CHI MUTATION IN A TRANSPOSON AND THE ORIENTATION-DEPENDENCE OF CHI PHENOTYPE

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ABSTRACT

Chi, an element that stimulates recombination *via* the *E. coli* RecBC pathway, can arise by spontaneous mutation in the transposon Tn*5*. When in phage λ in one orientation, the mutant transposon confers Chi+ phenotype (large plaque and a high rate of exchange near the transposon). In the other orientation, however, the transposon does not confer Chi+ phenotype. The mobility of the transposon allows us to show that the Chi+ orientation of the mutant Tn*5* is the same at different locations in λ. These include a site near gene *J*, one in *gam* at 69, one to the right of *gam* at 73 and several to the right of *R* between 95.7 and 99.5. To the right of *R*, the mutant transposon could be found in only one orientation, that which confers Chi+ phenotype. We speculate that the other orientation of Tn*5* in that locale is lethal to λ. The orientation-dependence of Chi+ phenotype also revealed that Tn*5* flip-flops *in* λ.

CHI is a genetic element that confers upon phage λ a high rate of Rec-mediated exchange in its neighborhood (STAHL, CRASEMANN and STAHL 1975). Chi promotes recombination only *via* the RecBC pathway (GILLEN and CLARK 1974; STAHL and STAHL 1977), so that Chi has the most conspicuous effect on exchange when λ is deficient for its own recombination pathway (Red) and for its RecBC-inhibiting function, Gam (STAHL and STAHL 1977). When λ is Red⁻ and Gam⁻ and growing in a *rec*+ cell, encapsidation is dependent upon Rec-mediated recombination (ENQUIST and SKALKA 1973; STAHL *et al.* 1972). Thus, the exchange-stimulating effect of Chi is manifested not only as a local increase in the recombination frequency, but also as an increase in burst (MALONE and CHATTORAJ 1975) and plaque size (HENDERSON and WEIL 1975).

Chi elements arise by mutation at four (or more) places in the λ chromosome and occur naturally in the *Escherichia coli* chromosome. When MALONE *et al.* (1978) examined *Eco*RI restriction fragments of *E. coli* DNA cloned in λ, they found that about half of the fragments, of average length 7 kb, conferred a Chi+ phenotype upon λ; *i.e.*, when such phages were made Red⁻ Gam⁻, they made

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relatively large plaques and had a high rate of exchange in the region encompassing the cloned fragments.

FAULDS *et al.* (1979) inverted some of these Chi-containing fragments, as well as a number of fragments that had been scored by MALONE *et al.* (1978) as Chi-less. They found that, for many of the fragments, the phenotype was reversed: some Chi$^+$ fragments became Chi$^-$ and some Chi$^-$ became Chi$^+$. They concluded that Chi is an asymmetric sequence active in only one of its two possible orientations in the region of lambda used for the cloning (from *red* leftward to near *J*; see Figure 1).

What feature of λ activates Chi in one orientation or, conversely, inactivates it in the other? Is this a local feature varying from one part of λ to another, or is it a global feature such that the active orientation of Chi is the same throughout λ? These alternatives could be distinguished by moving a given Chi to different positions within λ and examining its phenotype in each of its two possible orientations at each of those locations. To this end (and others), we isolated a Chi mutant of a transposon, and then "hopped" this transposon into various parts of λ. We then determined the Chi phenotype of the phage and the orientation of the transposon at each location.

This paper is in two parts: (1) the isolation and characterization of a Chi mutation on the kanamycin-resistant transposon Tn*5* (BERG 1977), and (2) the application of that Tn*5* Chi mutant to the problem at hand.

MATERIALS AND METHODS

Phage and bacterial strains: The phage mutations and bacterial strains used in this study are listed in Tables 1 and 2, respectively.

Transposition of transposons from E. coli *to* λ: Plate stocks of λ *b519 b515 int29 red3 gam210 cI857 nin5* phage, made on the transposon-donor strains, were used to superinfect the lysogen JC8679 (λ *b519 b515 int29 red3 gam210 cI857 nin5*) at a multiplicity of 5. After 20 min for phage adsorption, the culture was diluted 3-fold with broth and grown at 32° for 30 min to allow expression of drug resistance. The culture was then concentrated 50× and plated on rich plates containing the drug (20 μg/ml tetracycline, kanamycin, or ampicillin). Drug-resistant colonies were grown into cultures and induced by heat. Single plaques from the lysate were tested for the presence of a transposon by superinfecting JC8679 (λ*b519 b515 int29 red3 gam210 cI857 nin5*) at a multiplicity of 1 and spotting on a plate for drug resistance.

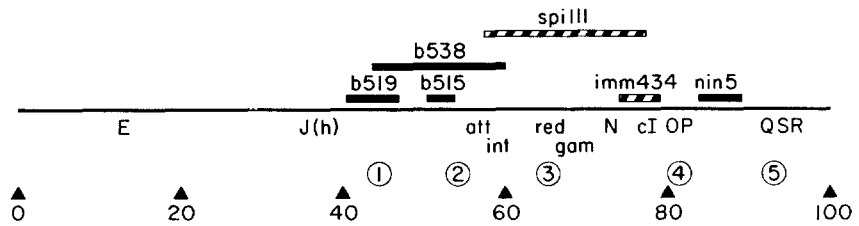

FIGURE 1.—Map of λ showing the locations of only the genes mentioned in this paper. The solid bars represent the deletions used, while the striped bars indicate regions of λ replaced by other DNA in the substitutions *spi*111 and *imm*^{434}. The positions of *EcoRI* sites are identified by the circled numbers.

TABLE 1

Phage mutations employed

Mutation	Properties	Reference
Deletions:		
*b*519	from 40.7 to 47.0	DAVIS and PARKINSON 1971
*b*515	from 50.4 to 53.8	DAVIS and PARKINSON 1971
*b*538	from 43.6 to 59.9	DAVIS and PARKINSON 1971
*nin*5	from 83.8 to 89.2; permits growth of N^- phage	FIANDT *et al.* 1971
Substitutions:		
*spi*111	*bio* transducing phage deleted from *att* to *cI*	SMITH 1975
imm^{434}	immunity region of phage 434 (crossed into λ)	KAISER and JACOB 1957
Recombination-related:		
*int*29	*sus*	ENQUIST and WEISBERG 1976
*red*3	unconditional	WEIL and SIGNER 1968
*gam*210	*sus*	ZISSLER, SIGNER and SCHAEFER 1971a,b
Others:		
*susP*80	blocks DNA replication on Su^- host	CAMPBELL 1961
*susE*4	blocks encapsidation on Su^- host	CAMPBELL 1961
*susS*7	blocks lysis on SuII+ host	GOLDBERG and HOWE 1969
h	host-range mutation (in *J*)	KAISER 1962 (?)
*cI*26	unconditional clear-plaque	MESELSON 1964
*cI*857	renders phage heat-inducible; makes clear plaques at temperatures $> 35°$	SUSMAN and JACOB 1962

Transposition of Tn5 from λ *into* E. coli: Cells of the Su^- strain JC9387 at about 2×10^9/ml were infected at 42° with λ *b*519 *b*515 *int*29 *red*3 *gam*::Tn5 *cI*857 *sus*P80 phage at a multiplicity of 5. After 10 min for phage adsorption, the cells were aerated for 10 min at 42°, diluted and plated on kanamycin plates as 37°. Kanamycin-resistant (Kan^R) colonies were checked for the absence of lysogeny by their sensitivity to infection by λ.

Selection for a Chi mutation in the transposon Tn5: Chi mutants of λ *b*519 *b*515 *int*29 *red*3 *gam*210::Tn5 *cI*857 *nin*5 (see RESULTS) were selected according to the methods of STAHL, CRASEMANN and STAHL (1975). Growth of the phage and detection of large plaque-forming mutants (Chi+ derivatives) were on the bacterial strain C600(P2).

To determine whether any of the phage became Chi+ by mutation of the Tn5, derivatives that had spontaneously lost Tn5 were selected by plating on the Su+ *recA* strain, QR48. Precise loss of Tn5 from the *gam* gene (with concomittant restoration of the suppressible *gam*210 allele) endows the phage with the ability to plate on QR48. Such cured phage strains were isolated and their genotypes verified by inability to plate on C600(P2) or on ED206 or to confer kanamycin-resistance. Those Chi+ phage isolates that made tiny plaques on 594(P2) when cured of Tn5 were saved.

Putting the Tn5 Chi mutant into λ *far from* gam: λ *spi*111 *nin*5 was grown on *E. coli* ($Tn5_\chi$+) in 15 parallel cultures. The phages from the resulting lysates were adsorbed to the *rec*+ lysogen AB1157 (λ *susE*4 *red*3 *gam*210), and the infected cells were plated on kanamycin plates. For each lysate, Kan^R colonies from 3 plates (20 to 250 colonies in total) were combined and grown overnight. Exponential subcultures of these bacteria were infected with λ *susE*4 *b*538 *imm*434, and the resulting lysates were plated on the Su^- *recA* lysogen, ED206 (λ*susE*4 *red*3 *gam*210).

TABLE 2

Strains of bacteria employed

Designation	Relevant properties	Source or reference
RM66	λ resistant, *recB*21 derivative of C600	MALONE *et al.* 1978
C600	*rec*+ SuII+	APPLEYARD 1954
C600(P2)	Su+ selective indicator for Red⁻ Gam⁻ λ	
JM1	*recB sbcA* SuIII+	STAHL *et al.* 1980b
ED206	*recA* Su⁻ selective indicator for λ Fec+*	HENDERSON and WEIL 1975
QR48	*recA* Su+	SIGNER and WEIL 1968
594	*rec*+ Su⁻	WEIGLE 1966
594(P2)	Su⁻ selective indicator for Red⁻ Gam⁻ λ	
JC8679	*recB recC sbcA* SuII+	J. GILLEN, Berkeley
JC9388	Su⁻ derivative of JC8679	A. J. CLARK, Berkeley
AB1157	*rec*+ Su+	BACHMAN 1972
NK5146	*su*+ *his*::*Tn*10	N. KLECKNER
EY16†	NK5146 *recB*21	see below
26/1163	F′ *proB lacZ*::Tn5/Δ(*proB lac*) *trp str*	D. BERG (*via* G. SMITH)
EY19‡	F′ *proB lacZ*::Tn5/chromosome of JC8679	see below
psc 304/C600	C6000 carrying plasmid (Tn*3*)	S. N. COHEN

* Fec+ phages plate on *recA* hosts by virtue of not being simultaneously Red⁻ and Gam⁻ (ZISSLER, SIGNER and SCHAEFER 1971a).

† The *recB*21 allele was co-transduced (*via* P1) with *thy*+ into a *thyA* (trimethoprim-resistant) derivative of NK5146. *recB* was identified by UV sensitivity, ability to support large-plaque formation by Red⁻ Gam⁻ λ, and inability to support growth of P2.

‡ The F′ (Tn5) plasmid in 26/1163 was transferred by conjugation to JC8679. Selection was for Kan^R Trp+.

Plates crowded with (turbid) plaques were replicated with velveteen onto kanamycin agar. Colonies were picked, purified and grown overnight in broth. These broth cultures were clarified by centrifugation and sterilized by shaking with chloroform. The titers of spontaneously released phage in these sterilized cultures were amplified by 2 successive cycles of overnight growth on plates seeded with ED206. These lysates, from which agar and cells had been removed by centrifugation, were predominantly Int⁻, signifying the deletion *b*538. Each lysate was centrifuged to equilibrium in a Cs-salt density gradient. The collected fractions were assayed on ED206(λ), revealing 2 density species of imm^{434} phages. Particles in the heavier peak proved to carry Tn5; their full genotype was λ *b*538 imm^{434} (Tn5χ+). The procedure ensured that the transposons are in λ to the left of *b*538 or to the right of imm^{434}.

Deletion mapping to determine locations of Tn5 in λ: The 12 independent isolates of λ *b*538 imm^{434} (Tn5χ+ far from *gam*) were made *red*3 *gam*210 by recombination. In this state, all had Chi+ phenotype (see RESULTS). Therefore, the Tn5 could be mapped by noting the appearance or the nonappearance of Chi⁻ phage following infection of heteroimmune lysogens bearing the set of cholorate-resistant prophage deletions described by ADHYA, CLEARY and CAMPBELL (1968).

Electron microscope heteroduplex analysis: Heteroduplex molecules were made and prepared for electron microscopy as described by CHATTORAJ and INMAN (1974) or by DAVIS, SIMON and DAVIDSON (1971). Enlarged projections of the molecules were measured by an electronic planimeter (Numonics, Landsdale, Penn.).

RESULTS

Part I: Isolation and characterization of a transposon carrying a Chi mutation

Do transposons carry Chi$^+$ sequences? Three transposable elements, Tn*10*, Tn5 and Tn*3*, were examined to determine whether or not they contain naturally occurring Chi sites. The phage λ *b*519 *b*515 *int*29 *red*3 *gam*210 *cI*857 *nin*5 was grown on *E. coli* transposon-carrying strains that were either Su$^+$ or *recB* (see Table 2) to prevent selection of Chi mutants. Rare phage particles that have acquired a transposon as result of "hopping" from the *E. coli* genome to λ were selected by their ability to confer drug resistance (Tet^R for Tn*10*, Kan^R for Tn5 and Amp^R for Tn3). Cultures from drug-resistant lysogenic colonies were then heat-induced to produce λ (Tn) phage stocks. These Red$^-$ Gam$^-$ phage were then tested for plaque size on the Su$^-$ rec$^+$ strain 594(P2). The λ(Tn*3*) and λ(Tn5) phage formed only tiny plaques; λ(Tn*10*) phage formed large plaques. We deduced from these results that Tn*3* and Tn5 are Chi$^-$, whereas Tn*10* is Chi$^+$.

The selection of a Chi$^+$ derivative of Tn5: Having found Tn5 to be Chi$^-$, we attempted to select a Chi$^+$ derivative. The strategy was to select for a mutation in a *red*3 *gam*210 phage carrying Tn5 and to determine whether the mutation was in the transposon or in λ. First, a phage was selected as described below in which Tn5 had hopped into the *gam* gene. Only Red$^-$ Gam$^-$ λ can grow on a P2 lysogen (ZISSLER, SIGNER and SCHAEFER 1971b). Since *gam*210 is a suppressible (*sus*) mutation, λ *red*3 *gam*210 cannot form plaques on the Su$^+$ P2 lysogen C600(P2). λ *red*3 *gam*210 into which Tn5 had hopped into *gam*, however, is expected to be able to plate on C600(P2) (*i.e.*, to be unconditionally Spi$^-$). Independent lysogens of λ *b*519 *b*515 *int*29 *red*3 *gam*210 *cI*857 *nin*5(Tn5) were heat induced and plated on C600(P2). Those phage that formed plaques were assumed to have Tn5 inserted into *gam*. This was verified from the heteroduplex made between one of them and wild type λ DNA. The transposon was found at 68.4 ± 0.6% from the left end of λ. Since the *gam* gene maps at 69% (HENDERSON and WEIL 1975), this result supports the phenotypic evidence that Tn5 has inserted into *gam*. The size of the stem and the loop parts were 3.1 ± 0.1% and 6.0 ± 0.3%, respectively. The size of the transposon was thus estimated to be 12.2 ± 0.4%, in agreement with the results of BERG (1977).

Sixty-six independent Chi mutations of λ *b*519 *b*515 *int*29 *red*3 *gam*210::Tn5 *cI*857 *nin*5 were selected by growing the phage on C600(P2) for several cycles and then plating on C600(P2) to identify large plaque formers, Chi$^+$ mutants (STAHL, CRASEMANN and STAHL 1975).

To determine if any of the 66 Chi mutations had arisen in Tn5, derivatives in which Tn5 had been precisely lost from the *gam* gene (see MATERIALS AND METHODS) were examined for their plaque sizes on 594(P2). Six of the 66 independent Chi$^+$ clones were found to lose Chi$^+$ phenotype simultaneously with the restoration of the *gam*210 allele. Further evidence that Chi mutations had occurred in the transposon is presented in the next section.

The Tn5x$^+$ large-plaque phenotype is orientation dependent: Experiments by FAULDS *et al.* (1979) indicate that the recombination-stimulating activity and

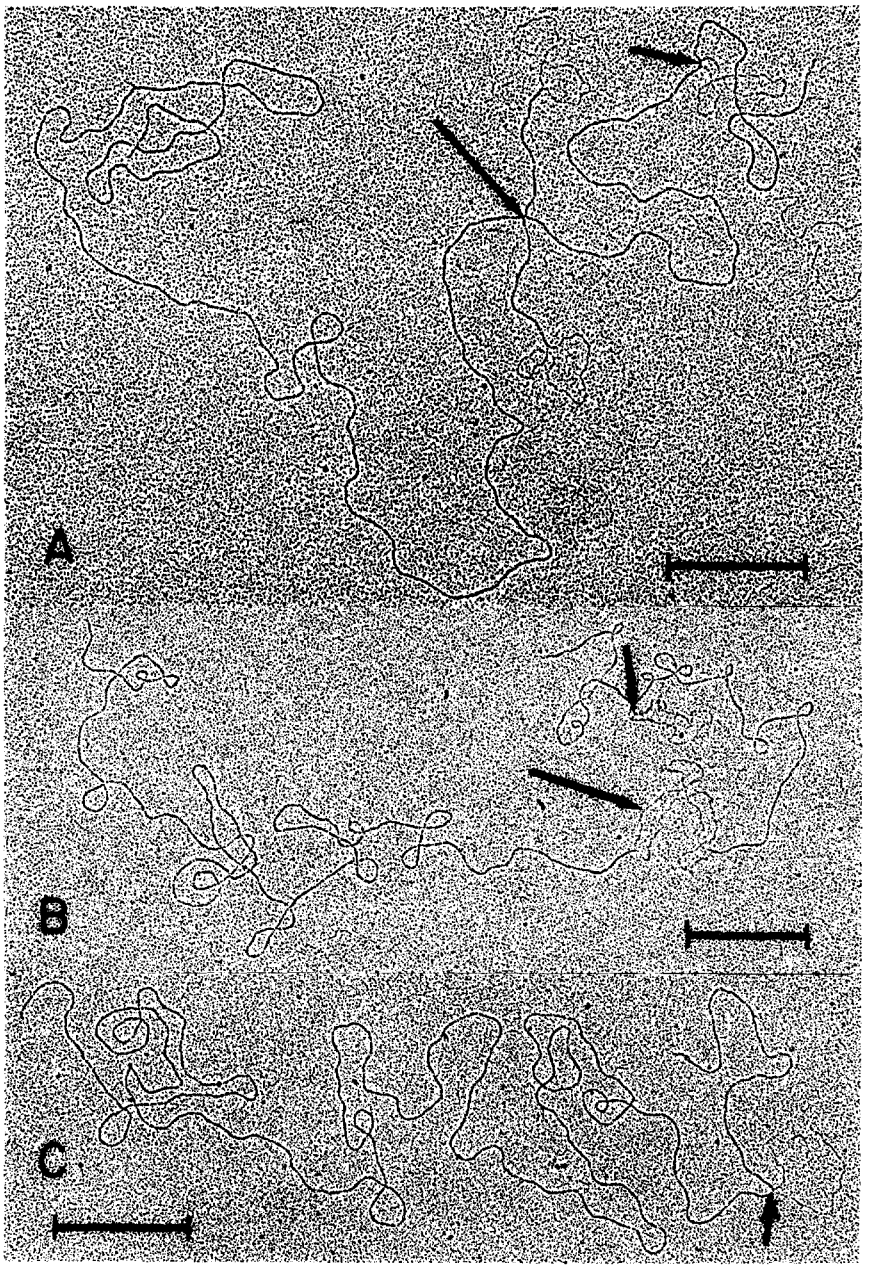

FIGURE 2.—(A) A typical heteroduplex between Chi+ λ (Tn*5*χ+) and Chi- λ (Tn*5*χ+) *nin5*. The big arrow indicates the cruciform junction, the position at which Tn*5* has inserted into χ. The transposons appear partly single-stranded (the "loops") and partly double-stranded (the "stems"). The stems result from intra-strand pairing of the inverted repeated sequences at the ends of the transposon. Also seen is the *nin5* deletion loop near the right end of the molecule (small arrow). (B) Same as 2A but further treated with GP32*I to convert the intra-strand pairing of stems to inter-strand pairing. The presence of a substitution loop (big arrow) indicates

large plaque size due to Chi can be orientation dependent. Many *EcoRI* fragments of *E. coli* DNA, cloned into λ between *Eco*RI sites 1 and 3 (see Figure 1), contained Chi sites (MALONE *et al.* 1978). When fragments conferring Chi$^+$ phenotype were inverted *in vitro*, some of them lost Chi activity. Similarly, some fragments originally classified as Chi$^-$ became Chi$^+$ upon *in vitro* inversion. Thus, "natural" *E. coli* Chi sites can operate in that part of λ when in one orientation, but not the other. In this section, we show that the activity of χ^+ arising by mutation in Tn*5* is also orientation dependent when the transposon is in *gam*, just to the right of restriction site 3.

The procedure was to transpose Tn*5*χ^0 and Tn*5*χ^+ from λ to the *E. coli* chromosome and then to hop each of these transposons back into the *gam* gene of λ. All the resulting λ *gam*::Tn*5* that originated from Tn*5*χ^0 retained their Chi$^-$ phenotype [tiny plaques on C600(P2)]. However, from among 20 independent λ isolates in which the original Tn*5*χ^+ element was transposed back into *gam*, 12 showed the Chi$^+$ phenotype [large plaques on C600(P2)] and eight did not; *i.e.*, in roughly half of the isolates, the Chi$^+$ phenotype did not manifest itself. The acquisition of the Chi$^+$ phenotype together with the transposon in 12 of 20 cases verifies our conclusion that Tn*5* had mutated to χ^+. To account for the other eight strains, which had no Chi activity associated with the transposon, we assumed that the Tn*5*$^+$ transposon had integrated into the *gam* gene in the reverse orientation to that of the original one. Inversion of the Chi mutation with respect to λ would make the Chi "cryptic." To obtain experimental support for this explanation we "heteroduplexed" DNA of Chi$^+$ λ *gam*::Tn*5*$^+$ strain with that of a Chi$^-$ λ *gam*::Tn*5*χ^+.

If the transposons in these two phages are in opposite orientations, we expect to observe at the position of *gam* an unannealed region (a single-stranded substitution loop) of the size of the Tn*5* loop.

When annealing of DNA single strands from these phage was carried out at 34° for 30 min, virtually every double-stranded molecule in the sample showed a cruciform structure in the *gam* region (as in Figure 2A). In order to test the noncomplementarity of the two transposons, we converted the cruciform structures to the normal double helical form. This was accomplished by heating the heteroduplexes to 76°, the melting temperature of λ DNA, and re-annealing at 51°. Duplex molecules without any cruciform junction could now be seen. Thirty-four such molecules were examined, four of which showed the expected substitution loop. As a control, when λ(Tn*5*χ^+) DNA was self-annealed and treated as above, only three molecules with the substitution loop were observed among 207 duplex molecules (without any cruciform junction) examined. The

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that the transposons have opposite orientations. Also seen is the *nin5* deletion loop (small arrow). (C) A heteroduplex between Chi+ λ (Tn*5*χ^+) and Chi+ λ (Tn*5*χ^+) *nin5* heat-treated at 76° (T_m) to favor inter-strand pairing of the inverted repeated sequences. The lack of a substitution loop indicates that the transposons have similar orientations. Also seen is the *nin5* deletion loop diagnostic of heteroduplexes (small arrow).

observation of some substitution loops was not totally unexpected (see *Tn5 spontaneously changes its orientation* below).

In order to be sure that the majority of the molecules showing a substitution loop in the heteroduplexed sample were indeed biparental, the experiment was modified to include an additional nonhomology marker. The *nin5* deletion was crossed out of the Chi$^+$ λ(Tn5x^+) parent, and this strains was heteroduplexed with the Chi$^-$ λ(Tn5x^+) parent, which still retained the *nin5* deletion. In the heteroduplex sample, all molecules with a *nin5* deletion loop also had the substitution loop at the expected place. In the control Chi$^+$/Chi$^+$ *nin5* sample, heteroduplex molecules had only the *nin* deletion loop. Three and 15 heteroduplex molecules, respectively, were analyzed in the two cases.

We would like to note that the heat treatment converted the Chi$^+$/Chi$^+$ *nin* heteroduplexes from cruciform to simple duplexes more effectively than it did Chi$^+$/Chi$^-$ *nin* heteroduplexes. The greater homology in the former case apparently assisted the conversion. As an alternative to heating, we attempted to denature partially the Chi$^+$/Chi$^-$ *nin* cruciform heteroduplexes with GP32*I, an *in vitro* cleaved form of the gene *32* product of phage T4 (MOISE and HOSADA 1976). The denaturation was carried out at pH 8.0 in 75 mM salt at 30° for 30 min at a DNA nucleotide:protein molecule ratio of 50:1. This was followed by annealing at room temperature in 0.2 M salt for 15. By this method, 50% of the cruciform molecules lost their cruciform appearance, half being converted to simple duplex forms, while the other half showed the expected substitution loop (see Figure 2). From observations on 16 heteroduplex molecules, we conclude that the x^+ transposons with Chi$^+$ and Chi$^-$ phenotypes, respectively, have inserted into λ at the same place, within the resolution of our micrographs, but in opposite orientations. This conclusion was further supported by a different property of heteroduplex molecules. In Chi$^+$/Chi$^+$ *nin5* heteroduplexes, the two loops of the cruciform transposon partially hybridized to each other (underwound loops: KLECKNER, SWAN and ZABEAU 1978; BERG *et al.* 1975; BROKER, CHOW and SOLL 1979) in all 11 molecules examined; whereas, in the Chi$^+$/Chi$^-$ *nin5* sample, no such overlap was observed in 23 molecules examined. Thus, underwound loops, whose appearance requires neither heating nor GP32*I, are conveniently diagnostic of the orientation of a transposon.

The Chi$^+$ Tn5x^+ *does stimulate recombination locally:* To verify that the Tn5x^+ mutation indeed stimulates exchange in its locale, we made crosses as described by MALONE *et al.* (1978). Two external markers, *h* and *S*$^+$, one in each parent, were selected for, and the segregation of an internal pair of alleles (*cIts* and *cI*$^-$) was scored as turbid and clear plaques at 34°, respectively. An increased ratio of turbids to clears in the presence of *gam*::Tn5x^+ would indicate that x^+ stimulates recombination. The results (Table 3A) show that, in the presence of the Chi$^+$ Tn5x^+, there is an approximately two-fold increase in the turbid:clear ratio compared to either Tn5x^o or the Chi$^-$ Tn5x^+. Since Chi-promoted recombination is RecB-dependent (STAHL and STAHL, 1977), this type of cross was also carried out in the *recB recC sbcA* strain, JC8679, to verify that the effect of Tn5x^+ was specific to the RecB recombination pathway (Table 3b).

TABLE 3

Recombinational activity of Tn5χ+

λ Type	Host	Number plaques counted	Turbid:clear plaques among *h S+*
A. *rec+* Crosses			
gam::Tn5χ°	594	921	1.13
Chi+ *gam*::Tn5χ+	594	1013	2.19
Chi- *gam*::Tn5χ+	594	861	1.28
B. *recB* Crosses			
gam::Tn5χ°	JC8679	522	0.68
Chi+ *gam*::Tn5χ+	JC8679	898	0.59

λ*b*519 *b*515 *int*29 *red*3 *gam*210::Tn5 *cI*857 *nin*5 phages were crossed with λ *h cI*26 *susS*7 (also Int- Red- Gam-) in the indicated hosts, and offspring were plated on strain RM66, which selects *h S+* recombinants. The plates were incubated at 32° to permit distinction between *cI*26 (clear) and *cI*857 (turbid). An elevated turbid-to-clear plaque ratio indicates Chi activity.

That no difference is seen in the ratios of turbids to clears for Chi+ Tn5+ and Tn5χ° supports the conclusion that the increase in turbid:clear ratio in the *rec+* host is due the presence of χ+ on the transposon.

Tn5 spontaneously changes its orientation: The plaque size of Red- Gam- λ carrying Tn5χ+ depends on the orientation of the transposon. We have used this orientation-dependent phenotype to identify phage particles in which the transposon has spontaneously inverted. Growth of the Red- Gam- λ (Tn5χ+) for only one overnight cycle on a plate seeded with a *rec+* bacterial strain [*e.g.*, C600(P2)] yields a population in which about 2 to 5 × 10^{-3} of the particles form large plaques on C600(P2). For several reasons, we believe that these large plaques arise from "flipping" of the transposon: (1) The acquisition of Chi+ phenotype occurs at a higher rate than that observed for mutation to Chi+. (2) The Chi+ derivatives have the same density in Cs-formate gradients as do the original Chi- Tn5χ+ phage, ruling out the possibility of a second tranposon inserted into the phage in the Chi+ orientation. (3) Hopping of the transposon from the Chi+ phage into *E. coli* and then again into λ gave rise to both small (Chi-) and large-plaque (Chi+) derivatives, indicating that the Chi+ phenotype was not due to an additional Chi mutation arising in the transposon. (4) Electron microscopy of heteroduplexes formed between the original Chi- Tn5χ+ and their Chi+derivatives revealed that the transposons are in opposite orientations.

Subsequently, we found that growth of a Red- Gam- Chi+ Tn5χ+ phage for five cycles on a RecB- bacterial strain, in which there is no selective advantage for the activity of Chi, yields some small-plaque formers. Chi- λ (Tn5+). These small-plaque derivatives were cycled to give Chi+ derivatives that were then cycled again to give Chi- phage. Microscopy confirmed that the transposon had flipped with each change of Chi phenotype.

Part II: Relative orientations of Tn5x$^+$ inserted at different places in the λ chromosome

Do all active Chi sites have the same orientation in the λ chromosome? Orientation dependence has been demonstrated for pieces of Chi-containing DNA inserted into the cloning vector λgt between *Eco*RI sites 1 and 3 (FAULDS *et al.* 1979). In a previous section, the phenotype of λ gam::Tn5x$^+$ was demonstrated to be dependent upon the orientation of the transposon. The experiments discussed in this section were carried out to determine whether our Tn5x$^+$ transposon inserted at various places in the λ chromosome must always be oriented in the same way to express the Chi$^+$ phenotype.

The procedure was to examine heteroduplexes formed between pairwise combinations of phage carrying transposons. During the renaturation process, the intramolecular formation of base pairs between the inverted repeats flanking the drug resistance genes precedes the intermolecular hybridization. The resultant heteroduplexes are molecules with two "lollipop" structures. When the transposons have the same orientation, one single-stranded loop is complementary to the other, and the loops are able to anneal partially. The resultant underwound double-stranded loop is shown in Figure 3. On the other hand, when the transposons are in the opposite orientation, there is no complementarity and no interaction is observed.

A preliminary experiment involved the formation of heteroduplexes between Chi$^+$ λ (Tn5x$^+$) and λ (Tn5x^0) (same orientation as Tn5x$^+$) inserted into the *gam* gene. The former phage carried the deletions *b*519 *b*515 and the latter was *b*538; thus, heteroduplex molecules could be readily identified. All of the 52 heteroduplexes examined showed the diagnostic interaction. The transposon loops in heteroduplexes formed between Chi$^-$ λ (Tn5x) in a *b*519 *b*515 background) and λ (Tn5x^0) (in a *b*538 background) showed no interaction among 60 (51 lollipops, nine substitution loops) heteroduplexes.

Next, the orientations of the transposons in 14 independent hops of Tn5x$^+$ into λ (see MATERIALS AND METHODS) were compared to that of the Tn5x$^+$ in the *gam* gene. Deletion mapping had located 12 of these to the right of *imm*^{434}. An *imm*$^{\lambda}$ derivative of one of the 12, λ (Tn5x$^+$) 3b, was constructed for use in this heteroduplex study. Each of the remaining 11 λ *imm*^{434} (Tn5x$^+$), with Tn5 mapping to the right of *imm*^{434} (see MATERIALS AND METHODS), were heteroduplexed to λ *imm*$^{\lambda}$ (Tn5x$^+$) 3b, and the orientations and approximate positions of their Tn5 with respect to Tn5x$^+$ 3b were determined. All 12 transposons were located between 95.7 ± 0.1 and 99.5 ± 0.01, in agreement with the deletion mapping, and all had the same orientation. Next ,the orientation of the λ *imm*^{434} (Tn5x$^+$) 3b transposon relative to the transposon in Chi$^+$ λ *imm*$^{\lambda}$ *gam*::Tn5x$^+$ was found to be the same. The orientations of two other Chi$^+$ Tn5x$^+$ transposons in λ *imm*^{434}, one located by deletion mapping near to the *J* gene and the other to the right of *gam*, were deduced by examining heteroduplexes formed with λ *imm*$^{\lambda}$ *gam*::Tn5x^0 (in the "Chi$^+$" orientation). They, too, have the same orientation. Therefore, for a Chi site located near *J*, several to the right of *R*, one in

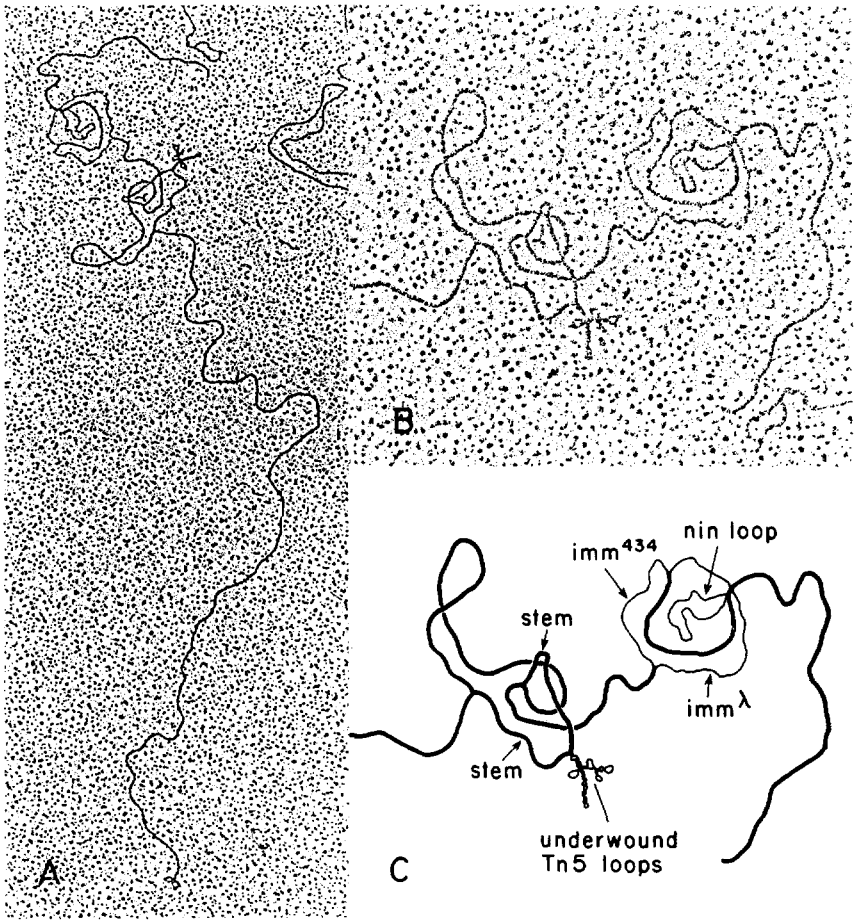

FIGURE 3.—Interacting "lollipops" of similarly oriented transposons located at different sites. (A) A heteroduplex molecule formed between Chi+ λ (Tn5χ+) *b*538 *red*3 *gam*210 *imm*434 (where Tn5χ+ is located near *J*) and λ *b*538 *red*3 *gam*210::Tn5χ° *imm*λ *nin*5. (The Tn5x° in *gam* has the same orientation as Tn5x+ in *gam*.) (B) An enlargement that better shows the interacting transposon loops. (C) An interpretive tracing of 3B, labelled to indicate the important features, notably the stems and the interacting "underwound" loops of the two transposons, the *imm*λ/*imm*434 substitution loop and the *nin*5+*nin*+ deletion loop. The heavy lines represent double-stranded DNA; the lighter ones denote single-stranded DNA.

gam, and one to the right of *gam* at 73, the Chi+ orientation of the Tn5χ+ is the same.

We attempted to recover flipped Chi- derivatives of some of the Tn5χ+ transposons. Red- Gam- phages carrying either of two Tn5+ located between 95.7 and 99.5, the Tn5χ+ near *J*, the one near *gam* and the one in *gam* were grown on the *recB* strain, JM1, for five cycles and then plated on C600(P2). The two transposons located to the right of *R* did not sport any Chi- λ (Tn5χ+), while the other three, at different sites, did. These three λ Chi- proved to have the

orientation of Tn*5* opposite to that of their Chi$^+$ precursors, as determined by electron microscopy.

Two explanations can be entertained for our inability to recover Chi$^-$ λ (Tn*5*x^+) to the right of *R*. One possibility is that Chi activity is not orientation dependent in this region of the λ chromosome, *i.e.*, Chi is active in both orientations. Cycling Tn*5*x^+ phage would not have yielded any Chi$^-$ flips. The more likely explanation is that one orientation of Tn*5* is lethal, perhaps by virtue of interfering with transcription of late genes. The lethality explanation is strongly supported by the fact that all 12 hops into this region of λ were in the same orientation. The Chi$^-$ orientation could be tolerated at the other three sites, where no genes essential for λ growth reside.

Since we have not been able to isolate phage carrying Tn*5*x^+ in the Chi$^-$ orientation to the right of *R*, we cannot say that Chi is active in only one orientation in this region. We can say, however, that when the transposon has the same orientation as the Chi$^+$ Tn*5*x^+ in *gam*, its Chi site is active.

DISCUSSION

Among the three transposons analyzed, only the largest (Tn*10*, 9300 base pairs) was found to harbor at least one "natural" *chi*$^+$, whereas the smaller ones (Tn*5*, 5300 base pairs, and Tn*3*, 4600 base pairs) were Chi$^-$.

Tn*10* can insert itself in λ in either orientation (KLECKNER, SWAN and ZABEAU 1978). Since all of the hops we observed into λ conferred a large-plaque phenotype on the Red$^-$ Gam$^-$ phage, Tn*10* probably has *chi*$^+$ on each of its DNA strands. These findings are in quantitative harmony with a previous estimate of one *chi*$^+$ per 5000 base pairs of *E. coli* DNA (FAULDS *et al.* 1979).

SPRAGUE, FAULDS and SMITH (1978) and SMITH, SCHULTZ and CRASEMANN (1980) have shown that x^+ in λ generally arises by single base-pair changes. Each of several independent x^+C's were shown to be due to a particular transversion, while x^+B was shown to arise on some occasions by a transversion and on others by a single base-pair deletion. We observed that six out of 66 Chi mutations arising in λ (Tn*5*) occurred in the transposon. Since the transposon is about 12% the size of the λ chromosome, it is likely that the Chi mutations occur in it by single base-pair changes.

FAULDS *et al.* (1979) showed that the phenotype of *E. coli* Chi elements cloned into λ on *Eco*RI fragments is dependent on the orientation of the fragment (and element) in λ. Our demonstration that a Chi element arising by mutation in (DNA carried in) λ has a phenotype that is likewise orientation dependent strengthens the speculation that the "natural Chi elements" of *E. coli* are fully identical with the Chi elements arising by mutation in λ.

Our demonstration that Tn*5* changes its orientation ("flips") is paralleled by work of D. E. BERG (personal communication), who showed that at least part of Tn*5* flipping is RecA-dependent and presumably the result of homologous exchange between the terminal inverted repeats of the transposon.

Tn5 exerts a strong polar depression on gene expression when inserted in either orientation in the *lac* operon of *E. coli* (BERG, WEISS and CROSSLAND 1980). We succeeded in finding Tn5x^+ inserted in λ to the right of gene *R*, upstream in an operon from the essential late-function genes. We were able to recover the transposon there in only one orientation, however. This suggests that the anti-transcription-terminating effect of λ's *Q* gene product (D. FORBES and I. HERSKOWITZ, personal communication) is able to overcome the transcription-termination signals of Tn5 in one, but not the other, of its two orientations.

Our studies using a Chi mutant of Tn5 have shown that the active orientation of Chi is the same throughout λ from a point just to the right of *J* to points to the right of *R*. These points fall on both sides of the origin of λ replication in gene *O*. The points in and near *gam* are in a region that is transcribed leftward, while the points to the right of *R* are in a region transcribed rightward. The point near *J* may be transcribed in both directions (HERSHEY 1971). Thus, the location of the transposon with respect to either the origin of λ replication or the promotors of λ transcription does not determine the sign of the orientation dependence of Chi phenotype. Chi's independence of nearby λ transcription was confirmed by the results of crosses in which one parent was repressed and one parent carried Chi (in a nontransactivated region). The Chi was equally active when in the repressed or the nonrepressed parent (STAHL *et al.* 1980a).

STAHL *et al.* (1980b) showed that the four Chi sites identified by mutation in λ, as well as *chi*$^+$*bio* on a transducing phage of spontaneous origin, act in a strongly directional way, with all five of those elements acting to their left. This directionality was clearly revealed when replication was blocked and there was a heterology near or at the Chi site in one of the parents in a cross. [With a different protocol, CHATTORAJ *et al.* (1979) showed a Chi-induced leftward "bias" in recombination stimulation, which was likely a reflection of the directionality observed in replication-blocked experiments.] The locations of sites examined spanned the same region of λ for which we have orientation-dependence data. Thus, these data bolster the suggestion of FAULDS *et al.* (1979) that directionality and orientation dependence are two aspects of a single phenomenon, with the most attractive explanation being that Chi elements oriented such that they "should" have rightward directionality in λ are unable to express Chi$^+$ phenotype.

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