Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8716–8720, November 1990
Biochemistry

A DNA gyrase-binding site at the center of the bacteriophage Mu genome is required for efficient replicative transposition

(supercoiling/topoisomerases)

MARTIN L. PATO[†‡§], MARTHA M. HOWE[¶], AND N. PATRICK HIGGINS[‖]

[†]Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262; [‡]Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206; [¶]Department of Microbiology and Immunology, University of Tennessee, Memphis, TN 38163; and [‖]Department of Biochemistry, University of Alabama, Birmingham, AL 35294

Communicated by Nicholas R. Cozzarelli, August 15, 1990

ABSTRACT **We have discovered a centrally located site that is required for efficient replication of bacteriophage Mu DNA and identified it as a strong DNA gyrase-binding site. Incubation of Mu DNA with gyrase and enoxacin revealed a cleavage site 18.1 kilobases from the left end of the 37.2-kilobase genome. Two observations indicate a role for the site in Mu replication: mutants of Mu, able to grow on an *Escherichia coli gyrB* host that does not allow growth of wild-type Mu, were found to possess single-base changes resulting in more efficient gyrase binding and cleavage at the site. Introduction of a 147-base-pair deletion that eliminated the site from a prophage inhibited the onset of Mu replication for >1 hr after induction.**

Bacteriophage Mu is one of the largest and most efficient transposable elements known. During the lytic cycle, the 37-kilobase (kb) genome undergoes 100 rounds of replicative transposition in <1 hr (for review, see ref. 1). Even though this large molecule transposes efficiently, insertions in Mu as small as 3 kb significantly reduce the frequency of transposition; large insertions have more dramatic effects, and doubling the size of the genome reduces frequency of transposition by $\approx 10^4$ (2). Transposons considerably shorter than Mu also show a length dependence for transposition (3, 4).

After infection of a sensitive host, the ends of Mu DNA are brought together to initiate the nonreplicative transposition that integrates the Mu genome into host DNA. The linear viral DNA is circularized by means of the coinjected N protein bound at the DNA ends (5); supercoiling of this DNA occurs, even though the molecule is not a continuous covalent circle (6). The integrated Mu genome then either persists as a prophage or continues into the lytic cycle, where it is amplified by replicative transposition. How the prophage ends are brought together for transposition after integration into the large circular bacterial chromosome is poorly understood. Models discussing the interaction of distant DNA sequences range from random collision of DNA–protein complexes to tracking of a protein bound to a DNA site along the DNA until the second site is encountered (for reviews, see refs. 7 and 8). Studies using small fragments of the Mu genome ends cloned into a supercoiled plasmid have ruled out the simple tracking model for Mu DNA (9), but the mechanism of juxtaposition of Mu ends is still unclear. Moreover, the large size of the intact Mu genome raises serious organizational problems that may not exist for shorter molecules.

In vitro, supercoiling is required for adopting the correct topological orientation to initiate transposition (10–12). *In vivo* studies with host gyrase mutants indicate a special role for supercoiling of Mu DNA (13, 14). We examined the Mu genome for sequences that might enhance replication by altering DNA supercoiling. Of particular usefulness in our studies was a class of mutants of Mu called *nuB* (15). These mutant phage were isolated for their ability to grow on a host *gyrB* mutant on which wild-type Mu does not grow. One hypothesis to explain this phenotype was that the *nuB* mutants have a gyrase-binding site with increased affinity for the host enzyme. We found a centrally located site that we have identified as a strong DNA gyrase-binding site. Two different *nuB* mutants have single base-pair changes that flank the gyrase-cut sites, and both *nuB* mutations strengthen gyrase binding and cleavage. Deletion of the gyrase-binding site inhibits normal Mu DNA replication after induction of a lysogen.

MATERIALS AND METHODS

Bacteria, Bacteriophage, and Plasmids. *Escherichia coli* K-12 DH5α (BRL) was used for transformations in the cloning procedures. An *E. coli* K-12 AB1157 derivative that is *recB recC sbcB* and monolysogenic for Mu *cts62* was used for construction of the deleted prophage. The phage used were Mu *cts62* and the *nuB* mutant derivatives *nuB1* and *nuB103* (15). Plasmid pBR322 was used for cloning of Mu DNA fragments.

Enzyme Reactions. Gyrase subunits A and B were purified as described (16, 17). Klenow fragment of DNA polymerase I was from New England Nuclear, T7 DNA polymerase was from United States Biochemical, and restriction enzymes were from Boehringer Mannheim or BRL.

Gyrase reaction mixtures (30 μl) contained 50 mM Tris·HCl (pH 7.6), 50 mM NaCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM spermidine, bovine serum albumin at 50 μg/ml, enoxacin at 15 μg/ml, and the indicated amounts of gyrase and DNA. After incubation at 30°C for 50 min, ATP was added at 333 μM, and incubation was continued at 30°C for 10 min. Gyrase cleavage was initiated by addition of 10 μl of solution containing 1% SDS and proteinase K at 10 μg/ml.

Mu fragments were end-labeled in 12-μl reactions containing 50 mM Tris·HCl; 10 mM $MgSO_4$; 0.5 mM dithiothreitol; bovine serum albumin at 50 μg/ml; 100 μM dATP, 100 μM dGTP, and 100 μM dTTP; 50 μCi of [α-^{32}P]dCTP (1 Ci = 37 GBq), 100 ng of purified DNA fragment, and 5 units of Klenow fragment of *E. coli* DNA polymerase I. Incubation at 37°C for 1 hr was followed by phenol/chloroform extractions (three times) and ethanol precipitation at −70°C.

DNA sequencing was carried out with [α-^{35}S]dATP by using the dideoxynucleotide chain-termination method (18). Chain-terminated products were separated on 6% sequencing gels and visualized by overnight autoradiography with Kodak X-Omat film.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

[§]To whom reprint requests should be addressed.

Other Materials. Enoxacin was a gift from Steve Gracheck and John Domagala at Warner–Lambert (Ann Arbor, MI). A concentrated drug solution was made by dissolving 5 mg of enoxacin in 1 ml of water, followed by addition of 10 μl of 6 M NaOH. The solution was stored frozen. Immediately before use, an aliquot of the concentrated suspension was diluted to 1 mg/ml in gyrase reaction buffer.

Site-Specific Deletion of the Gyrase-Binding Site. Deletion of 147 base pairs (bp) of Mu prophage DNA from the *Mlu* I site (18.0 kb) to the *Sca* I site (18.15 kb) was accomplished by a procedure similar to that of Winans *et al.* (19). A 1.4-kb *Bam*HI–*Cla* I Mu DNA fragment (17.2–18.6 kb) was filled in with Klenow polymerase and cloned into the *Sca* I site of pBR322, eliminating the *Sca* I site. The resulting plasmid DNA was cleaved with the enzymes *Mlu* I and *Sca* I and filled in with Klenow polymerase, removing 147 bp, and a 1.3-kb kanamycin (Kn) resistance cassette was inserted. The plasmid DNA linearized by cleavage with *Eco*RI was used to transform a *recB recC sbcB* host monolysogenic for Mu. The transformed DNA carries a Kn resistance marker at the site of the deletion and an ampicillin (Ap) resistance marker on the pBR322 DNA. Clones were screened for Kn resistance and Ap sensitivity, which selected for recombination of the 147-bp deletion and the Kn resistance cassette into the prophage DNA. The structure of the deleted prophage was verified by restriction analysis and Southern analysis: the 6.5-kb wild-type *Bam*HI–*Eco*RI fragment (17.2–23.7 kb) was replaced by a 7.6-kb fragment resulting from deletion of 147 bp and insertion of the 1.3-kb Kn resistance cassette; two *Dra* I sites located within the 147 bp were eliminated.

Measurement of Mu DNA Replication. Cultures of the Mu *cts62* lysogen and deleted derivative Mu *cts62* Δ147 were grown in L broth at 30°C to ≈10^8 cells per ml and induced by shifting to 42°C. Samples of 2 ml were labeled at intervals for 2 min with 0.2 mCi of [^{3}H]thymidine. Labeled DNA was prepared and hybridized to filters containing bound Mu DNA as described elsewhere (20). The data are expressed as the percent input counts per min hybridized to the filter.

RESULTS

A Strong Gyrase-Binding Site in the Middle of Mu. Gyrase-binding sites can be detected by cleavage of DNA in the presence of gyrase and oxolinic acid or more potent analogs of oxolinic acid, such as enoxacin (21–23). Gyrase can cleave DNA at many sites; however, some sites are particularly strong. When a DNA molecule has an exceptionally strong site, cleavage will occur predominantly at that site at appropriate concentrations of gyrase, yielding a cleavage resembling a restriction enzyme cut. To look for strong gyrase-binding sites, Mu *cts62* DNA was first cleaved with restriction enzymes *Eco*RI and *Bam*HI to generate five restriction fragments (Fig. 1) and then incubated with enoxacin and increasing concentrations of gyrase, followed by deproteination and electrophoresis in agarose gels (Fig. 2*A*). When the concentration of gyrase was increased, the 6.5-kb *Bam*HI–*Eco*RI fragment c spanning the central region of the genome (17.2–23.7 kb from the left end) disappeared, indicating a strong gyrase-binding site in that fragment. This experiment has been repeated several times, and whereas the preferential disappearance of fragment c was sometimes difficult to discern using wild-type DNA, it was always seen clearly in DNA analysis from the *nuB1* and *nuB103* mutants (Fig. 2*B*).

Close inspection of Fig. 2*B* showed the appearance of another band (marked c*). This product is ≈5.6 kb; hence, a cleavage site must be located ≈0.9 kb from either the *Bam*HI or the *Eco*RI restriction site in the Mu c fragment—i.e., at ≈18.1 kb or 22.8 kb. To determine whether the cleavage site is near 18.1 kb, a 1.4-kb *Bam*HI–*Cla* I fragment (17.2–18.6 kb) was prepared from *nuB103* DNA, as was a control 1.3-kb *Cla* I–*Cla* I fragment (18.6–19.9 kb). The fragments were end-labeled at the *Cla* I sites (Fig. 1). The labeled fragments were mixed with unlabeled DNA from *nuB1*, which had been cut with *Eco*RI and *Bam*HI as in Fig. 2, and cleaved with gyrase. The DNA fragments were then separated on agarose gel. The stained gel (Fig. 3 *Upper*) showed cleavage of the 6.5-kb fragment from *nuB1* DNA. Autoradiography of the dried gel (Fig. 3 *Lower*) showed that the labeled *Bam*HI–*Cla* I fragment was cleaved with the appearance of a labeled fragment of 0.5 kb, placing the cleavage site 0.5 kb from the labeled *Cla* I site or ≈18.1 kb from the Mu left end. The control *Cla* I–*Cla* I fragment remained essentially intact at levels of gyrase that cleaved nearly all of the *Bam*HI–*Cla* I fragment.

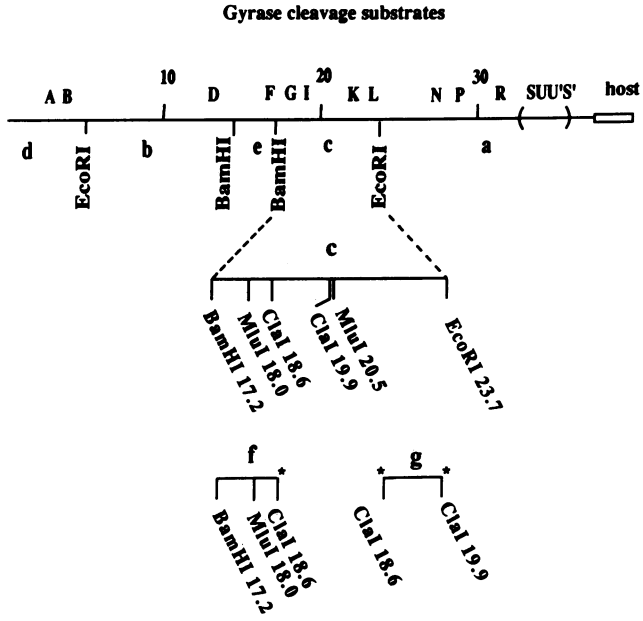

FIG. 1. Genetic map of phage Mu with a physical map of subgenomic fragments used to locate the gyrase-binding site. The Mu genome is ≈37.2 kb and, when digested with *Eco*RI and *Bam*HI, yields five fragments: a, ≈14.5 kb; b, 9.4 kb; c, 6.5 kb; d, 5.1 kb; and e, 2.7 kb. Enlargement of the 6.5-kb fragment c spanning the central portion of the genome is shown. Fragments used for fine mapping of gyrase-cleavage sites were a 1.4-kb *Bam*HI–*Cla* I fragment that extends from Mu coordinates 17.2–18.6 and a 1.3-kb *Cla* I fragment from 18.6–19.9 kb. Stars indicate the positions labeled by Klenow polymerase in the presence of [α-^{32}P]dCTP.

Placement of the gyrase-binding site at 18.1 kb locates it in the region of the genome that encodes phage head and tail proteins, between the late genes *G* and *I* (24), a location consistent with genetic mapping of the *nuB* mutants (15).

DNA Sequence of *nuB* Mutations. To sequence the gyrase-binding site, *Bam*HI–*Sca* I fragments (17.2–18.15 kb) from wild-type Mu and the *nuB* mutants were cloned into M13 and sequenced from the *Sca* I site. Sequence in the opposite direction was obtained by using synthetic primers complementary to the initial sequence. DNA sequence from wild-type Mu and from the *nuB* mutants was determined from the *Mlu* I site at 18.0 kb to the *Sca* I site at 18.15 kb (Fig. 4). The sequence for each of the *nuB* mutants differs from the wild-type sequence by a single-base change, and the changes are located 6 bp apart; the *nuB1* mutation is a G·C to C·G transversion, and the *nuB103* mutation is an A·T to G·C transition.

Sequencing of Gyrase-Cleaved DNA. To clone each gyrase-binding site for further sequence analysis, Mu DNA was digested with *Cla* I, the resulting 2-bp overhangs were filled in with the Klenow fragment of DNA polymerase, and *Bam*HI linkers were applied. After digestion with *Bam*HI the

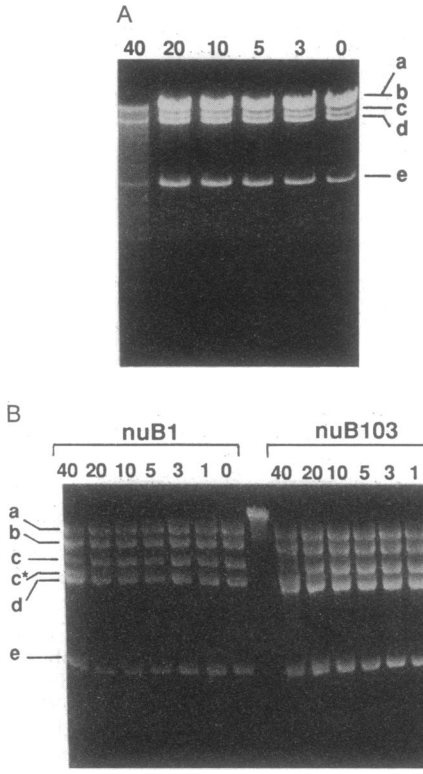

FIG. 2. Gyrase cleavage of Mu DNA. Reaction mixtures contained 1 μg of Mu *cts62* DNA (*A*) or *nuB1* and *nuB103* DNAs (*B*) cleaved with *Eco*RI and *Bam*HI were incubated with the indicated number of units (at top) of gyrase in the presence of enoxacin at 15 μg/ml. After inducing gyrase cleavage with SDS, DNAs were incubated for 30 min at 37°C with proteinase K and then subjected to electrophoresis through 1% agarose gel. After being stained with ethidium bromide, bands were photographed under UV illumination. DNA in the center lane of *B* is uncut Mu DNA; the band labeled c* is a cleavage product of band c.

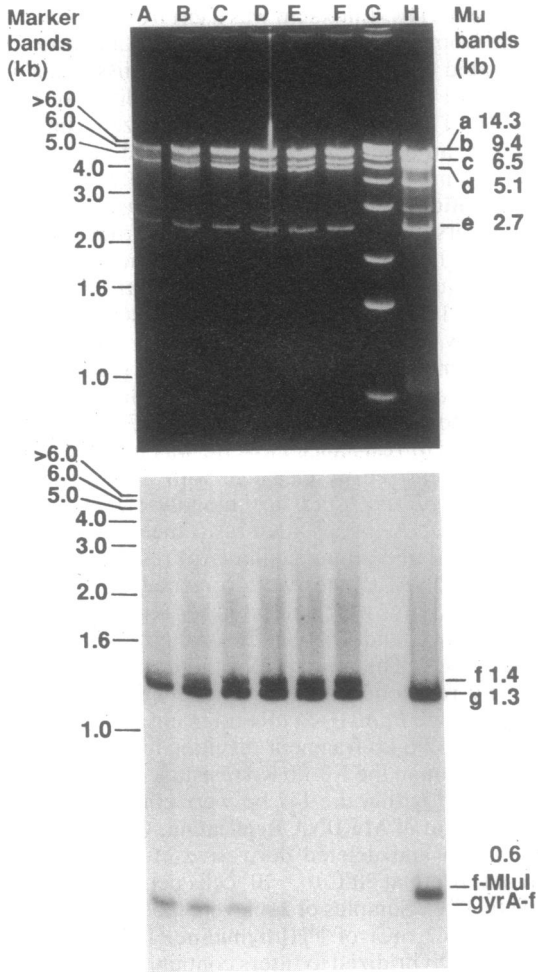

FIG. 3. Gyrase cleavage of Mu DNA and labeled fragments. Reaction mixtures were assembled with 1 μg of *nuB1* DNA cleaved with *Eco*RI and *Bam*HI (lanes A–F and lane H). Gyrase cleavage was carried out with enoxacin at 15 μg/ml and enzyme at 40 units (lane A); 20 units (lane B); 10 units (lane C); 5 units (lane D); and 2.5 units (lane E). No gyrase was added to reaction mixtures in lanes F, and 3 units of *Mlu* I was added in lane H. A 1-kb marker set (BRL) was loaded in lane G. All lanes except G also contained a submolar (15 ng) mixture of ^{32}P end-labeled *Bam*HI–*Cla* I fragment f and *Cla* I fragment g from *nuB103* (see Fig. 1). After electrophoresis through 1% agarose gel, bands were stained with ethidium bromide and photographed (*Upper*). After drying of the gel, the autoradiographic exposure was made to visualize the radioactive fragments (*Lower*). The 600-bp *Mlu* I fragment (f-MluI) and the gyrase cleavage product of band f (gyrA-f') are marked at right.

1.4-kb fragments corresponding to Mu sequence from 17.2 to 18.6 were cloned into pBR322 at the *Bam*HI site, generating plasmids pMP300, pMP310, and pMP320 from Mu wild type, Mu *nuB1*, and Mu *nuB103* DNAs, respectively. Each plasmid showed a single prominent gyrase-cleavage site within the cloned fragment, and the strength of gyrase cleavage was pMP320 > pMP310 > pMP300, reflecting the same relative cleavage strength observed for the Mu DNAs—namely, Mu *nuB103* > Mu *nuB1* > Mu wild type.

Next, the sequences of gyrase-cleavage products on each plasmid were analyzed. An oligonucleotide primer complementary to the region near 18.1 kb was used to prime dideoxynucleotide chain-terminating DNA sequencing reactions on plasmid DNAs cleaved with gyrase. Each plasmid gave its predicted sequence with the exception of one band that extended across all four lanes (Fig. 5). Chain termination at this position was due to gyrase cleavage. In all three plasmids T7 polymerase stopped at the same position corresponding to the A indicated as *A in Fig. 5. The relative strength of gyrase cleavage on the cloned DNAs was revealed in Fig. 5. Dideoxynucleotide sequencing reactions were set up so that the probability of incorporating a terminating nucleotide was ≈1/200 for the specified nucleotide. A position at which termination occurs ≈10% of the time (i.e., for the *nuB103* gyrase cleavage product) appears as a very strong band on the gel. From the sequencing termination frequency seen in Fig. 5 and from the results of several repetitions of experimental cleavage with gyrase of the DNAs containing the gyrase-binding sites, we estimate the frequency of gyrase cleavage of *nuB1* to be ≈2 times and *nuB103* to be ≈4 times greater than the wild type.

To precisely map the cut sites on both strands of the DNA, gyrase-cleaved DNA ends were filled in by incubation with Klenow fragment of DNA pol I and deoxynucleotide triphosphates, *Xho* I linkers were ligated to the repaired ends, and the plasmids were recircularized by using T4 DNA ligase (25). After selection of Ap-resistant transformants, seven plasmids (out of 12 that had a *Xho* I cleavage site) were found to contain linkers inserted at the gyrase-cleavage site. Sequence analysis showed that the *Xho* I linkers were located at precisely the same position in the gyrase-binding site and bounded by duplication of the sequence AATC. Thus, gyrase makes a 4-bp staggered cut with the sequence AATC at the center, and the *nuB1* and *nuB103* mutations are located on opposite sides of this gyrase-cleavage site. T7 polymerase must add one nontemplated nucleotide to the end of gyrase-cleaved molecules because the dideoxynucleotide sequenc-

```
W.T.    ACGCGTCAGC GCCGCTCTGA GGCAATAAAC AGAATCAGGC ATAAAATCAG  50
nuB1                                                      C
nuB103                                                G
W.T.    CCGCACAGAT TTTTTAAAAC GCGCCACGGG ATTTTTAAAC CGGTATTTAA 100
W.T.    CGGTGTATGA ATCCCGTTTT ATCTTCCTTT CACTTTCTTT CTCCAGTACT 150
```

FIG. 4. Nucleotide sequence around the gyrase-binding site. The *Bam*HI–*Sca* I fragments (17.2–18.15 kb) from wild-type, *nuB1*, and *nuB103* DNAs were cloned into M13 vectors, and the nucleotide sequence between *Mlu* I and *Sca* I sites was determined for each by using chain-termination procedures (18). The wild-type (W.T.) sequence is shown along with the single-base change observed for each *nuB* mutant.

ing gels showed the sequence AATCN (Fig. 4) and GATTN when primed in the opposite direction (data not shown).

Deletion of the Gyrase-Binding Site Blocks Replication. The phenotype displayed by the *nuB* mutants implies a function for the gyrase-binding site. To test the effect of the site on replicative transposition, we constructed a prophage (Mu *cts62* Δ147) with a deletion of 147 bp spanning the gyrase-cleavage site and an insertion of a Kn resistance cassette. Insertion of the Kn resistance cassette into the Mu genome by itself (at the *Hpa* I site at 21.5 kb) had no effect on replication (M.L.P., unpublished work). Replication of the wild-type and the deleted prophage was measured after induction of the respective lysogens. Replication of the wild-type prophage began within 10 min after induction, whereas replication of the deleted prophage was delayed by >1 hr (Fig. 6).

DISCUSSION

We have described a strong DNA gyrase-binding site in the middle of the Mu genome, shown that deletion of a 147-bp fragment containing the site inhibits normal Mu replicative transposition upon induction of a lysogen, and characterized the alterations in the *nuB* mutants as changes in the nucleotide sequence of the site.

Gyrase-Binding Site Near Center of Mu. Cleavage of Mu DNA and its restriction fragments with *E. coli* DNA gyrase and enoxacin revealed a gyrase-binding site near the center of Mu, located 18.1 kb from the left end of the 37.2-kb genome; this binding site has an associated gyrase phenotype (15). Morrison and Cozzarelli (25) showed that some gyrase-binding sites fit specific sequence rules, whereas others do not. A proposed consensus sequence (26) and the sequence of the bottom strand of the Mu gyrase-binding site are shown below:

```
consensus 5' RNNNRNR (T/g) } {GRYC(G/T)YNYN(G/t)NY 3'
Mu site   5' GTGCGGC   T   } {GATT  T  TATG  C  CT 3'
                 G(nuB1)     C(nuB103)
```

The cleavage position is shown by brackets. Bases in parentheses indicate alternative use, with bases in uppercase preferred over those in lowercase. The Mu sequence matches 10/13 positions of the Lockshon–Morris consensus; the *nuB1* and *nuB103* mutations alter the match to 11/13 and 9/13, respectively.

Location of the Gyrase-Binding Site. The location of the gyrase-binding site near the middle of the genome at 18.1 kb agrees with the deletion mapping of the *nuB* mutations to a region encoding phage head and tail proteins, close to genes *G* and *I* (15). The site lies in an untranslated region between

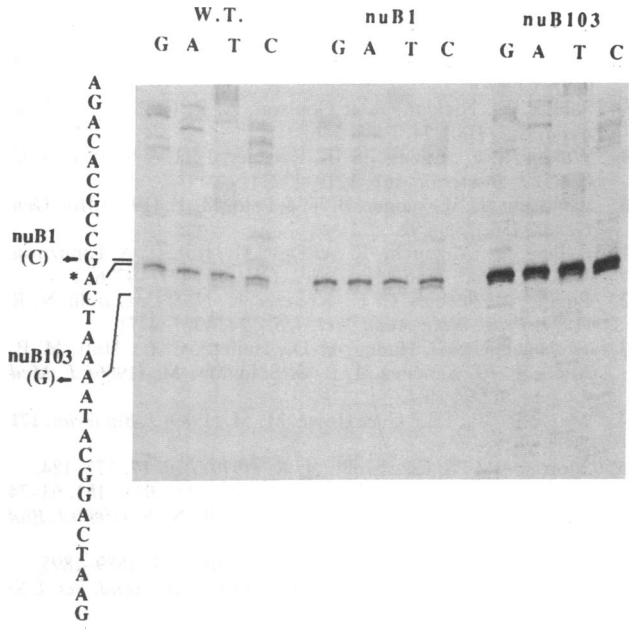

FIG. 5. Sequence of gyrase-cleavage sites. Plasmid DNAs cleaved with 40 units of gyrase were sequenced using dideoxynucleotide chain-terminating DNA sequencing reactions as described. The position of gyrase cleavage is indicated with an asterisk along with positions of the *nuB1* and *nuB103* mutations.

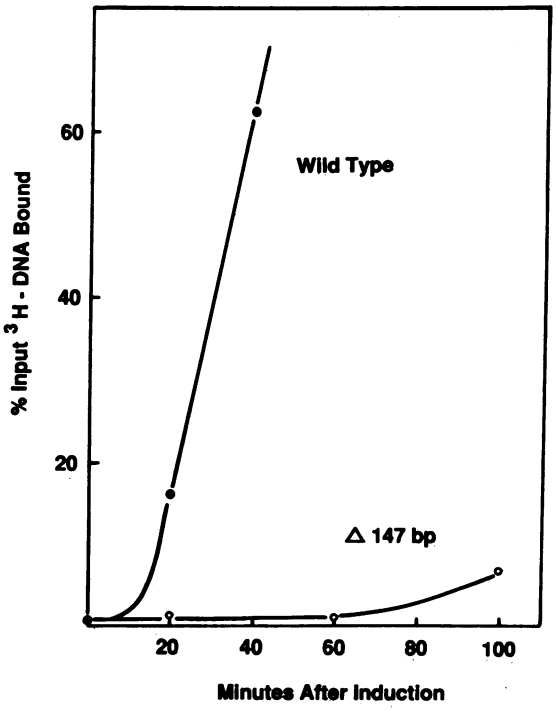

FIG. 6. Mu DNA replication with and without the gyrase-binding site. Lysogens of Mu *cts62* and Mu *cts62* Δ147 were thermoinduced and pulse-labeled at intervals with [³H]thymidine. Labeled DNA was isolated and hybridized to nitrocellulose filters carrying bound Mu DNA, and the percent of input labeled DNA that hybridized to the filters was determined.

the two genes. The operon containing the *I* gene is transcribed from a point slightly downstream from the *Sca* I site at 18.15 kb (24). Our sequence shows stop codons in all reading frames before the gyrase-binding site (Fig. 4 and data not shown), showing that the *G* gene translation must stop upstream of the gyrase-cleavage site. The precise termination point for the transcript encoding the *G* gene is as yet undefined. Because the site is untranslated, its function would be expected to be exerted only in cis; indeed, when a dilysogen of Mu *cts62* and Mu *cts62* Δ147 was induced, essentially all replication was of the wild-type prophage (M.L.P., unpublished work).

A Role for the Gyrase-Binding Site in Mu Replication. Deletion of a 147-bp segment spanning the gyrase-binding site inhibited normal Mu DNA replication after induction of a lysogen carrying the deleted prophage (Fig. 6). This finding and the phenotype displayed by *nuB* mutants show that the site is required for induction of normal replicative transposition. The Δ147 prophage retains the ability to replicate, as significant replication was seen after a delay of ≈100 min. We know that Mu early transcription, responsible for synthesis of the required replication proteins, is unaffected by deletion of the gyrase-binding site (M.L.P., unpublished work). The DNA replication delay observed with wild-type Mu in a *gyrB* host was eliminated with the *nuB103* mutant (S. H. Shore and M.M.H., unpublished work), showing that the site changes in the *nuB* mutants exert their effect on replication.

Cleavage efficiency of gyrase–DNA complexes formed *in vitro* can vary depending on site and on conditions (27). Therefore, a sequence change that increases gyrase cleavage *in vitro* may not necessarily signify an increase in gyrase binding or catalytic activity *in vivo*. Nonetheless, the *nuB1* and *nuB103* mutations that allow Mu growth in a *gyrB* host are both located only 6 bp apart at the strong central site (Fig. 4). We conclude that the central site is the most important gyrase-binding site in the 37.2-kb Mu genome. How could DNA gyrase acting at one site exert such a potent influence on Mu replication? One possibility is that transposition of Mu requires an unusual degree of supercoiling and that gyrase bound at the central site produces an exceptionally high superhelical density. Mu transposition *in vitro* does require supercoiled DNA. In the absence of host IHF protein, unusually high levels of supercoiling are required (12), although IHF protein should be present in the strains used in these studies.

A second possibility involves a model in which stable gyrase binding to the central site enhances replication by organizing Mu DNA structure to assist in bringing the ends of the genome together. This model explains the inhibitory effect of insertions on Mu transposition, as the site would be asymmetrically located in the prophage with an insertion and would, thus, interfere with the proper organization of the genome. In the absence of stable gyrase binding, the ends of the prophage, separated by 37.2 kb and constrained in the host genome, would have to be brought together by inefficient random collision.

The suggestion that topoisomerases organize DNA structure, perhaps in a manner tangentially related to their catalytic role in supercoiling, has been made both for prokaryotes and eukaryotes. Gyrase binding to the *par* locus of the plasmid pSC101 is required for partitioning, apparently independently of an effect on supercoiling (28). Binding of gyrase to certain repetitive sequences (REP) in the *E. coli* genome has been suggested to play a role in organizing nucleoid structure (29). Mutations in the T4 topoisomerase impede replication by causing a DNA delay phenotype (30). A role for topoisomerase II binding to DNA and to the nuclear matrix in organization of *Saccharomyces pombe* chromosome structure has been proposed (31). Even though substantial insights have been derived from studying protein and supercoiling requirements of shortened mini-Mu derivatives *in vitro* (9–12), the gyrase-binding site was not present in the constructs studied in soluble systems. These results demonstrate the importance of studying replication of large complete molecules. Understanding the role of gyrase in Mu replication may provide a key for defining the topological structure of other complex genomes.

We thank Steve Gracheck and Warner–Lambert (Ann Arbor, MI) for enoxacin and for providing travel funds. This work was supported by Grant GM24270 from the National Institutes of Health to M.L.P., Grants AI12731 and AI24774 to M.M.H., and Grant GM33143 to N.P.H.

1. Pato, M. L. (1989) in *Mobile DNA*, eds. Berg, D. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 23–52.
2. Faelen, M., Toussaint, A., Waggoner, B., Desmet, L. & Pato, M. (1986) *Virology* **153,** 70–79.
3. Chandler, M., Clerget, M. & Galas, D. (1982) *J. Mol. Biol.* **154,** 229–243.
4. Morisoto, D., Way, J., Kim, H.-J. & Kleckner, N. (1983) *Cell* **32,** 799–807.
5. Gloor, G. & Chaconas, G. (1986) *J. Biol. Chem.* **261,** 16682–16688.
6. Puspurs, A. H., Trun, N. J. & Reeve, J. N. (1983) *EMBO J.* **2,** 345–352.
7. Ptashne, M. (1986) *Nature (London)* **335,** 697–701.
8. Wang, J. C. & Giaever, G. N. (1988) *Science* **240,** 300–304.
9. Craigie, R. & Mizuuchi, K. (1986) *Cell* **45,** 793–800.
10. Leung, P. C., Teplow, D. B. & Harshey, R. M. (1989) *Nature (London)* **338,** 656–658.
11. Mizuuchi, M. & Mizuuchi, K. (1989) *Cell* **58,** 399–408.
12. Surette, M. G., Lavoie, B. D. & Chaconas, G. (1989) *EMBO J.* **8,** 3483–3489.
13. Resibois, A., Pato, M., Higgins, P. & Toussaint, A. (1984) in *Proteins Involved in DNA Replication*, eds. Hubscher, U. & Spadari, S. (Plenum, New York), pp. 69–76.
14. Ross, W., Shore, S. H. & Howe, M. M. (1986) *J. Bacteriol.* **167,** 905–919.
15. Yoshida, R. K., Miller, J. K., Miller, H. I., Friedman, D. I. & Howe, M. M. (1982) *Virology* **120,** 269–272.
16. Higgins, N. P., Peebles, C. L., Sugino, A. & Cozzarelli, N. R. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1773–1777.
17. Sugino, A. & Cozzarelli, N. R. (1980) *J. Biol. Chem.* **255,** 6299–6306.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
19. Winans, S. C., Elledge, S. J., Krueger, J. H. & Walker, G. C. (1985) *J. Bacteriol.* **161,** 1219–1221.
20. van Brunt, J., Waggoner, B. T. & Pato, M. L. (1977) *Mol. Gen. Genet.* **150,** 285–292.
21. Gellert, M., Mizuuchi, K., O'Day, M., Itoh, T. & Tomizawa, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4772–4776.
22. Sugino, A., Peebles, C. L., Kreuzer, K. N. & Cozzarelli, N. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4767–4771.
23. Domagala, J. M., Hanna, L. D., Heifetz, C. L., Hutt, M. P., Mich, T. F., Sanchez, J. P. & Solomon, M. (1986) *J. Med. Chem.* **29,** 394–404.
24. Margolin, W., Rao, G. & Howe, M. M. (1989) *J. Bacteriol.* **171,** 2003–2018.
25. Morrison, A. & Cozzarelli, N. R. (1979) *Cell* **17,** 175–184.
26. Lockshon, D. & Morris, D. R. (1985) *J. Mol. Biol.* **181,** 63–74.
27. Morrison, A., Higgins, N. P. & Cozzarelli, N. R. (1980) *J. Biol. Chem.* **225,** 2211–2219.
28. Wahle, E. & Kornberg, A. (1988) *EMBO J.* **7,** 1889–1895.
29. Yang, Y. & Ames, G. F.-L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8850–8854.
30. Stetler, G. L., King, G. J. & Huang, W. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3737–3741.
31. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. & Yanagida, M. (1987) *Cell* **50,** 917–925.