ACTIVITY OF CHI RECOMBINATIONAL HOTSPOTS IN *SALMONELLA TYPHIMURIUM*

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ABSTRACT

Chi sites have previously been shown to stimulate homologous recombination by the *Escherichia coli* RecBC pathway. To test the activity of Chi in another organism, bacteriophage λ crosses were carried out in *Salmonella typhimurium* strains bearing the *E. coli* λ receptor protein. Chi is active in these crosses in *S. typhimurium*, but is less active than in the same crosses carried out in *E. coli*. The lower Chi activity in *S. typhimurium* appears to be intrinsic to the *S. typhimurium* RecBC enzyme, since the Chi activity in *E. coli-S. typhimurium* hybrids depends on the species of origin of their RecBC enzyme. For these studies we constructed an F′ factor and a pBR322-derived plasmid carrying the *thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$ region of the *S. typhimurium* chromosome.

CHI sites in bacteriophage λ locally stimulate recombination by the *Escherichia coli* RecBC pathway. (For reviews, see STAHL 1979; SMITH 1983.) The action of Chi has been extensively studied in bacteriophage λ vegetative crosses in which recombination occurs by the host RecBC pathway. In addition, Chi is active in recombination of the repressed λ prophage following P1-mediated transduction and Hfr-mediated conjugation of *E. coli* (DOWER and STAHL 1981). Chi sites are determined by the nucleotide sequence 5′ G-C-T-G-G-T-G-G 3′ (SMITH *et al.* 1981b; PONTICELLI *et al.* 1985), and this sequence is found in the chromosomes of numerous organisms, such as yeast (CHATTORAJ *et al.* 1978) and mice (KENTER and BIRSHTEIN 1981). The occurrence of Chi in other organisms raises the possibility that Chi is active in recombination in organisms other than *E. coli*.

As a first test of this possibility, we have examined the activity of Chi in *Salmonella typhimurium*. Although the physiology and the genetic map of this bacterium are similar to those of *E. coli* and although the two organisms mate with high frequency, the nucleotide sequences of the two are only about 50% homologous, and genetic recombination between their chromosomes is infrequent (SANDERSON 1976). The results of the studies reported here show that Chi is active in *S. typhimurium* and suggest that Chi and the recombination-

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promoting factors interacting with it have been at least partially conserved during the evolution of these organisms.

The activity of Chi is readily measured in λ vegetative crosses, since there are available derivatives of λ with and without Chi sites in genetically marked intervals. Chi activity can be conveniently measured as the ratio of the frequency of recombinants with an exchange in a particular interval with Chi to that in the same interval without Chi. Since *S. typhimurium* is not the normal host for λ, the initial experiments reported here used derivatives of *S. typhimurium* in which derivatives of λ can grow. Additional experiments used derivatives of *E. coli* containing either the *E. coli recBC* genes or the *S. typhimurium recBC* genes.

MATERIALS AND METHODS

Bacterial and phage strains: Bacterial and phage strains, their genotypes and sources are listed in Tables 1 and 2, respectively.

Strain GS1007 was received from R. MAURER as strain DB4673. This strain was constructed by PALVA, LILJESTROM and HARAYAMA (1981), who designated it TS736. As described by them, TS736 is a derivative of *S. typhimurium* LT2 with a deletion of the *malB* region and containing F′112, which carries the *malB* region of *E. coli* K12. The *malB* region of *E. coli*, but not that of *S. typhimurium*, specifies the cell surface protein to which λ adsorbs. Although strain GS1007 is sensitive to λ, it proves to be a female by the following tests: (1) it is sensitive to the *S. typhimurium* female-specific phage SP6 (ZINDER 1961), (2) it fails to transfer the Mal^+ character to an *E. coli malB* mutant, (3) no Mal^- segregants have been observed even after extensive growth in the presence of acridine orange and (4) it readily accepts another *F* factor (data not shown). We presume that in the strain used here the *malB* region of *E. coli* is integrated into the *S. typhimurium* chromosome.

Culture media: Tryptone broth contains, per liter, 10 g Bacto-tryptone (Difco) and 5 g NaCl. Minimal medium contains, per liter, 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 2 g $(NH4)_2\ SO_4$, and 1 g Na_3 citrate; glucose (0.4% w/v) was added after autoclaving. LB agar contains tryptone broth plus 5 g Bacto-yeast extract (Difco), 15 g Bacto-agar (Difco) and 1 ml of 1 N NaOH.

Construction of F′ *thyA⁺ recC⁺ recB⁺ argA⁺* from *S. typhimurium*: Since recombination between *S. typhimurium* DNA and *E. coli* DNA is a rare event (SANDERSON 1976), mating between an *S. typhimurium* Hfr and an *E. coli* female should allow selection for an F′ factor bearing *S. typhimurium* genes near the point of transfer origin of the Hfr. Thus, *S. typhimurium* strain GS1036, with Hfr point of origin between *serA* and *argA* (SANDERSON *et al.* 1972), was mated with *E. coli* strain V82, carrying *argA81*::Tn*10 recB21 recC22*, to select F′ $argA^+$, which was then tested for the presence of *thyA*, *recC* and *recB*.

Cultures of GS1036 and V82 growing exponentially in L broth, at a density of about 2×10^8 cells/ml, were mixed (1 ml of each) and incubated without shaking for 2.5 h at 37°. The cells were collected by centrifugation and were resuspended in 1 ml of 10 mM $MgSO_4$. A sample of 0.1 ml was plated on minimal agar supplemented with 20 μg each of threonine and leucine per milliliter and was incubated overnight at 37°. Fifteen colonies appeared from the mating, but from the individual cultures, similarly treated, no colonies appeared. These colonies were purified by single colony isolation, once on supplemented minimal agar and twice on LB agar. All were sensitive to λ *cI60* (like V82 and unlike GS1036) but were resistant to UV light (like GS1036 and unlike V82). Of four strains tested, all were sensitive to phage T4 and resistant to phage P22 (like V82 and unlike GS1036) and were sensitive to the male-specific coliphage R17 (unlike V82). All of the 15 strains transferred at high frequency the Arg^+ character to strain

TABLE 1

Bacterial strains

Strain designation	Genotype	Source[a] or reference
Strains with *S. typhimurium* chromosome		
GS1007	*his-6165 ilv-452 metA22 metE551 trpB2 galE496 xyl-404 rpsL120 flaA66 hsdL6 hsdSA29 malB* (*E. coli*)	R. MAURER (strain DB4673); PALVA, LILJESTROM and HARAYAMA (1981) (strain TS736)
GS1010	*hisG recB*::Tn*10*	R. MAURER (strain DB4659)
GS1011	As GS1007 plus *thyA*	Spontaneous derivative of GS1007
GS1014	As GS1011 plus Tn*10* (location unknown)	GS1010 × GS1011
GS1015	As GS1011 plus *recB*::Tn*10*	GS1010 × GS1011
GS1036	*serA13 rfa-3058* (HfrK3)	K. SANDERSON (strain SA486); Sanderson *et al.* (1972)
Strains with *E. coli* chromosome		
AFT140	*recA56 srl-300*::Tn*10 thr-300 ilv-318 spc-300* (Hfr PO45)	See SCHULTZ, TAYLOR and SMITH (1983)
AFT228	*argA81*::Tn*10*	See SCHULTZ, TAYLOR and SMITH (1983)
AFT379	*thyA tonA his gal rpsL endA supE*	PONTICELLI *et al.* (1985)
S927	*thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 his-4 argE3 rpsL31 tsx-33 mtl-1 supE44 thyA* (F′ 15 *thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	See SCHULTZ, TAYLOR and SMITH (1983)
S928	*recA ton lac his trp thyA rpsL spc* (F′ 15 *thyA*$^+$ *recC*$^+$ *recB21 argA*$^+$)	See SCHULTZ, TAYLOR and SMITH (1983)
S930	As S928 but (F′ 15 *thyA*$^+$ *recC*$^+$ *recC22 argA*$^+$)	A. CLARK (strain JC5532)
V80	*thr-1 leu-6 thi-1 lacY1 supE44 tonA21 recB21 recC22* r^-m^- *lop-11*(?)	K. SPRAGUE (strain SF8)
V82	As V80 plus *argA81*::Tn*10*	AFT228 × V80
V186	*del(thyA-argA)232*[b]	CHAUDHURY and SMITH (1984b)
V199	As V82 plus (F′ *Sty thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	GS1036 → V82
V201	As V186 plus (F′ *Sty thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	V199 → V186
V203	As V186 plus (F′ 15 *thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	S927 → V186
V229	r^+_{SB} m^+_{SB} r^-_K m^-_K	R. MAURER (strain DB4734); (HATTMAN *et al.*, 1976; strain 3.21)
V230	r^-_K m^-_K (deletion)	R. MAURER (strain DB4934)
V241	*del(thyA-argA)232* (pBR322::*Sty thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	see MATERIALS AND METHODS
V243	*del(thyA-argA)232* (pBR322::*Eco thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$)	see MATERIALS AND METHODS
V261	*recA56 sr1–300*::Tn*10 del(thyA-argA)232*	AFT140 × V186
594	*lac-3350 galK2 galT22 rpsL179*	WEIGLE (1966)
C600	*thr-1 leu-6 thi-1 supE44 lacY1 tonA21*	APPLEYARD (1954)
D202	*argA81*::Tn*10 thyA tonA his gal rpsL endA supE*	AFT228 and AFT379 via intermediate strains

[a] *A* × *B*, phage P22 (for *S. typhimurium*) or P1 (for *E. coli*) mediated transduction, where *A* is the donor and *B* is the recipient; *A* → *B*, *F*-mediated conjugation, where *A* is the donor and *B* is the recipient.

[b] This deletion removes *recBC*, located between *thyA* and *argA*.

TABLE 2

Phage λ strains

Strain designation	Genotype
1081	*susJ6 b1453 cI857* χ^+D123
1082	*b1453* χ^+D123 *susR5*
1083	*susJ6 b1453* χ^+76 *cI857*
1084	*b1453* χ^+76 *susR5*
1395	*tsJ15 b1453* imm^{21} χ^+D123
1396	*b1453* imm^{21} cI^- χ^+D123 *tsR129*
1397	*tsJ15 b1453* χ^+76 imm^{21}
1398	*b1453* χ^+76 imm^{21} cI^- *tsR129*

Phages 1395–1398 were derived, respectively, from phages 1081–1084, obtained from F. and M. STAHL (STAHL and STAHL 1977), by vegetative crosses with imm^{21}, *tsJ15* and *tsR129* phages from our collection. The phage markers are written in the order of their occurrence on the λ map (see CAMPBELL 1971). *b1453* is a deletion removing *int, red* and part of *gam* (HENDERSON and WEIL 1975). The χ^+76 mutation was derived from a phage carrying the *b1453* mutation; it is inseparable from the *b1453* deletion (KOBAYASHI *et al.* 1982). The location of the χ^+D site is from SMITH *et al.* (1981a) and SANGER *et al.* (1982).

V186, carrying a deletion of the *thyA recC recB argA* region, and these derivatives were found to be Thy$^+$ and UV-resistant. We conclude that the strains contain F′ *thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$ from *S. typhimurium*; one of these strains was designated V199. This strain, the only one tested, transferred at high frequency to strain V261 (a *recA56* derivative of strain V186) the Arg$^+$, Thy$^+$ and RecB$^+$C$^+$ characters [determined by insensitivity to phage T4 2^- as described by CHAUDHURY and SMITH (1984a)].

Construction of plasmid pBR322 derivatives carrying *E. coli recB*$^+$*C*$^+$ or *S. typhimurium recB*$^+$*C*$^+$: We have reported (PONTICELLI *et al.* 1985) the molecular cloning into plasmid pBR322 of a 19-kb *Bam*HI fragment of the *E. coli* chromosome containing the *thy*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$ region, based on the work of SASAKI *et al.* (1982). A similar plasmid containing the *S. typhimurium thyA*$^+$-*argA*$^+$ region was constructed as follows. DNA from strain V201, containing F′ *thyA*$^+$ *recC*$^+$ *recB*$^+$ *argA*$^+$ from *S. typhimurium*, was digested with endonuclease *Bam*HI and was ligated with pBR322 DNA (BOLIVAR *et al.* 1977) cut with *Bam*HI. The ligated mixture was used to transform (MANDEL and HIGA 1970) strain D202, and Thy$^+$ Arg$^+$ Amp^R transformants were selected. As expected, these strains were found to be UV^R and to contain elevated levels of Exo V nuclease activity (D. W. SCHULTZ, unpublished observations). The plasmids in these strains were transferred by transformation to strain V186, bearing the *del(thyA argA)232* deletion.

Phage crosses and Chi activity measurement: For crosses in *E. coli* strains (see Table 4), bacteria growing exponentially in tryptone broth (with 0.1% maltose and 40 μg of thymine/ml) were infected with an average of five of each parental phage, incubated at 34° for 15 min for phage adsorption, diluted 100-fold into warm tryptone broth with maltose and thymine, aerated for 2 hr at 34° (crosses 1–6) or at 37° (crosses 7–10) and treated with $CHCl_3$ to promote lysis and kill residual bacteria. For crosses 1–6, recombinant progeny were determined by plating on strain 594 at 42°; Chi activity was calculated as $\sqrt{(c/t)_A \div (c/t)_B}$, where c/t is the ratio of clear to turbid plaques from cross *A* (1395 × 1396) and from cross *B* (1397 × 1398) as indicated (Figure 1). For crosses 7–10, using phages 1081 × 1082 and 1083 × 1084, recombinant progeny and Chi activity were determined from titrations on strain 594 at 42°. In this case, Chi activity was determined from the ratio t/c in the two crosses since the clear (*cI*) marker entered the cross in coupling with the *J* marker (see Figure 1).

For crosses in *S. typhimurium* (Table 3), the procedures were modified as follows.

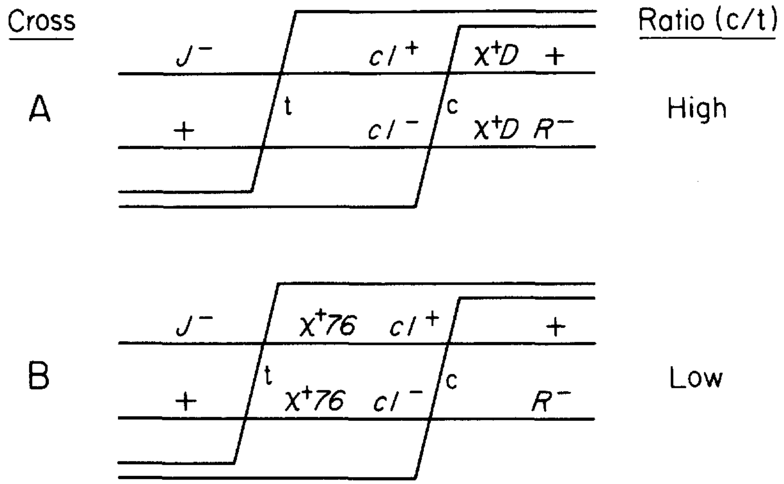

FIGURE 1.—λ crosses to measure Chi activity (after STAHL and STAHL 1977). In cross *A* each parental phage carried χ^+D, which due to its localized enhancement of recombination is expected to increase the frequency of exchanges in the *cI-R* interval (scored as clear plaques among selected J^+R^+ recombinants). Similarly, in cross *B*, χ^+76 is expected to increase the frequency of turbid plaques. The ratio (*c/t*) of clear to turbid plaques is measured for each cross. If Chi is active, this ratio is high in cross *A* and low in cross *B*. Chi activity is defined as the square root of the quotient of these ratios. In related crosses with phages 1081 × 1082 and 1083 × 1084, the c^- marker enters in coupling with the J^- marker; *t/c* ratios are therefore used to calculate Chi activity.

TABLE 3

Chi activity in *S. typhimurium* strains

		Chi activity			
			E. coli F′ *Eco recBC* genotype		
F^- strain designation	*S. typhimurium* chromosomal genotype	Haploid	*recB*$^+$*C*$^+$	*recB21*	*recC22*
GS1011	*recB*$^+$*C*$^+$	3.3, 3.0	5.5, 4.3	3.7, 3.5	3.8, 3.4
GS1014	*recB*$^+$*C*$^+$, Tn*10*	3.8, 2.5	4.9, 4.7	4.0, 3.4	3.8, 3.4
GS1015	*recB*::Tn*10*	0.8, 1.0	6.8, 7.1	ND, 1.0	4.8, 4.0

Chi activity was determined with phage crosses 1395 × 1396 and 1397 × 1398 as described in MATERIALS AND METHODS. Determinations were made with two independently constructed sets of F′ *recBC* strains in which crosses were performed on different days; values on the left are from one experiment, and those on the right are from another. F′ factors were introduced into strains GS1011, GS1014 and GS1015 by mating with strains S927 (F′ *recBC*$^+$), S928 (F′ *recB21*) or S930 (F′ *recC22*). ND, not determined.

Phage stocks were prepared on strain V229 to confer SB-specific host modification (COLSON and VAN PEL 1974). Because of slow adsorption of λ to strain GS1007 and its derivatives, ten of each parental phage were added for each bacterium in the adsorption mix, which was incubated for 20 min at 34°. Adsorption was found to be 50–80% complete. To reduce the number of unadsorbed phage, the cells were collected by centrifugation at 5°, washed once with an equal volume of tryptone broth, resuspended in tryptone broth and diluted for phage growth at 34°, as for crosses in *E. coli*. Recombinant progeny were determined by plating at 41° on strain V230, a nonrestricting *E. coli* strain on which the phage make large plaques. Chi activity was calculated as described above.

RESULTS

Chi activity in λ vegetative crosses in *S. typhimurium*: Chi activity was measured following infection of a derivative of *S. typhimurium* LT2 bearing the λ receptor coded by the *E. coli malB* region (PALVA, LILJESTROM and HARAYAMA 1981). The infecting phages bore the imm^{21} region to allow expression of phage lytic functions (FRIEDMAN and BARON 1974) and the *b1453* deletion to eliminate both the phage recombination functions and the *gam* product, which inhibits RecBC enzyme (HENDERSON and WEIL 1975; UNGER and CLARK 1972). The infecting phages bore complementing temperature-sensitive mutations in genes *J* and *R*, which allowed selection of J^+R^+ recombinants. Segregation of the *cI* clear marker, located between *J* and *R*, allowed determination of the ratio of the frequency of exchanges in the *J-cI* interval to that in the *cI-R* interval; this ratio is increased by χ^+76, located in the *J-cI* interval and decreased by χ^+D, located in the *cI-R* interval (STAHL and STAHL 1977; Figure 1). The square root of the quotient of these ratios determined in two crosses, one with χ^+76 in both parents and another with χ^+D in both parents, is defined as Chi activity (STAHL and STAHL 1977); a value of unity indicates that the distribution of exchanges in the two measured intervals is the same in the two crosses and that Chi is inactive, whereas a value greater than unity indicates that Chi increases the proportion of exchanges in the interval in which it is located. Chi activity is determined from the results of two crosses, each with Chi at a different location, so that the influence of Chi on λ multiplication is identical in both crosses; any observed differences in the two crosses can then be attributed to a local effect of Chi on recombination.

As shown in Tables 3 and 4, Chi is active in *S. typhimurium* $recB^+C^+$ derivatives GS1011 and GS1014 (values of ~3) but is less active than in *E. coli* $recB^+C^+$ (values of ~6) (crosses 4–6 in Table 4). Chi is not active in the *S. typhimurium recB*::Tn*10* strain GS1015; Chi has previously been shown to be inactive in *E. coli recB* mutants (MCMILIN, STAHL and STAHL 1974; GILLEN and CLARK 1974; STAHL and STAHL 1977).

The lower Chi activity in *S. typhimurium*, compared to that in *E. coli*, prompted us to search for the genetic factor(s) determining the level of Chi activity. Since RecBC enzyme has been shown by genetic and enzymological evidence to interact directly with Chi (SCHULTZ, TAYLOR and SMITH 1983; LUNDBLAD *et al.* 1984; PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985), we first examined *recBC* by introducing into the *S. typhimurium* strains an F′ episome bearing the *E. coli recBC* genes (designated here F′ *Eco* $recB^+C^+$). In these strains, Chi activity is elevated (Table 3): In a strain with the *S. typhimurium recB* gene inactivated by a Tn*10* insertion and with F′ *Eco* $recB^+C^+$, Chi activity is as high (~7) as in *E. coli* $recB^+C^+$ (crosses 4–6 in Table 4). In strains with both the *E. coli* and the *S. typhimurium* $recB^+C^+$ genes, Chi activity is intermediate (~5) between those in strains with only the *S. typhimurium* or the *E. coli* $recB^+C^+$ genes (~3 and 7, respectively) (Tables 3 and 4). That the increased

TABLE 4

Chi activity in *E. coli* strains

Cross no.	Strain designation	*E. coli* chromosomal genotype	Episomal or plasmid genotype	χ^+D cross (1395 × 1396)			χ^+76 cross (1397 × 1398)			Chi activity
				c	*t*	*c* ÷ *t*	*c*	*t*	*c* ÷ *t*	
1	V203	Δ*recBC*	F′ *Eco recB*$^+$*C*$^+$	336	104	3.23	93	788	0.12	5.2
2	V201	Δ*recBC*	F′ *Sty recB*$^+$*C*$^+$	221	141	1.57	97	242	0.40	2.0
2A	V201A	Δ*recBC*	F′ *Sty recB*$^+$*C*$^+$	173	106	1.63	114	188	0.61	1.6
2B	V201B	Δ*recBC*	F′ *Sty recB*$^+$*C*$^+$	217	203	1.07	127	326	0.39	1.7
2C	V201C	Δ*recBC*	F′ *Sty recB*$^+$*C*$^+$	248	150	1.65	119	251	0.47	1.9
3	V186	Δ*recBC*		69	129	0.54	58	113	0.51	1.0
4	594	*recBC*$^+$		273	51	5.35	30	252	0.12	6.7
5	C600	*recBC*$^+$		321	76	4.22	39	330	0.12	6.0
6	V230	*recBC*$^+$		188	37	5.08	35	203	0.17	5.4
				χ^+D cross (1081 × 1082)			χ^+76 cross (1083 × 1084)			
				t	*c*	*t* ÷ *c*	*t*	*c*	*t* ÷ *c*	
7	V203	Δ*recBC*	F′ *Eco recB*$^+$*C*$^+$	966	193	5.01	66	1029	0.064	8.8
8	V201	Δ*recBC*	F′ *Sty recB*$^+$*C*$^+$	514	211	2.44	90	400	0.23	3.3
9	V243	Δ*recBC*	pBR322::*Eco recB*$^+$*C*$^+$	614	94	6.53	66	842	0.078	9.1
9A	V243A	Δ*recBC*	pBR322::*Eco recB*$^+$*C*$^+$	367	99	3.71	50	519	0.096	6.2
10	V241	Δ*recBC*	pBR322::*Sty recB*$^+$*C*$^+$	443	155	2.86	117	487	0.24	3.5
10A	V241A	Δ*recBC*	pBR322::*Sty recB*$^+$*C*$^+$	257	114	2.25	89	317	0.28	2.8

Chi activity was determined with phage crosses 1395 × 1396 and 1397 × 1398 (crosses 1–6) or with phage crosses 1081 × 1082 and 1083 × 1084 (crosses 7–10), as described in MATERIALS AND METHODS. Data are the numbers of clear (*c*) and turbid (*t*) plaques counted for each cross, the ratios of these numbers, and the Chi activity calculated from these ratios, as described in the MATERIALS AND METHODS section. Strains designated A, B or C have genotypes identical to the strains without the suffix, but were independently constructed.

Chi activity in these strains is due to the *E. coli* $recB^+C^+$ genes was shown by introducing F′ *Eco recB21* or F′ *Eco recC22*; in these strains, Chi activity is comparable to that in F^- *S. typhimurium* $recB^+C^+$.

Chi activity in λ vegetative crosses in *E. coli* containing the $recB^+C^+$ genes from *S. typhimurium* on an F′ factor or on a pBR322-derived plasmid: To test further the view that the level of Chi activity is determined by the species of origin of the *recBC* genes in the cell, we constructed an F′ episome and a pBR322-derived plasmid bearing the *S. typhimurium* $thyA^+$ $recC^+$ $recB^+$ $argA^+$ region (designated here F′ *Sty* $recB^+C^+$ and pBR322::*Sty* $recB^+C^+$) as described in MATERIALS AND METHODS, and transferred these constructs to an *E. coli* strain deleted for the corresponding region. As controls, similar *E. coli* strains bearing F′ *Eco* $recB^+C^+$ or pBR322::*Eco* $recB^+C^+$ were constructed. Chi activity in λ vegetative crosses is high (~5) in the F′ *Eco* $recB^+C^+$ strain (cross 1) and is comparable to that in haploid *E. coli* strains 594, C600 and V230 (crosses 4–6 in Tables 4). On the other hand, Chi activity is low (~2) in the F′ *Sty* $recB^+C^+$ strain (cross 2) and is comparable to that in haploid *S. typhimurium*. Similarly, Chi activity is high (~8) in the pBR322::*Eco* $recB^+C^+$ strain (cross 9) but low (~3) in the pBR322::*Sty* $recBC^+$ strain (cross 10). These results indicate that the genetic factor responsible for the level of Chi activity is present on the F′ factor and on the *Bam*H1 chromosomal fragment containing the *thyA recC recB argA* region. From the data described in the preceding section, we conclude that this genetic factor is *recBC*.

DISCUSSION

The main result reported here is that Chi sites are active in *S. typhimurium*. This result indicates that Chi sites and the recombinational factors interacting with them have been at least partially conserved during the evolution of *S. typhimurium* and *E. coli*, in which Chi activity has been previously studied. This result encourages the search for Chi activity in more distantly related organisms in which Chi sites have been observed.

A secondary result is that Chi is not as active in *S. typhimurium* as it is in *E. coli* and that this difference in activity is at least partly due to a difference in the RecBC enzymes of the organisms. In these studies, Chi activity was measured as the ratio of the frequency of recombination in a genetic interval with Chi to that in the same interval without Chi (STAHL and STAHL 1977). The observed lower Chi activity might stem either from a lesser activity of Chi *per se* or from a higher frequency of Chi-independent recombination.

The view that the lower Chi activity stems from a lesser activity of Chi *per se* is consistent with the observation that RecBC enzyme directly interacts with Chi and with the hypothesis that the two species of RecBC enzyme differ in this interaction. The interaction between RecBC enzyme and Chi has been shown both genetically and enzymologically. Certain mutational changes in the *E. coli* RecBC enzyme reduce or abolish Chi activity while leaving the enzyme recombinationally proficient (SCHULTZ, TAYLOR and SMITH 1983; LUNDBLAD *et al.* 1984; CHAUDHURY and SMITH 1984a). Purified RecBC enzyme cuts one DNA strand, that containing 5′G-C-T-G-G-T-G-G3′, as the enzyme unwinds

DNA from right to left [relative to the Chi sequence as written here (PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985)]. Chi-dependent cutting is also manifest in crude extracts; mutations reducing or abolishing Chi genetic activity correspondingly reduce or abolish Chi cutting activity in crude extracts (PONTICELLI *et al.* 1985). Chi cutting activity is also manifest in crude extracts of *S. typhimurium* (D. W. SCHULTZ and G. R. SMITH, unpublished results), but at present this activity cannot be precisely quantitated. Thus, the available evidence is consistent with the hypothesis that the *S. typhimurium* RecBC enzyme is stimulated by Chi to promote recombination, but not to as high a level as is the *E. coli* RecBC enzyme. Lesser stimulation by Chi would be reflected by low Chi activity, as measured in the crosses reported here (Tables 3 and 4).

An alternative hypothesis for the lower Chi activity manifest by the *S. typhimurium* RecBC enzyme states that the Chi-*in*dependent recombination promoted by the *S. typhimurium* RecBC enzyme is more frequent than that promoted by the *E. coli* RecBC enzyme. More frequent Chi-independent recombination would lower the measured Chi activity by increasing the frequency of exchange in the non-Chi-containing (control) intervals used in the crosses to measure Chi activity (Figure 1). Thus, even though the RecBC enzyme-Chi interaction might be equally strong for both species of RecBC enzyme, the measured Chi activity would be lower for *S. typhimurium* RecBC enzyme if its Chi-independent recombination were higher. During these studies, we obtained evidence supporting this view. λ $Red^-Gam^-\chi^0$ phages make large plaques on *E. coli* strains containing the *S. typhimurium* $recB^+C^+$ genes; with respect to this phenotype the *S. typhimurium* $recB^+C^+$ genes are dominant to the *E. coli* $recB^+C^+$ genes (data not shown). Large plaque formation of λRed^-Gam^- phages is indicative of enhanced recombination, such as that promoted by a Chi site, or of decreased inhibition of replication (for a review, see SMITH 1983). The dominance of the *S. typhimurium* $recB^+C^+$ genes in this regard favors the former possibility, but a firm conclusion requires precise measurements of the frequencies of Chi-dependent and Chi-independent recombination promoted by the two species of RecBC enzyme.

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