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Nucleotide Sequence of the Chi Recombinational Hot Spot χ^+D in Bacteriophage Lambda

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Chi sites in bacteriophage λ stimulate recombination promoted by the RecBC pathway of *Escherichia coli*. Mutations which create these sites occur at four widely separated loci in λ. We report here the nucleotide sequence surrounding the site of one of these loci, χD, located near the *S* gene. The mutations creating the active Chi site, designated χ^+D, are transversions from $\frac{G}{C}$ to $\frac{T}{A}$. This mutation, like the χ^+B and χ^+C mutations previously analyzed, leads to a nucleotide sequence common to all three active Chi sites.

Generalized recombination in bacteriophage λ is stimulated by special sites, called Chi, when recombination proceeds via the RecBC pathway of the host of λ, *Escherichia coli* (see reference 24 for a review). One hypothesis of the mechanism of this stimulation supposes that Chi sites are special nucleotide sequences recognized by one of the enzymes in the RecBC pathway. To substantiate this hypothesis, we have examined the nucleotide sequences surrounding Chi sites to determine whether Chi is a unique nucleotide sequence (22, 23). Extensive sequence homology was found in the sequences of two widely separated Chi sites in λ (22). From those analyses we could not conclude how much of the homology was essential for Chi activity. A comparison of other sequences containing Chi would aid in revealing the extent of the common sequence necessary for Chi. In this paper we report the nucleotide sequence of a third Chi site in λ and compare that sequence with the two Chi sequences previously determined.

Wild-type λ is devoid of Chi, but mutations which create Chi occur at the four well-separated loci shown in Fig. 1 (10, 25). Sequence analysis of the χB and χC loci was possible because of the availability of deletion mutations with endpoints near the Chi sites. These deletions allowed the genetic and physical mapping essential for locating an interval small enough for convenient nucleotide sequence analysis. The mutations creating Chi at the χB and χC loci were found to be single-base-pair changes (22, 23).

A similar analysis of the χA and χD loci was not immediately possible since these loci are in regions of genes essential for λ growth. The availability of λ DNA fragments with endpoints near χD and inserted into self-replicating plasmids allowed the genetic and physical mapping and the nucleotide sequence analysis of χD reported here.

(We use here the nomenclature of Malone et al. [12]. χ^+ designates the genotype of the active form of Chi sites in λ. χ^0 designates the inactive form. χA, χB, χC, and χD designate the loci in λ where the χ^+ mutations may arise. Chi refers to the phenotype of the sites.)

MATERIALS AND METHODS

Bacterial strains. *E. coli* strains with λ prophage deletions extending from *gal* through *attL* and into the *Q* to *S* region were isolated and characterized by Shapiro and Adhya (19). More detailed mapping was described by Herskowitz and Signer (11). Strains MS5061 and MS509 were obtained from the collection of Frank Stahl (University of Oregon), and strains MS1118 and MS1153 were obtained from the collection of Jeffrey Roberts (Cornell University). Strain 594 (28) is *lac gal* Str^r and does not suppress amber (*sus*) mutations.

E. coli strains with plasmids into which fragments of λ DNA were inserted were obtained from other investigators as follows. From David Wilson (Cornell University) we obtained strain D264, which contains plasmid pDW1, a derivative of plasmid pBR322 (26) carrying *Eco*RI fragment F of λ (27) with a *Bam*HI linker attached to the right cohesive end. In addition to bearing this plasmid, strain D264 is HfrC *thi-1 trpR rbs-115 leu*. From Jeffrey Roberts and Michael Schechtman (Cornell University) we obtained strain Q, which contains plasmid pOPII-λPQ, a derivative of plasmid pMB9 (3) carrying a fragment of the *lac* operon with UV5 mutation (21) and *Eco*RI fragment E of λ (27) bearing the *nin-5* mutation (5). In addition to bearing this plasmid, strain Q carries a deletion of the *lac-pro* region of the chromosomeand an episome

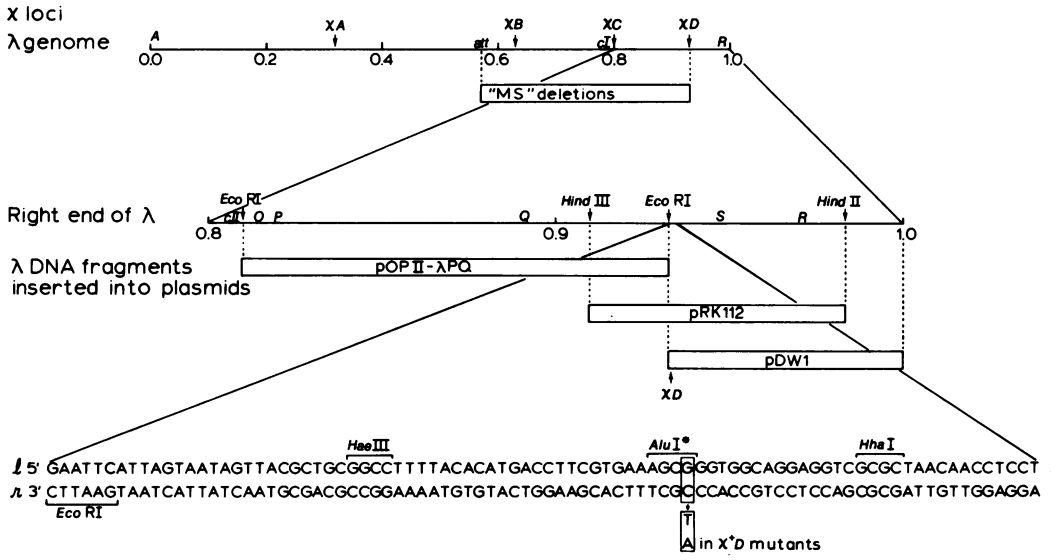

FIG. 1. *Genetic and physical map of phage λ, showing the location and nucleotide sequence of the χD locus. On each line, physical distances are drawn to scale. The top line shows the four loci at which χ^+ mutations arise, genes A, J, cI, and R, and the prophage attachment site att at the indicated fractional distances from the left end of the λ chromosome (7). The extents of prophage deletions in strains MS509, MS1118, MS1153, and MS5061, used to map χD (Table 1), are drawn relative to the positions indicated on the top line. The second line shows the right end of λ, including the χD locus, genes cII through R, and the positions of certain endonuclease cleavage sites. The extents of λ DNA fragments generated by these endonucleases are shown relative to the second line. These fragments are present in the indicated plasmids, which were used to map χD (Table 2). The bottom line shows the nucleotide sequence surrounding the χ^+D mutations, which alter the base pair outlined (Fig. 2). Hyphens are omitted for clarity. Brackets above and below the line indicate recognition sequences for the indicated endonucleases. AluI* is a cleavage site present only in DNA from χ^+D mutants (Fig. 3). l and r refer to the conventional strand designations in λ.*

with the *lacI*^{q} mutation (15). From William Reznikoff (University of Wisconsin) we obtained plasmid pRK112λ, a derivative of plasmid ColE1 bearing the kanamycin and neomycin resistance determinants of Tn*5* (1) and *Hin*dIII-*Hin*dII fragment A2 of λ (18) bearing the *susS7* mutation (9). Strain C600 (*thr-1 leu-6 thi-1 lacY1 supE44 tonA21*) was transformed with this plasmid DNA to generate strain FB1034.

Phage strains. λ*int4 red3 gam*210 χ^+D123 *susS7* and λ*tsJ*15 *red3 gam*210 *c*I26 χ^+D124 were obtained from Jean Crasemann (University of Oregon). λ*b*1453 χ^+D123 *susR*5 was obtained from Frank and Mary Stahl (University of Oregon). Other phages were from our collection or were from crosses between them and those listed above. *susP* and *susR* mutations are described by Campbell (4).

Phage crosses. Cells containing plasmids or partially deleted prophages were grown at 37°C in a medium containing 1% (wt/vol) tryptone (Difco Laboratories, Detroit, Mich.) and 0.5% (wt/vol) NaCl in water, supplemented with 0.1% (wt/vol) maltose. At a cell density of about 3×10^8/ml, five phages per cell were added. After 20 min at 37°C without shaking, to allow phage adsorption, the infected cells were diluted 1:1,000 in the above medium. After aeration at 37°C for 2 h, $CHCl_3$ was added to kill remaining cells. Sus$^+$ recombinants were selected on strain 594 on plates containing 1% (wt/vol) Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.5% NaCl, and 1% agar (Difco). Sus$^+$ recombinants were scored for Chi as described in footnote *a* of Tables 1 and 2.

Enzymes. Sources of some of the enzymes have been described previously (22, 23). In addition, endonuclease *Msp*I was purchased from New England BioLabs, Beverly, Mass.

Preparation of DNA and DNA sequence analysis. Wild-type (χ^0D) DNA was prepared from λ*c*I60 *susS7* (22). Mutant (χ^+D) DNA was prepared from λ*c*I857 χ^+D123 *susS7* and from λ*b2 red3 gam*210 *c*I26 χ^+D124 *susS7*. Samples of approximately 100 μg were digested for 18 h at 37°C with 20 U of endonuclease *Eco*RI in a reaction volume of 1.0 ml. After phenol extraction and ethanol precipitation, the DNA was labeled with polynucleotide kinase by the exchange procedure (2), using approximately 5 U of kinase in a reaction volume of 100 μl. Reaction was for 90 min at 37°C. After precipitation of the DNA with ethanol in the presence of ammonium acetate (14), the DNA was digested for 12 h at 37°C with 8 U of endonuclease *Msp*I in a reaction volume of 50 μl. After the addition of sucrose and marker dyes, the DNA was subjected to electrophoresis in a 7% polyacrylamide gel (Bio-Rad Laboratories, Richmond, Calif.) in E buffer (20) until xylene cyanol FF had migrated about 7 cm. The third most rapidly migrating band, at a position about two-thirds of the way from the origin to xylene cyanol

FF, was detected by autoradiography, electroeluted, and subjected to nucleotide sequence analysis (13, 14).

RESULTS

Genetic and physical location of χD locus. The precise location of the χD locus was essential to identify a region of λ sufficiently small for convenient DNA sequence analysis. Stahl et al. (25) mapped the λ^+D123 allele to a position in or between genes *Q* and *S*. We mapped this and another χ^+D allele (χ^+D124), both of spontaneous origin (J. Crasemann, personal communication), between the endpoints of two deletions by using a variation of the procedure described by Stahl et al. (25). χ^+D phages with *susS* or *susR* mutations were grown in cells containing partially deleted prophages from which S^+ or R^+ could be rescued (Fig. 1). The simultaneous rescue of the χ^0 allele from a given prophage indicated that the deletion in that prophage had not removed the χD locus.

Table 1 shows that the χD locus is located between the endpoints of deletions MS1153 (or MS1118) and MS509. χ^0 was rescued from deletions MS5061, MS1118, and MS1153, but not from MS509, whether S^+ or R^+ was the selected rescued allele. Similar results were obtained in crosses with χ^+D123 and χ^+D124, which suggest that these two χ^+ mutations are located at the same site or near each other. (The nucleotide sequence analysis reported below demonstrated that these two mutations occurred at the same base pair.) These results did not, however, allow us to conclude whether χD is located in *Q*, in *S*, or between the two genes.

To locate the χD locus on the physical map of λ, we utilized plasmids containing fragments of λ DNA from the *Q–S* region (Fig. 1). The endpoints of these fragments were generated by endonucleases whose cleavage sites have been located on the physical map (6, 16, 18, 27). The rescue of the χ^0 allele from these fragments would locate χD relative to these cleavage sites. This rescue was accomplished similarly to the rescue from the prophages, via linkage to a selectable marker (*susP*$^+$, *susS*$^+$, or *susR*$^+$).

TABLE 1. *Rescue of χD from partially deleted prophages*[a]

Prophage deletion strain	Deletion ends between[b]:	Fraction of Chi^0 among following selected recombinants:		
		S^+		R^+ (χ^+D123)
		χ^+D123	χ^+D124	
MS5061	*Q21* and *Qts7*	74/141	317/469	189/347
MS1118	*Qts7* and *S3*	176/310	280/419	72/119
MS1153	*Qts7* and *S3*	67/117	409/592	116/182
MS509	*S3* and *S2*	0/370	0/448	0/111

[a] χ^+D *susS* or χ^+D *susR* phages were grown in the indicated bacterial strains as described in the text. Sus^+ recombinants were selected on strain 594 and scored by an inspection of the plaque size for the Chi phenotype (10). The data indicate the number of Chi^0 (small-plaque phenotype) recombinants out of the total number of recombinants inspected. Representative plaques from each cross were restreaked on fresh plates to verify the Chi phenotype. Complete genotypes of the infecting phage were *int4 red3 gam210* χ^+D123 *susS7*, *b1453* χ^+D123 *susR5*, and *b2 red3 gam210 cI857* χ^+D124 *susS7*.

[b] From reference 11.

Table 2 shows that χD is located very near *Eco*RI site 5 and to the right of it. Among $SusR^+$ phages generated by recombination with plasmid pRK112, 12% have rescued χ^0. This result shows that χD is located on the 3,600-base-pair fragment A2 (18) generated by endonucleases *Hin*dIII and *Hin*dII. This fragment contains *Eco*RI site 5 (18, 27). We crossed χ^+D Sus^- phages with the two *Eco*RI-generated fragments extending to the left (pOPII-λPQ) and to the right (pDW1) of this site. No χ^0 recombinants were found among 5,000 P^+ recombinants generated by recombination with plasmid pOPII-λPQ. On the other hand, a small number of χ^0 recombinants were found with plasmid pDW1. These results show that χD is located on *Eco*RI fragment F (27), which extends to the right from *Eco*RI site 5. Taken together, the results in Table 2 show that χD is between the *Eco*RI site and the *Hin*dII site located about 2,800 base pairs to its right. From the low frequency of χ^0 rescue from plasmid pDW1 and the high frequency of χ^0 rescue from plasmid pRK112, we concluded that χD is located very near the right of *Eco*RI site 5.

Nucleotide sequence of the χD locus. To determine the nucleotide sequence of the χD locus, we prepared DNA labeled at *Eco*RI site 5 and extending rightward about 350 base pairs to an *Msp*I site. This fragment contains the region deduced above to contain the χD locus.

Figure 2 shows the results of nucleotide sequence analysis from wild-type (χ^0), χ^+D123, and χ^+D124 DNAs. The sequences obtained are identical for all three DNAs, except for the 55th nucleotide to the right of the *Eco*RI cleavage site (5′ G-A-A-T-T-C 3′; measured on the *l* strand). In wild-type DNA (λcI60 *susS7*), this nucleotide is G, whereas in χ^+D123 and χ^+D124 DNAs, this nucleotide is T. Sequence analysis of another wild-type (χ^0) strain (λcI72) showed a G at nucleotide 55 (data not shown). In addition, our wild-type sequence in this region agreed with that determined by F. Sanger and A. Coulson for their strain of λcI857 *susS7* (personal communication). We concluded that the λ^+D mutation is the change G → T 55 nucleotides from the *Eco*RI site on the *l* strand.

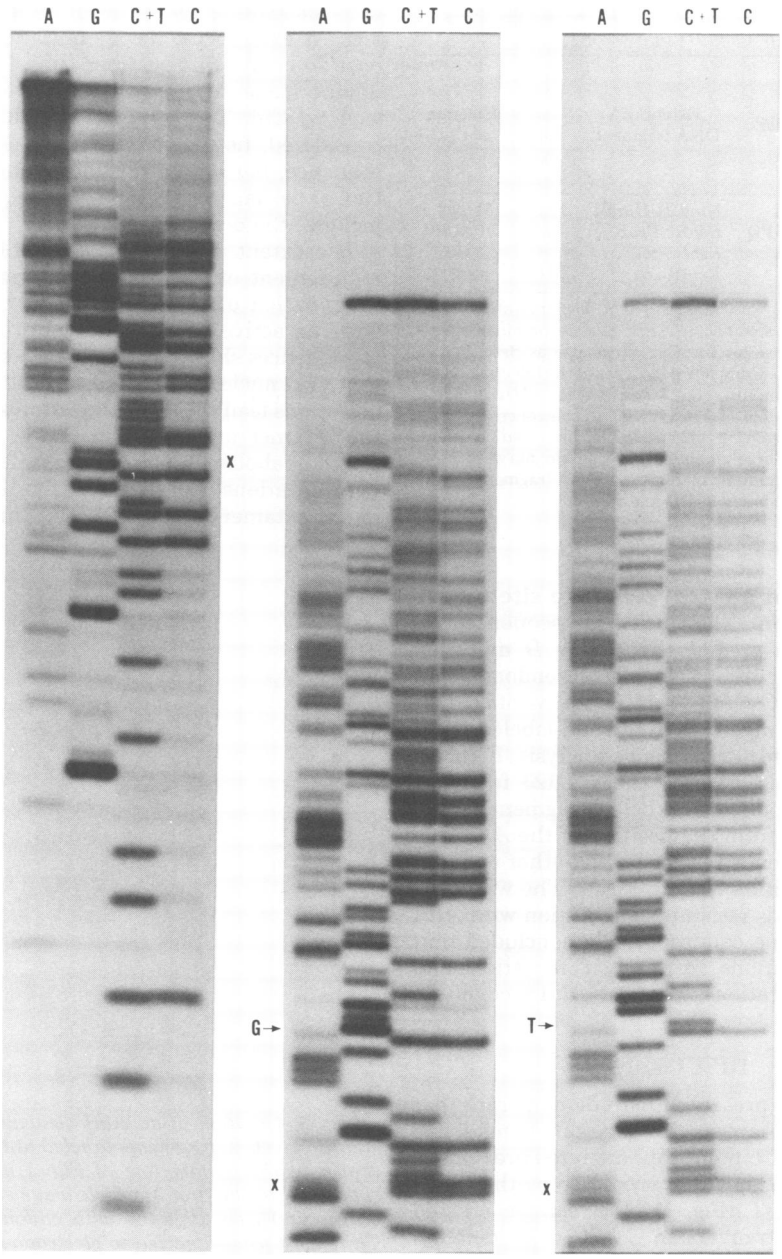

FIG. 2. *Nucleotide sequence determination. (Left) χ^+D124 sequence extending about 40 nucleotides rightward from the EcoRI site shown in the bottom line of Fig. 1. Identical sequences were obtained with wild-type (χ^0) and χ^+D123 DNAs (data not shown). (Middle) Wild-type (χ^0) sequence extending rightward from nucleotide 38 to about nucleotide 85, relative to the EcoRI cleavage site. (Right) χ^+D124 sequence corresponding to the wild-type sequence in the middle panel. An identical sequence was obtained with χ^+D123 DNA (data not shown). Arrows indicate the point of the χ^+D mutation. The sequence determination was by the method of Maxam and Gilbert (13, 14). Cleavage at specific bases is indicated at the top of each lane. For the left panel, cleaved samples were subjected to electrophoresis through a 20% polyacrylamide gel until xylene cyanol FF (designated X) had migrated 11.5 cm. For the middle and right panels, electrophoresis was through a 12% polyacrylamide gel until xylene cyanol FF had migrated 34 cm. Migration was from top to bottom. Further details on the preparation of the labeled DNA and on the sequence determination are given in the text and references cited there.*

TABLE 2. *Rescue of χD from plasmids carrying fragments of λ DNA*[a]

Bacterial strain	Plasmid	Extent of λ DNA fragment[b]	Recombinants selected	Fraction of Chi[0]
FB1034	pRK112	*Hin*dIII-*Hin*dII	R+	51/432
Q	pOPII-λPQ	*Eco*RI-*Eco*RI	P+	0/5,000
D264	pDW1	*Eco*RI-*cos*[c]	S+	3/500
D264	pDW1	*Eco*RI-*cos*	R+	4/4,000

[a] χ^+D123 phage with *susP*, *susS*, or *susR* mutations were grown in the indicated bacteria, and Sus+ recombinants were selected and scored for the Chi phenotype as described in Table 1, footnote *a*. Complete genotypes of the infecting phage were *int4 red3 gam210* χ^+D123 *susS7*, *b1453* χ^+D123 *susR5*, and *red3 gam210 cI26 susP80* χ^+D123. In control crosses with χ^0 phage (*red3 gam210 cI26 susS7* or *int4 red3 gam210 susP80*), no Chi+ (large-plaque) Sus+ recombinants were found (data not shown), indicating that the λ DNA fragments in the plasmids did not contain a χ^+D mutation.

[b] See Fig. 1.

[c] The right cohesive end of λ.

Creation of an *Alu*I cleavage site by the χ^+D mutation. The nucleotide sequences reported above predict that the χ^+D mutation creates a recognition sequence for endonuclease *Alu*I, d(5′ A-G-C-T 3′) (17). We verified this prediction by digestion of the end-labeled DNA fragments used for sequence analysis. Figure 3 shows that digestion of the χ^+D123 fragment with *Alu*I produces a labeled fragment of the size expected from the position of the *Alu*I recognition sequence relative to the other endonuclease recognition sites in Fig. 1. The wild-type (χ^0) fragment is resistant to digestion with *Alu*I under the same conditions. We concluded that the χ^+D mutations, like the χ^+C mutations and some χ^+B mutations, create an *Alu*I recognition site (22, 23).

DISCUSSION

The results presented here demonstrate that χ^+D mutations are single-base-pair changes, like the χ^+B and χ^+C mutations analyzed previously. The active χ^+D hot spot is created by the transversion G → T (Fig. 1). χ^+B can be created either by the tranversion C → G or by deletion of this C (22), whereas χ^+C is created by the transversion A → T (23). The χ^+ mutations change sequences in λ which are almost the Chi sequence into the correct, active Chi sequence.

A comparison of the nucleotide sequences at the χ^+B, χ^+C, and χ^+D loci sheds some light on the sequence which defines Chi. As shown in Fig. 4, the octamer 5′ G-C-T-G-G-T-G-G 3′ is present at all three loci.When the two possible active Chi sequences at the χB locus are taken into account, this octamer is the longest continuous nucleotide sequence present at all three loci. At each locus, the χ^+ mutation changes a base to create this octamer (Fig. 4). If Chi is a unique nucleotide sequence, this octamer may well be Chi. Two other possibilities must be considered, however. Perhaps Chi is a unique sequence, but only a portion of the octamer is Chi; or perhaps Chi is a variable sequence which includes the octamer.

The extent and precise nucleotide sequence requirement of Chi can be determined via an examination of mutations which create or destroy an active Chi sequence. In the alignment of sequences shown in Fig. 4, we could conclude that two nucleotides are essential to Chi. χ^+B mutations lead to a G at the leftmost position in the octamer underlined in Fig. 4. A C at this position abolishes Chi activity (22). χ^+C and χ^+D mutations lead to a T at the third position in the octamer. An A or a G at this position is

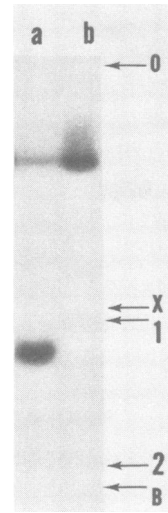

FIG. 3. *Creation of an AluI cleavage site by χ^+D mutations. DNA fragments labeled at the EcoRI site shown in the bottom line of Fig. 1 and extending rightward to the first MspI cleavage site (about 350 base pairs) were digested with endonuclease AluI. The digest was subjected to electrophoresis through an 8% polyacrylamide gel in E buffer (20). Migration was from top to bottom. (a) χ^+D123 DNA; (b) wild-type (χ^0) DNA. Arrows indicate the positions of the origin (0), migration of xylene cyanol FF (X), and migration of bromophenol blue (B). The positions of labeled fragments produced by digestion with endonuclease HhaI (arrow 1) and with endonuclease HaeIII (arrow 2) were determined in a separate experiment under identical conditions by noting their migration relative to the dyes. The relative sizes of these fragments are shown in Fig. 1. A small amount of labeled DNA from χ^+D123 ramained undigested.*

χ^+B121 5' ACAATGAGTGGCAGATATAGGCTGGTGGTTCAGGCGGCGCATTTTTAT 3'
χ^+B132 5' AACAATGAGTGGCAGATATAGCTGGTGGTTCAGGCGGCGCATTTTTAT 3'
χ^+C157 5' CGTTGATAAGTCGCAGATCAGCTGGTGGAAGAGGGACTGGATTCCAAA 3'
χ^+D123 5' TACACATGACCTTCGTGAAAGCTGGTGGCAGGAGGTCGCGCTAACAAC 3'

FIG. 4. *Comparison of nucleotide sequences at the χ^+B, χ^+C, and χ^+D loci. Portions of the l-strand sequences surrounding the indicated χ^+ mutations are shown. Two different sequences, exemplified by the χ^+B121 and χ^+B132 mutations, are found at the χ^+B locus (22). The χ^+C sequence is from reference 23, and the χ^+D sequence is from Fig. 1 and 2. The sequences are aligned such that the underlined octamer is coincident for the four sequences (see text). Hyphens are omitted for clarity.*

ineffective (23; Fig. 2). These observations are consistent with the view that Chi is, or contains, the unique octamer indicated in Fig. 4. Verification of this hypothesis could be achieved by analysis of more mutations which create Chi or by the isolation and analysis of mutations which destroy Chi. If Chi is limited to the octamer, all of these base changes would occur within the octamer.

It is interesting that the octamer is oriented in the same direction at the three χ^+ loci presently analyzed. That is, the octamer shown is present on the *l* strand of λ at all three active Chi sites. Faulds et al. (8) have shown that the inversion of Chi within λ inactivates Chi. Yagil et al. (E. Yagil, N. Dower, D. Chattoraj, M. Stahl, C. Pierson, and F. W. Stahl, Genetics, in press) have shown that the active orientation of the Chi site that they used, resulting from a spontaneous mutation in the transposon Tn*5*, is the same throughout the right 60% of λ. If the octamer is at least part of Chi, we would anticipate that it would be present in the same orientation at each position in λ, as we have observed.

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