Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8220–8224, December 1987
Biochemistry

Specific binding of transposase to terminal inverted repeats of transposable element Tn*3*

(transposon/DNA binding protein/DNase I "footprinting")

HITOSHI ICHIKAWA*, KAORU IKEDA*, WILLIAM L. WISHART†, AND EIICHI OHTSUBO*‡

*Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Yayoi 1-1-1, Tokyo 113, Japan; and †Department of Molecular Biology, Princeton University, Princeton, NJ 08544

Communicated by Norman Davidson, July 27, 1987

ABSTRACT **Tn*3* transposase, which is required for transposition of Tn*3*, has been purified by a low-ionic-strength-precipitation method. Using a nitrocellulose filter binding assay, we have shown that transposase binds to any restriction fragment. However, binding of the transposase to specific fragments containing the terminal inverted repeat sequences of Tn*3* can be demonstrated by treatment of transposase–DNA complexes with heparin, which effectively removes the transposase bound to the other nonspecific fragments at pH 5–6. DNase I "footprinting" analysis showed that the transposase protects an inner 25-base-pair region of the 38-base-pair terminal inverted repeat sequence of Tn*3*. This protection is not dependent on pH. Interestingly, binding of the transposase to the inverted repeat sequences facilitates DNase I to nick at the end of the Tn*3* sequence. It was also observed that the transposase protects the end regions of restriction fragments with a cohesive sequence at their 5′ end or with a flush end from DNase I cleavage. The specific and nonspecific binding of transposase to DNA is ATP-independent.**

Transposons are distinct segments of DNA that are capable of movement from one site to many different sites. Many transposons have been identified in bacteria (for review, see ref. 1). Tn*3* is an ampicillin-resistance transposon, which is 4957 base pairs (bp) in length and contains a 38-bp terminal inverted repeat at the left terminus (IRL) and at the right terminus (IRR) (2). Tn*3* has two genes, *tnpA* and *tnpR*, necessary for its transposition, which occurs by a two-step mechanism (2–4). In the first step of transposition, a donor replicon carrying Tn*3* and a recipient replicon fuse to form a cointegrate, in which Tn*3* is duplicated and appears at the junction between the two replicons in a direct orientation. Cointegrate formation requires transposase, the *tnpA* gene product, which has a molecular mass of 113 kDa (2, 5–7). For this step, the terminal inverted repeats are also essential. These repeats are thought to be the recognition sites for the transposase (2, 7). In the second step, the *tnpR* gene product, called resolvase, catalyzes a site-specific recombination at the internal resolution site (IRS or *res*) of Tn*3* between the two Tn*3*s in the cointegrate to give a recipient replicon containing Tn*3* as well as the donor replicon (7–11). The tnpR protein is also a repressor that inhibits synthesis of both transposase and itself at the level of transcription (5, 12).

The purification of Tn*3* transposase was first reported by Fennewald *et al.* (13). The purified protein exhibits a heat-sensitive binding activity toward single- or double-stranded DNA. Subsequently, we have purified Tn*3* transposase by another method and have reported that the transposase binds nonspecifically to all double-stranded DNA fragments in the absence of ATP but binds specifically to the DNA fragments containing the Tn*3* terminal regions when 8 mM ATP is present (14).

In this paper, we study the interaction of Tn*3* transposase, which has been purified by a low-ionic-strength-precipitation method, with DNA. We demonstrate that the purified transposase is an ATP-independent DNA binding protein, which has interesting properties that may be involved in the actual transposition event of Tn*3*.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The bacterial strains used were *Escherichia coli* K12 derivatives C600 and a minicell-producing strain, DS410. pMB8::Tn3no.5 was a plasmid carrying the Tn*3* *tnpR* mutant (Tn3no.5) (2). The plasmids pUC18L1 and pUC18R1 were constructed by inserting *Hpa* II restriction fragments, named L (364 bp) and R (453 bp), containing IRL and IRR of pMB8::Tn3no.5, respectively, into an *Acc* I site in pUC18 (15), as shown in Fig. 1. Plasmid DNAs in C600 were prepared according to Ohtsubo *et al.* (16).

Purification of Transposase. DS410 harboring pMB8::Tn3no.5 was grown in 1.8 liters of Bacto antibiotic medium 3 (Difco) at 17.5 g/liter with gentle aeration at 37°C for 14 hr and was further incubated at 27°C for 8 hr. Cells were harvested, washed with 100 mM Tris Cl (pH 7.5), frozen in liquid nitrogen, and stored at −70°C.

The frozen cells were thawed and suspended in 150 ml of lysis buffer [100 mM Tris Cl, pH 7.5/20 mM EDTA/10 mM 2-mercaptoethanol/20% (wt/vol) sucrose/250 μM phenylmethylsulfonyl fluoride]. Lysozyme (Seikagaku Kogyo, Tokyo) was added at a final concentration of 2 mg/ml, and the suspension was kept on ice for 30 min with gentle stirring. Fifty milliliters of 5 M NaCl and 50 ml of 10% (wt/vol) Triton X-100 was added. After 30 min of stirring and mixing several times on a Vortex mixer, cell debris was removed by centrifugation at 100,000 × *g* for 30 min. The cleared lysate was dialyzed against two changes of 5 liters of TEM-A buffer (50 mM Tris Cl, pH 7.5/1 mM EDTA/10 mM 2-mercaptoethanol/250 μM phenylmethylsulfonyl fluoride) for 6 hr each. The resulting precipitate was pelleted by centrifugation at 10,000 × *g* for 20 min and washed with 100 ml of TEM-A buffer. The pellet was dissolved in 200 ml of TEM-A buffer containing 300 mM NaCl. It was passed through a DEAE-Sephadex A-50 (Pharmacia) column (3 × 20 cm) previously equilibrated with the same buffer. The eluate was dialyzed against two changes of 5 liters of TEM-B buffer (25 mM Tris Cl, pH 7.5/100 μM EDTA/10 mM 2-mercaptoethanol/250 μM phenylmethylsulfonyl fluoride) for 4 hr each. The precipitate was pelleted by centrifugation at 10,000 × *g* for 10 min and washed successively with 10 ml of TEM-B buffer and 5 ml of

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.

Abbreviations: IRL and IRR, inverted repeats at the left and right terminus, respectively, of Tn*3*.

‡To whom reprint requests should be addressed.

TEM-B buffer containing 1 M NaCl. The second wash was passed through a Sephacryl S-300 (Pharmacia) column (2.6 × 100 cm) equilibrated with the same buffer. Transposase was eluted with a K_{av} of 0.3. The transposase fractions were dialyzed against three changes of 1 liter of PEM buffer (20 mM potassium phosphate, pH 6.5/100 μM EDTA/10 mM 2-mercaptoethanol/250 μM phenylmethylsulfonyl fluoride) containing 200 mM KCl for 1 hr each and loaded on a CM-Sephadex C-50 (Pharmacia) column (0.9 × 20 cm) equilibrated with the same buffer. The column was washed with 40 ml of PEMG buffer [PEM buffer plus 10% (wt/vol) glycerol] containing 200 mM KCl and was eluted with an 80-ml linear gradient of 200 mM–1 M KCl in PEMG buffer. Transposase was eluted at ≈400 mM KCl. It was dialyzed against three changes of 500 ml of TEDG buffer [25 mM Tris Cl, pH 7.5/100 μM EDTA/500 μM dithiothreitol/10% (wt/vol) glycerol] containing 100 mM KCl for 1 hr each. The precipitate was pelleted by centrifugation at 10,000 × *g* for 5 min and dissolved in 200 μl of storage buffer (TEDG buffer containing 300 mM KCl). The transposase solution was frozen in liquid nitrogen and stored at −70°C.

Protein concentration was determined using the method described by Bradford (17) with bovine serum albumin as standard. Electrophoresis of proteins was performed using an 8% $NaDodSO_4$/polyacrylamide gel according to the system described by Laemmli (18).

Nitrocellulose Filter Binding Assay. The nitrocellulose filter binding assay used was essentially the same as that used previously (15). pMB8::Tn3no.5 DNA was digested with *Hpa* II (Takara Shuzo, Kyoto), and the ends of the DNA fragments were labeled with [α-^{32}P]dCTP (Amersham) using the DNA polymerase I large fragment (Takara Shuzo). The ^{32}P-labeled fragments (0.1 pmol) were incubated with 1 μg of transposase for 10 min at 27°C in 50 μl of binding buffer [100 mM KCl/25 mM Tris Cl/10 mM $MgCl_2$/100 μM EDTA/500 μM dithiothreitol/bovine serum albumin (100 μg/ml)] containing 8 mM ATP or 25 mM potassium biphthalate. The pH of the buffer was adjusted to between 4 and 9 with NaOH. Heparin (Sigma) was added at 10 μg/ml, followed by further incubation for 10 min. The samples were filtered through nitrocellulose filters (BA85, Shleicher & Schuell). The filters were then washed five times with 1 ml of binding buffer containing 8 mM ATP or 25 mM potassium biphthalate. DNA fragments retained on the filter were eluted by soaking in 1 ml of elution buffer [25 mM Tris Cl, pH 7.5/0.2% (wt/vol) $NaDodSO_4$] for 30 min with gentle shaking. The eluted DNA fragments were ethanol-precipitated and electrophoresed in a 4% polyacrylamide gel.

DNase I "Footprinting." Restriction fragments L'-H, L'-B, R'-H, and R'-B (see Fig. 1), which were labeled with ^{32}P at the 5' end of one strand of the duplexes, were prepared as described in Fig. 1. The DNase I footprinting assay used was a modification of the method described by Galas and Schmitz (20). Approximately 0.2 pmol of the ^{32}P-labeled fragment was mixed with various quantities of transposase (0–2 μg) in 40 μl of binding buffer and incubated for 10 min at 27°C. Ten microliters of DNase I (10–80 units/ml; Takara Shuzo) in the binding buffer was added, and the incubation was continued for 2 min. After extraction with phenol, the DNA samples were ethanol-precipitated and electrophoresed in 6% polyacrylamide sequencing gels.

RESULTS

Purification of Transposase. Tn*3* transposase was purified from a culture of the *E. coli* minicell-producing strain DS410 harboring plasmid pMB8::Tn*3*no.5, which was known to overproduce Tn*3* transposase (14). Purification of the transposase protein was monitored by $NaDodSO_4$/polyacrylamide gel electrophoresis. Tn*3* transposase forms aggregates

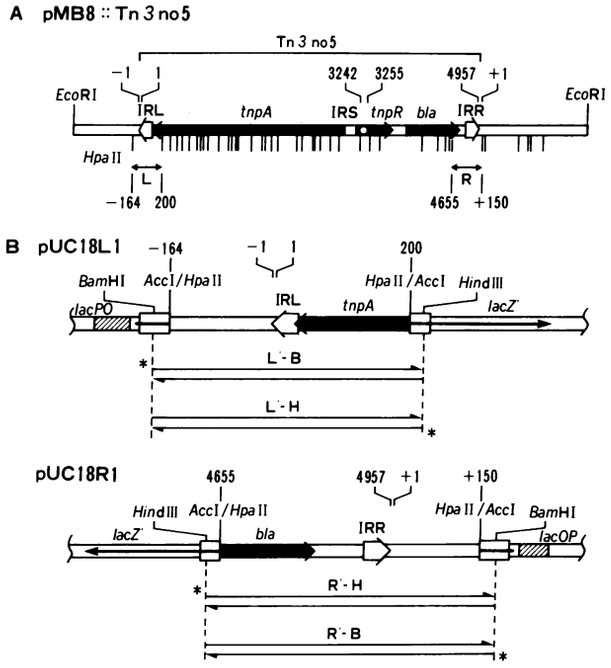

FIG. 1. Physical and genetic structures of plasmids and ^{32}P-labeled fragments used for DNase I footprinting analysis. (*A*) pMB8::Tn3no.5 DNA linearized by cutting at an *Eco*RI site. The thick open and closed arrows indicate the inverted repeats (IRL and IRR) and the three genes (*tnpA*, *tnpR*, and *bla*) of Tn*3*, respectively. The white dot in the *tnpR* gene indicates the position of the *Eco*RI linker insertion in Tn3no.5. Numbers on Tn3no.5 represent the coordinates of the nucleotide sequence of Tn*3* (2). Numbers with a plus or minus sign represent the coordinates of the pMB8 sequence. Vertical bars below the plasmid sequence indicate restriction sites for *Hpa* II. L and R, *Hpa* II restriction fragments; IRS, internal resolution site. (*B*) Diagram of the inserts in pUC18L1 and pUC18R1 that were constructed by inserting fragments L or R, respectively, of pMB8::Tn3no.5 into the *Acc* I site of pUC18. Open boxes indicate multiple restriction sites of pUC18 for enzymes such as *Acc* I, *Hin*dIII, *Bam*HI, *Sma* I, and *Kpn* I. The positions of the *lacPO* region (hatched box) and the *lacZ'* gene (thin horizontal arrow) in the pUC18 sequence are indicated. The restriction fragments, designated L'-H, L'-B, R'-H, and R'-B, contain the terminal regions of Tn*3*. Half arrows show the direction of a DNA strand from 5' to 3'. Asterisks at the 5' end of a strand indicate the position labeled with ^{32}P. To prepare fragment L'-H, pUC18L1 was digested with *Hin*dIII (Takara Shuzo). The 5' ends of the digested DNA were labeled with ^{32}P using polynucleotide kinase (Takara Shuzo) and [γ-^{32}P]ATP (Amersham), and the labeled DNA was digested with *Bam*HI (Takara Shuzo). The small fragment L'-H was separated by electrophoresis in a 5% polyacrylamide gel and eluted from the gel according to the procedure of Maxam and Gilbert (19). Fragment R'-H was prepared from pUC18R1 in an analogous manner. To prepare fragment L'-B (or R'-B), pUC18L1 (or pUC18R1) was first cleaved with *Bam*HI. After labeling the 5' ends of the DNA, the sample was cleaved with *Hin*dIII, and fragment L'-B (or R'-B) was purified, as described above.

in low-ionic-strength solutions (13) to give a precipitate. We used this property to fractionate and concentrate transposase.

The actual purification method is described in detail in *Materials and Methods*. Our method consists of essentially four steps: (*i*) cell lysis is performed by using Triton X-100 in 1 M NaCl; (*ii*) the lysate, which is cleared by centrifugation, is dialyzed to eliminate NaCl, and the precipitate generated is harvested; (*iii*) gel filtration with a Sephacryl S-300 column is performed, after elimination of contaminant nucleic acids by passing through a DEAE-Sephadex column and concentration of transposase by dialysis at low ionic strength to yield a precipitate; and (*iv*) by using cation exchange chromatog-

raphy with a CM-Sephadex column, transposase is purified to near homogeneity (Fig. 2). Two hundred and thirty micrograms of transposase has been obtained from 4.7 g of wet cells (1.8 liters of culture) by this method.

Transposase Is an ATP-Independent DNA Binding Protein. A nitrocellulose filter binding assay of the purified transposase with restriction fragments of pMB8::Tn3no.5 DNA was carried out. Fig. 3 (lanes 2 and 3) shows that all of the restriction fragments were bound by transposase and were retained on the nitrocellulose filter in the presence or absence of ATP. This result suggests that the transposase is an ATP-independent DNA binding protein, as previously reported (14). Although the above experiments were carried out under acidic conditions, the DNA binding activity was observed in a pH range of 4–9 (data not shown). However, when the transposase–DNA complexes were treated with heparin (10 μg/ml), the DNA fragments containing terminal regions of Tn*3* were retained on the nitrocellulose filter, but the other fragments were not; heparin dissociated transposase from the fragments containing no terminal regions of Tn*3* (Fig. 3, lanes 4 and 5). This dissociation was the most effective around pH 5.4 (data not shown). Fig. 3 (lanes 4 and 5) also shows that ATP was not required for binding of transposase to the specific fragments. This result is not consistent with that reported previously (14).

Transposase Binds Specifically to the Terminal Inverted Repeat Sequences. To determine actual binding sites of transposase, DNase I footprinting experiments were carried out using a fragment that contained IRL of Tn*3*. As shown in Fig. 4*A*, the protected region on fragment L′-H, which was labeled with ^{32}P at the 5′ end of one strand of the duplex (see Fig. 1), was found to be within the 38-bp inverted repeat sequence in the absence of ATP at pH 5.4. The nucleotide sequence protected by transposase is represented schematically in Fig. 5. Enhancement of cleavage by DNase I in the presence of transposase was also observed in the protected region (Figs. 4*A* and 5). By using fragment L′-B, which was the same fragment as fragment L′-H except that the 5′ end of the complementary strand was labeled (see Fig. 1), the protected region on the complementary strand was also found to be within the inverted repeat, as shown in Figs. 4*B* and 5. In addition to protection, three bands of enhanced cleavage by DNase I were also observed in this strand (Figs. 4*B* and 5).

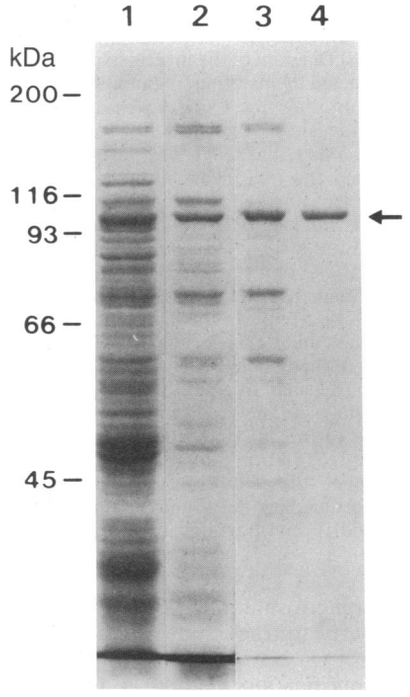

FIG. 2. Electrophoresis of transposase purification fractions in an 8% NaDodSO$_4$/polyacrylamide gel. Lanes: 1, whole cells; 2, precipitate at the first dialysis; 3, Sephacryl S-300 gel filtration fraction; 4, CM-Sephadex chromatography fraction. The gel was stained with Coomassie brilliant blue. The arrow indicates the position of the transposase protein. The molecular mass standards used were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (93 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa).

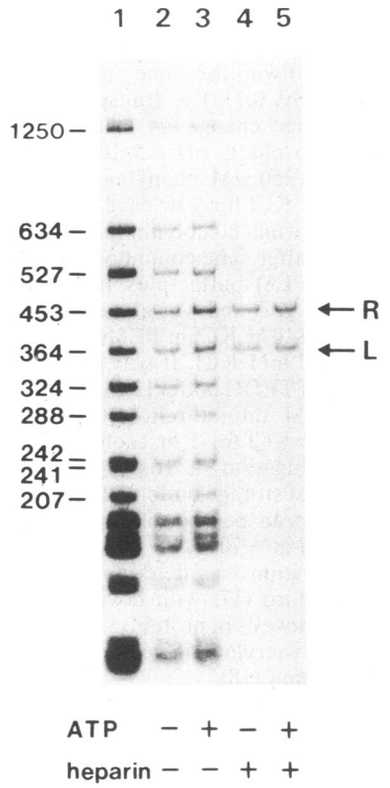

FIG. 3. Autoradiogram of a polyacrylamide gel showing the results of the nitrocellulose filter binding assay. Lanes: 1, total *Hpa* II digests of pMB8::Tn3no.5 DNA; 2–5, *Hpa* II DNA fragments retained on nitrocellulose filters at pH 5.4. The presence or absence of 8 mM ATP and heparin at 10 μg/ml is indicated. The arrows mark the bands of the fragments containing IRL (L) or IRR (R) (see Fig. 1). The sizes (in bp) of the respective fragments are indicated.

The DNase I footprinting experiments described above were carried out at pH 5.4. At this pH, the transposase was effectively dissociated from DNA fragments containing no terminal inverted repeats by heparin. When the footprinting experiments were carried out at pH 7.5, at which the nonspecific binding was not effectively eliminated by heparin, the same region in the inverted repeat sequence as that shown in Fig. 5 was protected by transposase, regardless of the presence or absence of ATP (Fig. 4 *C* and *D*). The protection pattern also remained the same regardless of the absence or presence of heparin (data not shown).

DNase I footprinting of IRR of Tn*3* was also carried out at neutral pH using fragments R′-H and R′-B, in which the 5′ end of one or the other strand of the duplex was respectively labeled with ^{32}P (see Fig. 1). As shown in Figs. 4*E* and 5, transposase protected the same sequence as that within IRL and enhanced the cleavage at the same sites within IRL.

An interesting observation is that transposase enhanced cleavage at the junction between the terminal sequence of Tn*3* (3′ . . . TGGGG 5′) and the sequence of the vector plasmid (3′ ATTAA . . . 5′), as indicated in Fig. 4 *B* and *D* and

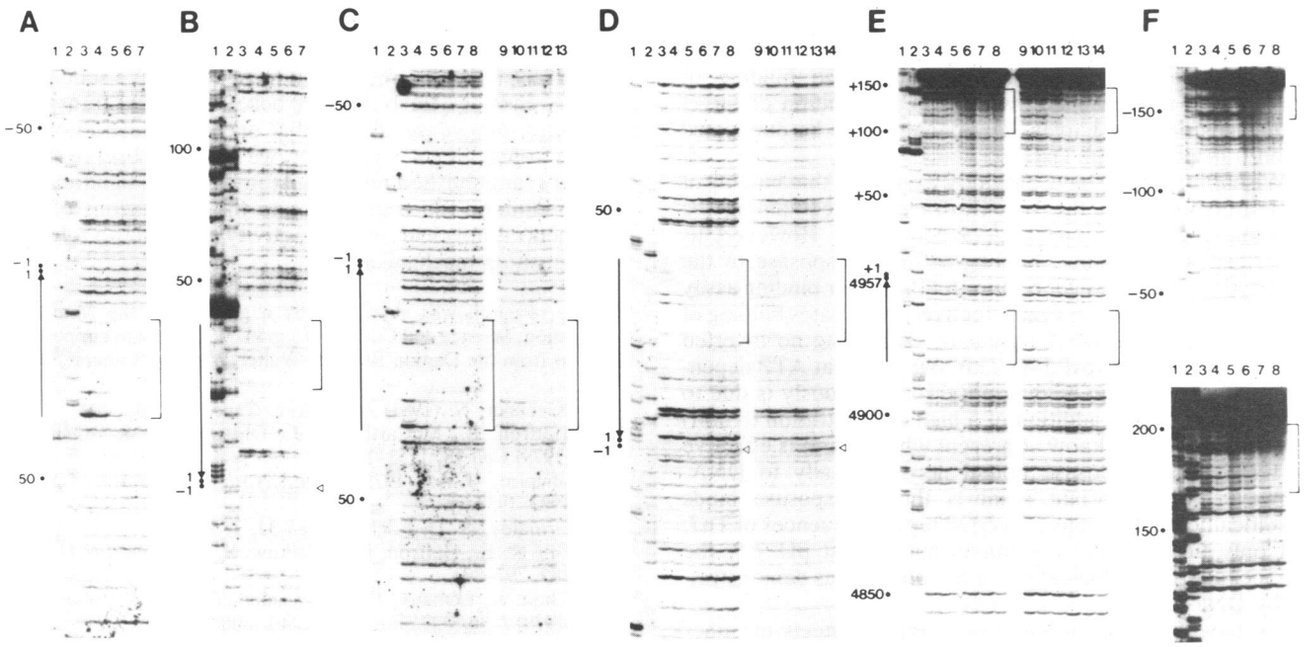

FIG. 4. DNase I footprints in the Tn*3* terminal regions. (*A* and *B*) Footprints at pH 5.4 using fragment L′-H or L′-B, respectively. (*C–E*) Footprints at pH 7.5 using L′-H, L′-B, or R′-H, respectively. (*F*) Footprints at pH 7.5 of transposase on the end region of L′-H (*Upper*) and L′-B (*Lower*) (see Fig. 1). Lanes 1 and 2 in each panel show markers prepared as described below. Lanes 3–8, fragments partially digested with DNase I in the presence of transposase at 0, 2, 4, 8, 20, or 40 μg/ml, respectively, and in the absence of ATP. Lanes 9–14, the same as lanes 3–8, respectively, except that the digestion was performed in the presence of 8 mM ATP. In some panels, lanes 8 and 14 are missing. Numbers indicate the positions of bases (see the coordinate systems shown in Figs. 1 and 5). Arrows correspond to the region of the inverted repeats of Tn*3*. Brackets mark the region protected by transposase. Open arrowheads mark enhanced digestion with DNase I at the Tn*3* end. Markers for identification of the bands on DNase I footprints on fragment L′-H (or R′-H) were prepared as follows: The DNA sequencing reaction was performed by the dideoxynucleotide method (21, 22) using denatured DNA from plasmid pUC18L1 (or pUC18R1) as a template and 15-mer M13 primer M1 (Takara Shuzo), which hybridized at the *lacZ′* region of the template and initiated DNA synthesis toward the insert in the plasmid (see Fig. 1). The ^{32}P-incorporated reaction products were ethanol-precipitated, rinsed with 70% (vol/vol) ethanol, dried, and then digested with *Hin*dIII. To prepare markers for the footprinting using fragment L′-B (or R′-B), we used pUC18L1 (or pUC18R1) as a template and 17-mer reverse primer (Amersham) that hybridized at the *lacPO* region and initiated DNA synthesis toward the insert in the plasmid (see Fig. 1). The reaction products were digested with *Bam*HI. The two marker samples shown were those obtained in the presence of ddGTP (lane 1) and ddATP (lane 2).

Fig. 5. This enhancement of cleavage by DNase I at the end of Tn*3* was clearer in the presence of ATP. This cleavage was not due to the nicking activity associated with transposase itself (data not shown).

Transposase Protects the End Regions of Particular Restriction Fragments. We noticed that transposase protected regions other than those in the inverted repeats (for example, a region shown at the top of gels in Fig. 4*E*). This region was positioned at the end of fragment R′-H and was approximately 100 bases outside the Tn*3* sequence. We observed that the other end region of fragment R′-H was also protected (data not shown). Similarly, the end regions of fragment L′ (L′-H or L′B; see Fig. 1) were also found to be protected by transposase (Fig. 4*F*). Binding of transposase to the end regions of the restriction fragments was not sensitive to pH and was ATP-independent. The sequences of these four protected regions had no homology with the sequence of the inverted repeat, and there was no common sequence among these four regions. These fragments, which had been digested with *Bam*HI or *Hin*dIII, had a cohesive sequence at their 5′ ends. We, therefore, examined fragments prepared by digestion with *Sma* I (to generate flush ends) or with *Kpn* I (to generate a cohesive sequence at the 3′ ends) and found that the terminal region with a flush end was bound by transposase, but the terminal region with a cohesive sequence at the 3′ end was not (data not shown).

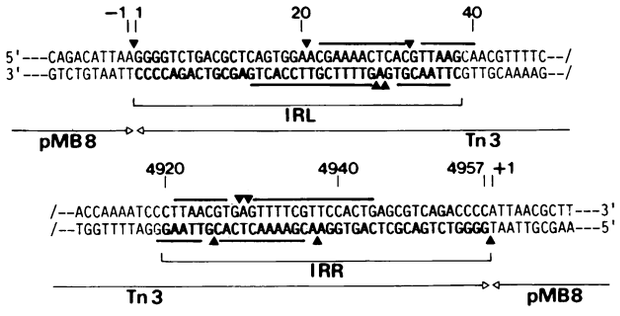

FIG. 5. Nucleotide sequence of the terminal regions of Tn*3* showing the sequences protected by transposase from DNase I digestion. The sequences protected by transposase are indicated by the lines above and below the DNA strands. Arrowheads indicate the positions where digestion is enhanced. The numbers represent the same coordinates as in Fig. 1.

DISCUSSION

Transposition of Tn*3*, as well as other transposons, is generally dependent on the presence of the terminal inverted repeats in these elements. It is thought that the inverted repeats of a transposon provide the recognition sites for the transposase encoded by the element. In this paper, we have shown that the Tn*3* transposase actually binds specifically to the inverted repeats of Tn*3*. Tn*3* transposase has also been shown to bind nonspecifically to DNA (this paper and ref. 14). This property may be essential for recognition of the terminal inverted repeat sequences of Tn*3* by a mechanism where transposase binds first to nonspecific DNA sequence and somehow finds the specific sequences located far from

the sequence first bound by transposase, as discussed by Jack *et al.* (23) for recognition of specific sequences by restriction enzyme *Eco*RI. The nonspecific binding of transposase may also be involved in recognition of target sequences on the recipient DNA that are to be duplicated upon transposition of Tn*3*.

Wishart *et al.* (14) have previously reported that binding of Tn*3* transposase to the specific fragments containing the terminal regions of Tn*3* is ATP-dependent. However, as described in this paper, binding of the transposase to the inverted repeats does not require ATP. A filter binding assay has shown that heparin very effectively eliminates binding of transposase to the DNA fragments containing no inverted repeat sequences at pH 5–6. This suggests that ATP dependency of the specific binding observed previously is due to acidification by the addition of a high concentration (8 mM) of ATP. We do not know at present why heparin is effective in removing transposase bound nonspecifically to DNA. However, an important point is that transposase binds specifically to the terminal inverted repeat sequences of Tn*3*, not only under acidic conditions but also at pH 7.5, the intracellular physiological pH of *E. coli* cells, as demonstrated by DNase I footprinting analyses.

We have shown that Tn*3* transposase protects an inner 25-bp region in the 38-bp inverted repeat sequence. Recently, Huang *et al.* (24) have shown genetically that deletion of a 4-bp sequence within the binding region that we have observed greatly reduces the frequency of transposition. This is consistent with the assumption that the inverted repeats are bound by transposase to initiate the transposition event of Tn*3*. Huang *et al.* (24) have also reported that the same deletion causes loss of transposition immunity—namely, the phenomenon that Tn*3* does not transpose to the other genome containing Tn*3* or the inverted repeat sequence of Tn*3*. This result strongly suggests that binding of transposase to the inverted repeat sequences is related to transposition immunity.

Tn*3* transposase has been shown to enhance cleavage with DNase I at the two ends of Tn*3* on one of the strands to give the 5′ ends of the Tn*3* sequence, 5′ GGGGT. . . . This indicates that binding of the transposase to the inner part of the inverted repeats changes the conformation near the Tn*3* ends to facilitate DNase I to nick at the Tn*3* ends. In previously proposed molecular models, the transposition event is initiated by nicking a strand at the end of the transposon, followed by transferring the nicked strand of the transposon to the target site, which is subjected to a staggered cut (3, 25). It is, therefore, interesting to assume that transposase causes the conformational change of the Tn*3* terminal regions *in vivo* to facilitate a nuclease(s) that may be involved in the actual transposition event to nick at the Tn*3* ends. The transposase of bacteriophage Mu (Mu A protein) has also been purified (26) and has been shown to bind specifically to the terminal regions of Mu using the DNase I footprinting method (27). Mu A protein has three binding sites at each terminus in which Mu has only 2 bp of the terminal inverted repeat, but enhancement of cleavage with DNase I at the end of Mu has not been clearly demonstrated.

We have observed that, in addition to the inverted repeat sequences of Tn*3*, Tn*3* transposase protects the end regions of the restriction fragments that have a cohesive sequence at their 5′ ends or a flush end. We believe that Tn*3* transposase may have a helicase activity that opens the restriction fragments from their ends; however, we failed to observe such an activity (unpublished results). At present the significance of the binding of Tn*3* transposase to the end regions of DNA fragments is not clear, but this activity might play a role in the transposition mechanism.

This research was supported by a grant from the Ministry of Education, Science and Culture of Japan. W.L.W. was supported by a grant from the Damon Runyon–Walter Winchell Cancer Fund.

1. Kleckner, N. (1981) *Annu. Rev. Genet.* **15,** 341–404.
2. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18,** 1153–1163.
3. Shapiro, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1933–1937.
4. Grindley, N. D. F. (1983) *Cell* **32,** 3–5.
5. Gill, R. E., Heffron, F. & Falkow, S. (1979) *Nature (London)* **282,** 797–801.
6. Chou, J., Lemaux, P. G., Casadaban, M. J. & Cohen, S. N. (1979) *Nature (London)* **282,** 801–806.
7. McCormick, M., Wishart, W. L., Ohtsubo, H. L., Heffron, F. & Ohtsubo, E. (1981) *Gene* **15,** 103–118.
8. Arthur, A. & Sherratt, D. (1979) *Mol. Gen. Genet.* **175,** 267–274.
9. Kostriken, R., Morita, C. & Heffron, F. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4041–4045.
10. Reed, R. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3428–3432.
11. Reed, R. R. & Grindley, N. D. F. (1981) *Cell* **25,** 721–728.
12. Chou, J., Casadaban, M. J., Lemaux, P. G. & Cohen, S. N. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4020–4024.
13. Fennewald, M. A., Gerrard, S. P., Chou, J., Casadaban, M. J. & Cozzarelli, N. R. (1981) *J. Biol. Chem.* **256,** 4687–4690.
14. Wishart, W. L., Broach, J. R. & Ohtsubo, E. (1985) *Nature (London)* **314,** 556–558.
15. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
16. Ohtsubo, E., Rosenbloom, M., Schrempf, H., Goebel, W. & Rosen, J. (1978) *Mol. Gen. Genet.* **159,** 131–141.
17. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
20. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Messing, J. (1983) *Methods Enzymol.* **101,** 20–79.
23. Jack, W. E., Terry, B. J. & Modrich, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4010–4014.
24. Huang, C.-J., Heffron, F., Twu, J.-S., Schloemer, R. H. & Lee, C.-J. (1986) *Gene* **41,** 23–31.
25. Grindley, N. D. F. & Reed, R. R. (1985) *Annu. Rev. Biochem.* **54,** 863–896.
26. Craigie, R. & Mizuuchi, K. (1985) *J. Biol. Chem.* **260,** 1832–1835.
27. Craigie, R., Mizuuchi, M. & Mizuuchi, K. (1984) *Cell* **39,** 387–394.