157	JC5519	JC8679
405	1.2×10^{-1}	9.5×10^{-3}	7.1×10^{-3}
315	1.2×10^{-1}	5.1×10^{-3}	9.3×10^{-3}
242	9.0×10^{-2}	4.4×10^{-3}	3.2×10^{-3}
239	5.9×10^{-2}	4.1×10^{-3}	1.5×10^{-3}
166[a]	3.7×10^{-2}	2.3×10^{-3}	2.4×10^{-3}
166[b]	6.1×10^{-2}	1.8×10^{-3}	3.1×10^{-3}
90	1.9×10^{-2}	6.3×10^{-4}	5.5×10^{-4}
44	3.6×10^{-3}	4.7×10^{-5}	5.3×10^{-5}
38	2.1×10^{-3}	1.6×10^{-5}	ND
31	9.4×10^{-4}	3.9×10^{-6}	ND
27	3.5×10^{-4}	1.7×10^{-6}	ND
23	5.8×10^{-5}	2.2×10^{-7}	ND
21	4.8×10^{-5}	1.6×10^{-7}	ND
19	2.0×10^{-5}	ND	ND
0	$<9.7 \times 10^{-7}$	ND	ND

ND = not determined.
[a] Plasmid BP2213
[b] Plasmid BP2223

squares linear regression of the data gives a slope of 3.5×10^{-4}/bp for 27–405 bp. There is a break between 23 and 27 bp in the graph. The recombinant frequencies for 19-, 21- and 23-bp substrate lengths are about 10-fold lower than the frequency at 27 bp, and they constitute a different linear dependence of recombinant frequency on substrate lengths, having a slope of 1×10^{-5}/bp.

Recombinant frequencies measured using the *recBC*$^-$ strain JC5519 are also linearly dependent on substrate lengths from 90–405 bp (slope of 2.7×10^{-5}/bp, Figure 6b). There is a break between 90 and 44 bp in the graph. From 166 to 90 bp the frequency decreases threefold, whereas the frequency decreases 13-fold from 90 to 44 bp (Table 3). From 44 bp to 21 bp the graph appears to be nonlinear.

The data show the existence of threshold substrate lengths, between 23–27 bp in rec$^+$ and 44–90 bp in *recBC*$^-$ strains. In addition, recombination over the entire range of substrate lengths (19–405 bp) are *recBC*-dependent in the *recBC*$^+$ strain. Thus, the threshold length of 23–27 bp is a property of the RecBC pathway itself; it is not due to some other pathway becoming apparent at short substrate lengths, as suggested by SINGER *et al.* (1982) for T4 recombination. The threshold length of 44–90 bp observed in the *recBC*$^-$ strain is a property of the RecF-dependent pathway, since the *recBC*$^-$*recF*$^-$ strain gives at least 10-fold lower recombinant frequencies over the range 38–405 bp (results not shown).

Strains JC8679 (*recBC*$^-$*sbcA*$^-$) and the JC5519 (*recBC*$^-$) give similar recombinant frequencies for substrate lengths of 44–405 bp (Table 3). We do not

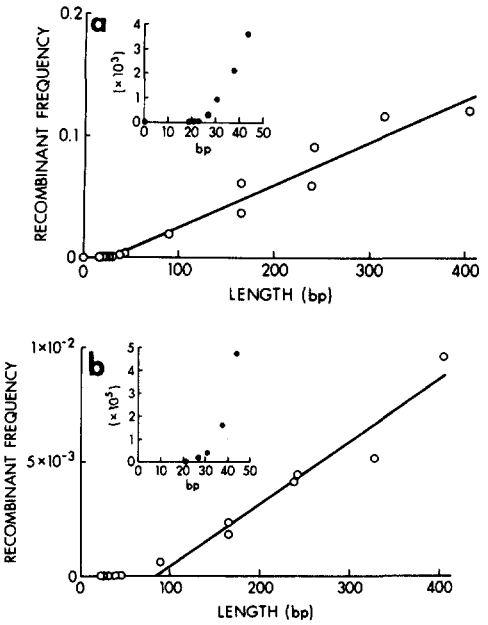


FIGURE 6.—The recombinant frequencies as a function of the length of perfectly homologous substrates. The least-square linear regression is shown for each strain. Insets show expanded views of the region involving very short substrates: a, AB1157—wild-type strain; *b*, JC5519—*recB21 recC22* strain.

detect any additional phage-plasmid cointegrates formed by the RecE pathway. Strain JC8679, compared to JC5519, gave more variable recombinant frequencies, possibly reflecting the effect of variable multimerization of the plasmid (results not shown; FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982) on the recombinant frequencies (H. V. HUANG, unpublished results).

Homology dependence of phage-plasmid recombinations: We measured the frequency of recombination of defined substrate homologies ranging from 65 to 90% [defined as the number of sequence matches with V3 divided by the total sequence length (Figure 2; METHODS AND MATERIALS)].

We used three independent Charon 4A phage clones of the same V13 sequence (λV2, λV13, and λV18, see METHODS AND MATERIALS)—that differed in clone size, position and orientation of the V sequence—to control for effects other than substrate homology. We measured the recombinant frequencies of BP2213 (Figures 2 and 3) crossed with λV3 (100% homology) and with λV2, λV13, and λV18 (92% homology) and the recombinant frequencies of pV1305 (Figure 2) crossed with λV2, λV13 and λV18 (100% homology) and with λV3 (92% homology). The results of the crosses in the wild-type AB1157 strain are shown in Table 4.

pV1305 recombines with λ2, λV13 and λV18 (168 bp of perfect homology) at an average frequency of 4.5×10^{-2}, similar to the recombinant frequency of 3.5×10^{-2} between BP2213 and λV3 (166 bp of perfect homology, Table

TABLE 4

Effects of substrate homology *vs.* other effects

Wild type—A. Recombinant frequencies[a]

Plasmid	Phage λV3	λV2	λV13	λV18
BP2213	3.5×10^{-2}	1.9×10^{-3}	4.5×10^{-4}	1.9×10^{-3}
		mean = 1.4×10^{-3}, range[b] = fourfold		
pV1305	1.0×10^{-3}	4.1×10^{-2}	2.5×10^{-2}	6.9×10^{-2}
		mean = 4.5×10^{-2}, range = threefold		
πMT1	3.5×10^{-7}	ND	ND	ND

B. Summary

Homology (%)	Crosses	Recombinant Frequency	Relative Frequency[c]
100	BP2213 × λV3	3.5×10^{-2}	≡1
	pV1305 × λV2, λV13, λV18	4.5×10^{-2}	0.78
92	BP2213 × λV2, λV13, λV18	1.4×10^{-3}	25
	pV1305 × λV3	1.0×10^{-3}	35
None	πMT1 × λV3	3.5×10^{-7}	10^5

ND = not determined.

[a] Mean recombinant frequencies from three to six crosses.

[b] Range = highest recombinant frequency/lowest recombinant frequency.

[c] Relative frequency = recombinant frequency of BP2213 × λV3/recombinant frequency of the indicated crosses.

4). Thus, recombination is equally efficient for these two pairs of 100% homologous sequences. There is no evidence for sequence specificity that distinguishes the V3 from the V13 sequence pairs. The threefold range of recombinant frequencies between λV2 λV13 and λV18 when crossed with pV1305 gives an indication of the amount of variability due to factors other than substrate homology.

When homology was decreased to 92%, in the crosses between BP2213 and λV2 λV13, or λV18, the average recombinant frequency was 1.4×10^{-3}. Similarly, the pV1305 and λV3 cross (92% homology) gave a frequency of 1.0×10^{-3}. Thus, the decrease of homology resulted in a 25- to 45-fold decrease of recombinant frequency, compared to the crosses using 100% homologous substrates. Effects other than homology, as indicated by crosses involving λV2, λV13 and λV18, are minor in comparison.

Given that substrate homology is indeed the major determinant of recombinant frequencies, we measured the recombination between 65–90% homologous substrates (Table 5). First, decreased homology has a dramatic effect on the recombinant frequency in all three strains (Table 5). For example, *rec*$^+$ cells give decreases of 42-, 88-, 246- and 300-fold for 90, 89, 84 and 70–65% substrate homologies, respectively. In comparison, different phage carrying the same V sequence (for example, the λV2, λV13, λV18 group) give, at most, a

TABLE 5

Effects of substrate homology

Homology %	BP221 crossed with	Relative frequencies[a]		
		AB1157 (rec$^+$)	JC5519 (recBC$^-$)	JC8679 (recBC$^-$ sbcA$^-$)
100	λV3	≡1 (1.2×10^{-1})[b]	≡1 (8.7×10^{-3})[b]	≡1 (7.1×10^{-3})[b]
90	λV9	17	38	76
	λV11	67 mean = 42	67 mean = 53	178 mean = 127
89	λV2	80	62	37
	λV13	146	223	209
	λV18	38 mean = 88	126 mean = 137	111 mean = 119
84	λV10	245	121	394
	λV19	150	126	473
	λ3207	43 mean = 146	290 mean = 179	108 mean = 325
65, 70[c]	λV14	300	435	888

[a] Relative frequencies = recombinant frequency of the 100% homologous cross/recombinant frequency of the indicated crosses.

[b] Recombinant frequencies of the 100% homologous crosses in the respective strains.

[c] λV14 contains the V14A and V14B sequences that are, respectively, 70 and 65% homologous to the V3 sequence.

sixfold difference in recombinant frequencies, reaffirming the dominant effect of homology on recombination. Second, at all levels of homology, the recombination in wild-type cells is *recBC*-dependent, since *recBC*$^-$ cells give lower recombinant frequencies at all homologies tested. Third, we see no convincing differences between the results for the *recBC*$^-$ strain and the *recBC*$^-$ *sbcA*$^-$ strain, indicating that the RecE pathway has no detectable effects on the homology sensitivity in these crosses. Fourth, all three strains show comparable reductions in recombinant frequencies for any given decrease in substrate sequence homology. Thus, there is no convincing difference in homology dependence between the three strains in contrast to the distinct difference in the threshold lengths of wild-type and *recBC*$^-$ strains.

DISCUSSION

Genetics of phage-plasmid recombination: Homologous recombination between *int*$^-$ *red*$^-$ *gam*$^-$ phage and plasmid (Table 2) is mediated by the RecBC pathway, requiring *recA* and *recBC*. There is a low level of RecF-mediated recombination detectable in a *recBC*$^-$ cell, which requires *recA* and *recF*.

Strain JC8679 (*recBC*$^-$ *sbcA*$^-$) is UV-resistant and gives wild-type levels of conjugational recombination. However, cells from the same cultures give recombinant frequencies comparable to the *recBC*$^-$ strain for all substrate lengths (44–405 bp) and substrate homologies (65–100%) tested. The reason for this surprising result is unclear. It is possible that RecE-mediated phage plasmid recombination is predominantly nonreciprocal and therefore fails to give cointegrates. Alternately, the RecE pathway might require much more than 405 bp of perfect homology to act efficiently. This seems unlikely, since RecE-

mediated, plasmid-plasmid recombination proceeds efficiently for closely spaced markers separated by 275 bp (JAMES, MORRISON and KOLODNER 1982), although it is not clear if flanking homologies, present in the plasmid-plasmid recombination system and absent in the phage-plasmid recombination system, might be needed by RecE. Further, it is conceivable that phage-plasmid recombination involves structural or topological constraints that are not met by the RecE pathway.

Length thresholds and minimal efficient processing segments (MEPS): There is a linear dependence of recombinant frequencies on substrate lengths for both the RecBC and RecF-dependent pathways. Both pathways have a *threshold substrate length* that we shall call MEPS, for minimal efficient processing segment, below which the recombinant frequency decreases sharply. The length of MEPS is between 23 and 27 bp for the RecBC pathway and between 44 and 90 bp for the RecF-dependent pathway. Similar thresholds have been found in other studies. For T4 recombination, BAUTZ and BAUTZ (1967) found a threshold at a length of five mutation sites, corresponding to *ca.* 50 bp (PRIBNOW *et al.* 1981), and SINGER *et al.* (1982) found a change in the slope of the length-dependence curve at *ca.* 50 bp. WATT *et al.* (1985) showed that a minimum of *ca.* 20 bp of homology is required for phage-plasmid recombination, consistent with our results showing that 19–21 bp long homologies give recombinant frequencies at least 20-fold higher than crosses where there is no homology between phage and plasmid (Table 3). *In vitro* studies show that RecA promotes heteroduplex formation when the substrates have 151 bp of homology, but no detectable heteroduplex formation was found with 30 bp of homology (GONDA and RADDING 1983).

MEPS as a recombination unit: We propose that the MEPS length and its associated recombination frequency can serve as unit measures for genetic lengths. Any pair of substrates is considered to be a collection of MEPS at which recombination can occur. For a linear pair of perfect homologies L bp long, there are $L - M + 1$ MEPS of M bp long. If all MEPS have equal probabilities of serving as targets for recombination, then substrates L bp long will recombine at a frequency, f_L, equal to $(L - M + 1)F$, where F is the frequency of recombinants for a single MEPS. If the linear length dependence of recombinant frequency is expressed by the linear regression: $f = a + bL$, where a is the y-intercept and b is the slope, then $F = b$, and $M = 1 - a/b$. For the wild-type strain, the calculated length of MEPS is 29.6 bp, slightly longer than the observed 23–27 bp. Its associated recombinant frequency, F_{RecBC}, is 3.5×10^{-4} MEPS^{-1}. For the *recBC*$^-$ strain, MEPS is calculated to be 86 bp, and $F_{\text{RecF}} = 2.7 \times 10^{-5}$ MEPS^{-1}.

The molecular bases of MEPS: The RecBC pathway for phage-plasmid recombination requires *recA* and *recBC* (Table 2) both above and below the MEPS length (Table 3). The RecF-dependent pathway requires *recA* and *recF*. At present we do not know the precise roles or the stage of recombination at which each of these enzymes might act in determining the length of MEPS of each pathway. However, given that the length of MEPS of the RecBC pathway and the RecF-dependent pathway is different, it is possible that the length of

MEPS is determined by multiple components, each characterized by its length requirements: there might be a component specific to the RecBC or the RecF pathway, and a component common to both pathways, perhaps RecA. We cannot exclude the participation of other enzymes that might be involved in recombination. These include single strand-binding protein, DNA polymerase, DNA ligase and mismatch-repair enzymes.

The existence of threshold substrate lengths or MEPS may be interpreted by two classes of models. First, recombination above and below the threshold might be due to distinct recombination pathways, as proposed by SINGER *et al.* (1982) for T4 recombination. The *recBC*-dependence of recombination at all lengths of homology makes this unlikely (Table 3). Thus, the MEPS of wild-type cells is a property of the RecBC pathway. Similarly, in *recBC*$^-$ cells, recombination is dependent on *recF* both above and below the length of MEPS, and therefore, the MEPS of *recBC*$^-$ cells is a property of the RecF-dependent pathway.

The second class of models involves physical constraints on recombination when the substrate length is short. Recombination might require a certain minimum amount of physical space to operate efficiently. The amount of space required depends on the respective sizes and the stabilities of the enzyme-DNA complexes during initiation of recombination, branch migration and resolution of the recombination intermediates. When substrate lengths are shorter than the length of MEPS, recombination becomes inefficient or ineffective. One possibility is that independent recombination events interfere with each other, and the substrate length at which this interference becomes significant gives rise to the observed length of MEPS. Alternately, the individual steps of a single recombination event (RADDING *et al.* 1982; DRESSLER and POTTER 1982) interfere with each other if the substrate is too short. For example, successful completion of one step (*e.g.*, initiation) takes up so much space on short substrates that a subsequent step (*e.g.*, resolution) is inhibited because the amount of space the latter requires is not available.

In addition, recombination may be inefficient if the intermediates are too unstable or if they exist too transiently. The RecF pathway seems to operate much slower than the RecBC pathway (MAHAJAN and DATTA 1979; LLOYD and THOMAS 1983). Perfect homologies that are too short might give intermediates that are too unstable for the relatively slow RecF-dependent pathway to convert efficiently into stable recombinants. It is also possible that intermediates having only short heteroduplexed regions are rapidly destroyed by some enzyme activity in the cell. Thus, the length of the RecF MEPS could reflect the kinetics of the RecF-dependent pathway and the half-life of any unstable recombination intermediates.

Homology dependence: Both the RecBC and RecF-dependent pathways are extremely sensitive to substrate homology (Table 5; SEED 1983; WATT *et al.* 1985). We propose that, during recombination of mismatched substrates, the rate-limiting step, perhaps initiation, depends on regions of perfect homology. Support for this comes from comparing the data for homology dependence and for length dependence of recombination. For both RecBC and RecF-

dependent pathways, the recombinant frequency between mismatched substrates (Table 5) resembles the recombinant frequencies for short, perfect homologies (Table 3). For example, the longest region of perfect homology in the BP221-V9 or V11 crosses is 64 bp (Figure 2). The recombinant frequency, via the RecBC pathway, is 1.8 to 6.9 $\times$ 10^{-3} (Table 5). It is comparable to, or higher than the recombinant frequency for 44 bp long, 100% homologous substrates and is lower than that for 90 bp long, 100% homologous substrates (Table 3). Thus, homology detection might effectively be a search for perfect sequence matches.

The RecBC and RecF-dependent pathways show similar dependence on substrate sequence homology (Table 5). This is in contrast to the different length of MEPS for the RecBC and RecF-dependent pathways. We think that the similarity of the homology dependence of the RecBC and the RecF-dependent pathways reflects the properties of a component sensitive to mismatches and common to both pathways. The most likely candidate is RecA, required by both pathways. The rate of RecA-dependent strand exchange for mismatched substrates *in vitro* is lower than that for perfect homologies (BIANCHI and RADDING 1983). If this *in vitro* property of RecA is also true *in vivo*, then mismatched substrates should be used relatively slowly by RecA. The dramatic decreases of recombinant frequencies with decreased substrate homology for both RecBC and RecF-dependent pathways might therefore reflect primarily the properties of RecA.

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