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Identification of a transposon Tn7-dependent DNA-binding activity that recognizes the ends of Tn7

(transposable element/DNA recombination/*tnsB* gene)

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ABSTRACT **The bacterial transposon Tn7 is distinguished by its capacity for high-frequency transposition to a specific site in the *Escherichia coli* chromosome. *tnsB* is one of the five Tn7-encoded transposition genes. We have identified *in vitro* a *tnsB*-dependent DNA binding activity that interacts specifically with cis-acting transposition sequences at the Tn7 termini. Although the left and right termini of Tn7 are structurally distinct, each end contains several copies of a closely homologous 22-base-pair sequence. We present results indicating that this 22-base-pair repeat sequence is recognized by the *tnsB*-dependent binding activity.**

Transposable elements are DNA segments that move from one genetic location to another. Analysis of many transposons has revealed that they usually encode two types of transposition information: (*i*) DNA sequences at the ends of the element, which participate directly in transposition, and (*ii*) proteins, which promote transposition (1, 2). It is generally presumed, and in a few cases has been demonstrated (3, 4), that these element-encoded proteins interact directly with the cis-acting sites at the ends of the element.

We are studying the bacterial transposon Tn7 (ref. 5 and Fig. 1*a*), which is distinguished by its capacity to transpose to specific sites in the genomes of many bacteria (5–8). Tn7 transposes at high frequency to a specific site at minute 84 of the *Escherichia coli* chromosome, called *attTn7* (5, 13, 16, 17). When Tn7 inserts into *attTn7*, it does so in a unique orientation (16). Tn7 can also transpose at low frequency to sites other than *attTn7* (18, 19). The Tn7-encoded cis-acting sequences required for transposition lie at the ends of Tn7 (9, 20–22). The termini of Tn7 are highly related inverted repeats of about 30 base pairs (bp) (refs. 13 and 14; Fig. 1*b*), but considerably more sequence information than these inverted repeats is required for transposition (refs. 20 and 21; L.K.A. and N.L.C., unpublished data). The ends of Tn7 also contain several copies of a closely homologous 22-bp sequence (13); the right end of Tn7 (Tn7*R*) contains four closely spaced 22-bp repeats, whereas the three repeated 22-bp sequences in the left end of Tn7 (Tn7*L*) are separated by unrelated sequences (Fig. 1*b*). A deletion analysis of the ends of Tn7 (L.K.A. and N.L.C., unpublished data) suggests that the 22-bp repeat elements are important cis-acting transposition sequences. The Tn7-end derivative shown in Fig. 1*c*, which contains the terminal 166 nucleotides of Tn7*L* and the terminal 199 nucleotides of Tn7*R*, can transpose efficiently in the presence of Tn7-encoded proteins (L.K.A. and N.L.C., unpublished data). Thus, these end segments contain all the cis-acting sites required for Tn7 transposition. Tn7 encodes a complex array of transposition proteins (9, 20, 21). Rogers *et al.* (12) and we (C.S.W. and N.L.C., unpublished data) have determined that Tn7 encodes five transposition genes:

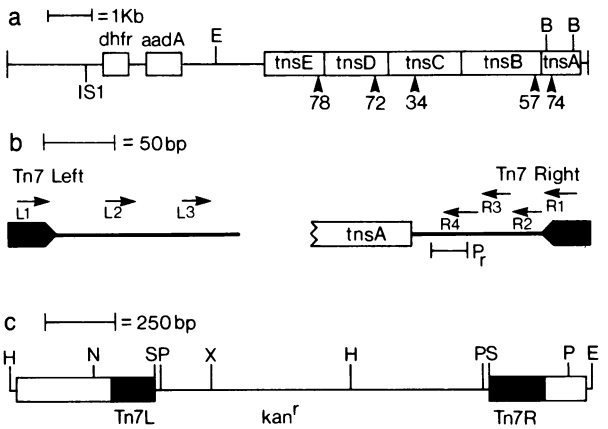

FIG. 1. Physical maps of Tn7 and derivatives. (*a*) Tn7. Tn7's *Eco*RI site and the position of IS*1* (insertion sequence 1) in Tn7*S* (9) are shown. *dhfr* encodes a dihydrofolate reductase (10) that provides resistance to trimethoprim, *aadA* encodes a 3″(9)-*O*-nucleotidyltransferase (11) that provides resistance to streptomycin/spectinomycin, and *tnsA–E* are Tn7's transposition genes (ref. 12; C.S.W. and N.L.C., unpublished data). Positions of the *tns* mini-Mu insertions used are indicated by arrowheads. (*b*) Detailed view of Tn7 ends. The thick block segments are Tn7's terminal inverted repeats (13, 14), the arrows (L1–L3 and R1–R4) are the 22-bp repeats (13), the open box is the *tnsA* NH_2-terminal coding region, and the P_r region is the proposed *tnsA* promoter (15). (*c*) A Tn7-end derivative. The segment shown is contained within the polylinker of plasmid pLA1. *kan*^{r} is a gene that provides resistance to kanamycin. The filled boxes are the ends of Tn7 and the open boxes are *attTn7* sequences. Only the *Nla* III site in *attTn7L*/Tn7*L* is shown. Restriction enzyme sites: B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; N, *Nla* III; P, *Pst* I; S, *Sal* I; X, *Xho* I.

tnsA, *tnsB*, *tnsC*, *tnsD*, and *tnsE* (Fig. 1*a*). Genetic analyses have revealed two distinct but overlapping Tn7 transposition pathways: *tnsA*, *tnsB*, *tnsC*, and *tnsD* are required for transposition to *attTn7*, whereas *tnsA*, *tnsB*, *tnsC*, and *tnsE* are required for transposition to sites other than *attTn7*. We report here that *tnsB* provides a specific DNA-binding activity, which recognizes sequences at both ends of Tn7 that participate directly in transposition.

MATERIALS AND METHODS

Bacteria and Plasmids. LB broth was as described by Miller (23); supplements were carbenicillin (100 μg/ml) and tetracycline (5 μg/ml). *E. coli* NLC51 [F$^-$ *araD139A* Δ(*argF–lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR val*^{R} *recA56*] was constructed from MC4100 (ref. 24; S. Michaelis, University of California, San Francisco) by phage P1 transduction as described by Miller (23) to *val*^{R} with AW76 (A. Wright, Tufts University, Health Sciences Campus, Boston), then to *srl300*::Tn*10-recA56* with JC10240 (A. J. Clarke,

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University of California, Berkeley), and then to *srl*$^+$ *recA56* with MC4100. LA3 is NLC51 *attTn7*$_{84}$::Tn*7* and was obtained by isolation of a trimethoprim-resistant, tetracycline-sensitive derivative of NLC51 with a plasmid containing Tn*7S* (pXJS5287) (9). The Tn*7* derivative used in this work, Tn*7S* (9), contains an IS*1* insertion (Fig. 1*a*); the transposition properties of Tn*7S* are indistinguishable from those of canonical Tn*7* (9).

pRM2 contains active *attTn7* (38) and was obtained by insertion of the *Taq* I (see ref. 13 for location of restriction sites) segment of plasmid pAR6 (25), containing the Tn*7* insertion site, into the *Acc* I site of pUC18 with the *glmS* sequences adjacent to the vector *Eco*RI site. The Tn*7*-end derivative shown in Fig. 1*c* was constructed by *in vivo* transposition of the Tn*7*-end derivative Tn*7S*::Tn*9*Δ*Pst*I (9) into pRM2, digestion of the resulting plasmid with *Hpa* I and *Bal* I [which cut in Tn*7L* and Tn*7R*, respectively (13)], ligation in the presence of a *Sal* I linker (Pharmacia), and subsequent introduction of a fragment encoding kanamycin resistance with terminal *Sal* I sites (Pharmacia) to give pLA1. pLA26 contains the *attTn7L*/Tn*7L Nla* III–*Pst* I fragment of pLA1 (Fig. 1*c*) inserted into the polylinker of pUC18. pRM8 contains a single copy of the 22-bp L1 repeat (Fig. 1*b*) and was obtained by insertion of a synthetic 22-bp double-stranded oligodeoxynucleotide with the sequence 5′ GACAAAATAG-TTGGGAACTGGG 3′ (Biomolecular Resource Center, University of California, San Francisco) into the *Sma* I site of pUC18, with the 3′ end of the sequence shown adjacent to the vector *Eco*RI site. pCW4 contains the transposition (*tns*) genes of Tn*7*. It was constructed by *in vivo* transposition of Tn*7* into pRM2 and subsequent introduction of this plasmid's Tn*7*/*attTn7R Eco*RI fragment into the *Eco*RI site of pACYC184. pCW4 was mutagenized by *in vitro* introduction (26) of a polar mini-Mu transposon (C.S.W. and N.L.C., unpublished work). The positions of the mini-Mu insertions used in this study are indicated in Fig. 1*a*. pCW12 contains *tnsB–tnsE* and was constructed by insertion of the large *Eco*RI–*Bgl* II fragment of Tn*7* (Fig. 1*a*) into the *Eco*RI–*Bam*HI sites of pUC19.

Manipulation and Analysis of DNA. DNA-modifying enzymes were obtained from commercial sources and used as recommended by the vendor. Plasmid growth, isolation, transformation, restriction enzyme analyses, and cloning were performed as described by Maniatis *et al.* (27), except that DNA fragments in slices from low-melting-point agarose gels (Sea Plaque, FMC, Rockland, ME) usually were used directly in the assembly of recombinant molecules as described by Struhl (28). DNA fragments used in the binding reactions were prepared by digestion with appropriate restriction enzymes, purified by electroelution from agarose gels, and 5′-end-labeled with deoxynucleoside 5′-[α-^{32}P]triphosphate and the Klenow fragment of DNA polymerase I.

DNA Fragments Used in Binding Assays. Fragments containing the following sequences were tested: *attTn7*, the 554-bp pRM2 *Eco*RI–*Hin*dIII fragment; Tn*7R*, the 402-bp pLA1 *Sal* I–*Eco*RI fragment; Tn*7L*, the 231-bp pLA26 *Eco*RI–*Hin*dIII fragment; kanamycin-resistance, the 524-bp pUC-4K (Pharmacia) *Xho* I–*Hin*dIII fragment; L1, the 73-bp pRM8 *Eco*RI–*Hin*dIII fragment; pUC18, the 2.7-kbp plasmid pUC18 linearized with *Eco*RI.

Preparation of Cell Extracts. Cells were grown to an OD_{600} of 1 in LB broth at 37°C, harvested by centrifugation, and resuspended (2 ml per g of cells) in 150 mM Tris·HCl, pH 8.0/100 mM KCl/10 mM magnesium acetate/1 mM EDTA/5 mM dithiothreitol/10% (vol/vol) glycerol. Cells were lysed by sonication, and cell debris was removed by centrifugation at 5000 × *g* for 20 min at 4°C. The resulting supernatant was dialyzed against 25 mM Tris·HCl, pH 8.0/1 mM EDTA/4 mM dithiothreitol/200 mM KCl/20% (vol/vol) glycerol for 2 hr at 4°C and used directly or stored at −80°C. A typical preparation yielded about 18 mg of total protein per ml, determined as described by Bradford (29).

DNA Binding Assays. Reaction mixtures (20 μl) contained 17.5 mM Tris·HCl (pH 8.0), 1 mM EDTA, 2 mM dithiothreitol, 100 mM KCl, 10% (vol/vol) glycerol, 1–5 ng of end-labeled DNA fragment, 5 μg of poly(dI-dC)·poly(dI-dC) (Pharmacia) to prevent nonspecific binding (30), and crude extract as indicated. Reaction mixtures were incubated at 4°C for 10 min and then loaded onto 6% (29:1 acrylamide/*N*,*N*′-methylenebisacrylamide weight ratio) polyacrylamide gels in 89 mM Tris/89 mM boric acid/2 mM EDTA and electrophoresed at 250 V for about 90 min. The gels were dried under vacuum and autoradiographed.

RESULTS

A Tn*7*-Encoded Protein Promotes Specific Binding to Tn*7* Ends. We have used a sensitive gel retardation assay (31, 32), which exploits electrophoretic differences between protein–DNA complexes and free DNA, to identify a Tn*7*-dependent DNA-binding activity that specifically recognizes Tn*7L* and Tn*7R*. We found that extracts derived from cells containing Tn*7* in chromosomal *attTn7* (*attTn7*$_{84}$::Tn*7*) promoted the formation of discrete complexes of retarded mobility with Tn*7L* (Fig. 2*a*, lane 3) and with Tn*7R* (Fig. 2*b*, lane 3) that were not observed with extracts derived from cells lacking Tn*7* (Fig. 2 *a* and *b*, lanes 2). We examined the specificity of formation of these complexes in several ways. When a large excess of unlabeled Tn*7L* (lane 4) or Tn*7R* (lane 5) was included in reaction mixtures with end-labeled Tn*7L* (*a*) or with end-labeled Tn*7R* (*b*), no Tn*7*-dependent bands were observed. We observed this competitive effect when excess unlabeled DNA was added to reaction mixtures with the end-labeled DNA (as shown) or when it was added to the reaction mixtures after prior incubation of the end-labeled DNA and crude extract (data not shown). Addition of excess unlabeled pUC18 to reaction mixtures containing end-labeled Tn*7L* (*a*, lane 7) or end-labeled Tn*7R* (*b*, lane 7) did not block Tn*7*-dependent complex formation. These results indicate that the observed bands of retarded mobility reflect specific, noncovalent protein–DNA interactions. This view is supported by our observation that no bands of retarded mobility were detected after addition of $NaDodSO_4$ (0.2% final concentration) to a reaction mixture containing end-labeled Tn*7L* and extract derived from cells containing Tn*7* (data not shown).

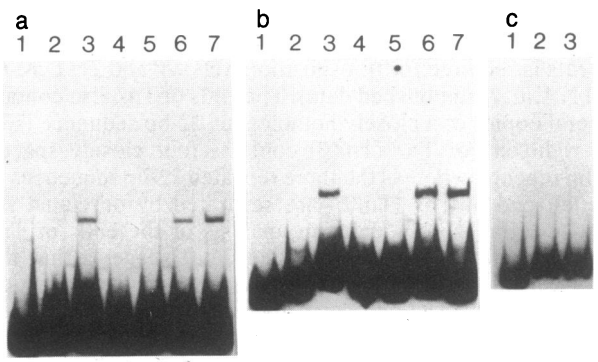

FIG. 2. Tn*7*-dependent binding to Tn*7L* and Tn*7R*. Tn*7L* (*a*), Tn*7R* (*b*), and kanamycin-resistance (*c*) DNA fragments were isolated, end-labeled, and incubated in reaction mixtures containing crude extract protein and competitor DNA as indicated below. After incubation, reaction mixtures were analyzed by gel electrophoresis and autoradiography. LA3 contains Tn*7* and NLC51 does not. (*a* and *b*) Lanes 1: no extract. Lanes 2: NLC51 (6 μg of extract protein). Lanes 3–7: LA3 (6 μg of extract protein) plus 70 ng of unlabeled competitor fragments (Tn*7L*, Tn*7R*, *attTn7*, and pUC18, respectively). (*c*) Lane 1: no extract. Lane 2: NLC51 (60 μg). Lane 3: LA3 (60 μg).

The Tn*7L* and Tn*7R* fragments used in these experiments also contain flanking *attTn7* sequences (Fig. 1*c*). Do these sequences participate in specific binding? We found that the presence of excess unlabeled *attTn7* in reaction mixtures containing end-labeled Tn*7L* (Fig. 2*a*, lane 6) or Tn*7R* (Fig. 2*b*, lane 6) had no effect on Tn*7*-dependent complex formation, suggesting that *attTn7* sequences are not involved in this reaction. Moreover, we were unable to detect Tn*7*-dependent complexes with end-labeled *attTn7* (data not shown) or with a DNA fragment lacking Tn*7* transposition sequences (Fig. 2*c*, lane 3).

These experiments identified a Tn*7*-dependent binding activity that specifically recognizes Tn*7* ends. The observation that specific binding to either end is inhibited by the presence of the other end suggests that the same binding activity recognizes similar sequences in both ends of Tn*7*.

Specific Binding to the Ends of Tn*7* Is Dependent on *tnsB*. To determine if the Tn*7*-encoded transposition genes direct specific binding, we examined crude extracts derived from cells bearing pCW4, a plasmid containing the *tns* genes (*tnsA–E*). When extracts derived from pCW4 cells were incubated with end-labeled Tn*7R* (Fig. 3, lanes 16 and 17), we observed the same retarded bands seen with extracts derived from $attTn7_{84}$::Tn*7* cells (lanes 4 and 5). Therefore, specific binding to Tn*7R* can be attributed to the *tns* genes. We also noted in these experiments the presence of several *tns*-dependent complexes, particularly at higher extract concentrations (compare lanes 4 and 5). One explanation for multiple complexes is that there are multiple protein–DNA interactions with the ends of Tn*7*. We occasionally observed bands of retarded mobility that were not dependent on Tn*7*-encoded genes (lanes 2 and 3). Although it is attractive to suggest that host-encoded proteins may be involved in Tn*7* transposition, there is no other direct evidence to support this hypothesis.

Which *tns* gene(s) directs binding to the ends of Tn*7*? We have genetically defined the Tn*7*-encoded transposition genes by insertional mutagenesis using a polar mini-Mu transposon (C.S.W. and N.L.C., unpublished data). When extracts derived from cells containing disrupted *tnsC*, *tnsD*, or *tnsE* were incubated with end-labeled Tn*7R* DNA, the same Tn*7*-dependent retarded bands (Fig. 3, lanes 10–15) seen with extracts derived from cells containing intact *tns* genes (lanes 16 and 17) were observed. In contrast, when extracts derived from cells with an insertion in *tnsB* were examined, no Tn*7*-dependent retarded bands were detected (lanes 8 and 9).

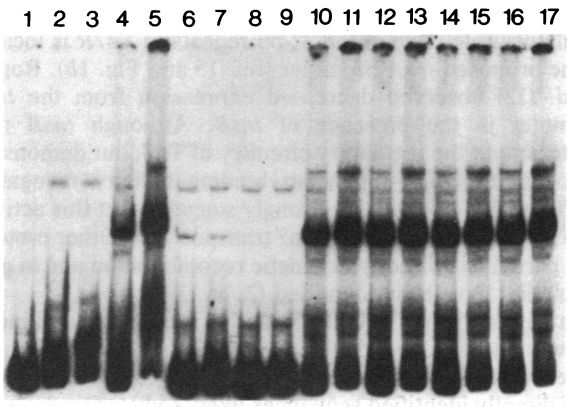

FIG. 3. Binding analyses with mutant *tns* genes. Reaction mixtures containing crude extract protein [17 μg (even-numbered lanes) or 52 μg (odd-numbered lanes except lane 1)] as indicated below with end-labeled Tn*7R* were analyzed as described for Fig. 2. LA3 contains Tn*7* and NLC51 does not; all pCW4 derivatives were in NLC51. Lanes: 1, no extract; 2 and 3, NLC51; 4 and 5, LA3; 6 and 7, pCW4*tnsA*::mini-Mu-74; 8 and 9, pCW4*tnsB*::mini-Mu-57; 10 and 11, pCW4*tnsC*::mini-Mu-34; 12 and 13, pCW4*tnsD*::mini-Mu-72; 14 and 15, pCW4*tnsE*::mini-Mu-78; 16 and 17, pCW4.

Assays with Tn*7L* gave similar results (data not shown). Therefore, *tnsB* is required for Tn*7*-end binding, whereas *tnsC*, *tnsD*, and *tnsE* are not.

Genetic analysis of the *tns* genes has shown that *tnsA* and *tnsB* comprise an operon with *tnsA* nearer the promoter (C.S.W. and N.L.C., unpublished data). Examination of *tnsB*-dependent binding activity in crude extracts derived from cells containing a polar insertion in *tnsA* supports this view: specific binding to Tn*7R* (Fig. 3, lanes 6 and 7) was substantially reduced. To determine whether *tnsA* has a direct role in binding, we also examined binding activity in crude extracts derived from cells containing the plasmid pCW12, which lacks *tnsA* and contains *tnsB–E*. When extracts from pCW12 cells were incubated with end-labeled Tn*7L* (Fig. 4*a*) and end-labeled Tn*7R* (Fig. 4*b*), we observed the same Tn*7*-dependent bands (lanes 6 and 7) seen with extracts derived from $attTn7_{84}$::Tn*7* cells (lanes 3). We also found that extracts derived from cells containing a plasmid encoding only *tnsB* promote formation of these Tn*7*-dependent complexes (data not shown). We conclude that specific binding to Tn*7L* and Tn*7R* requires *tnsB* but does not require *tnsA*, *tnsC*, *tnsD*, or *tnsE*.

The amount of *tnsB*-dependent binding activity in pCW12 extracts is about 30-fold greater than the activity in $attTn7_{84}$::Tn*7* extracts (compare lanes 6 and 3 in Fig. 4 *a* and *b*). Furthermore, more complex is apparently formed with Tn*7R* than with Tn*7L* at equivalent amounts of extract (compare lanes 3 in *a* and *b*, and compare lanes 6 in *a* and *b*). One explanation for these results is that *tnsB*-dependent specific binding activity has a higher affinity for Tn*7R* than for Tn*7L*.

Binding to Common Recognition Sites in Both Ends of Tn*7*. Which nucleotides in the ends of Tn*7* direct *tnsB*-dependent binding? Although the ends of Tn*7* are not identical, they are related: each end of Tn*7* contains several copies of a closely homologous 22-bp sequence (ref. 13 and Fig. 1*b*). When an end-labeled DNA fragment containing the 22-bp L1 sequence was incubated with a pCW12 extract, a discrete band of retarded mobility was observed (Fig. 5, lane 6), whereas no retarded bands were detected with extracts derived from cells lacking Tn*7* (lanes 2 and 3). In control experiments, no bands of retarded mobility were detected when excess unlabeled Tn*7L* was included in reaction mixtures with end-labeled L1 fragment, when excess unlabeled plasmid DNA containing the synthetic L1 sequence (pRM8) was included in reaction mixtures with end-labeled Tn*7R*, or when a 55-bp end-labeled fragment lacking the synthetic L1 sequence was used (data not shown). These results suggest that the 22-bp repeated sequence element present in both ends of Tn*7* contains at

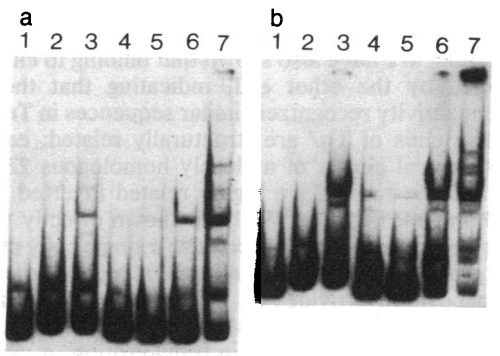

FIG. 4. Binding analysis with extracts lacking *tnsA*. Reaction mixtures containing crude extract protein as indicated below with end-labeled Tn*7L* (*a*) or Tn*7R* (*b*) were analyzed as described for Fig. 2. LA3 contains Tn*7* and NLC51 does not; pCW12 was in NLC51. Lanes: 1, no extract; 2, NLC51 (17.2 μg); 3, LA3 (17.2 μg); 4, pCW12 (0.06 μg); 5, pCW12 (0.17 μg); 6, pCW12 (0.52 μg); and 7, pCW12 (1.72 μg).

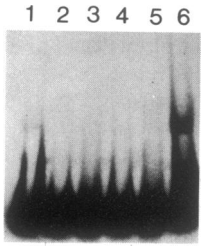

FIG. 5. Binding to the L1 22-bp repeat. Reaction mixtures containing crude extract protein as indicated below with end-labeled L1 fragment were analyzed as described for Fig. 2. LA3 contains Tn*7* and NLC51 does not; pCW12 was in NLC51. Lanes: 1, no extract; 2, NLC51 (17 μg); 3, NLC51 (86 μg); 4, LA3 (17 μg); 5, LA3 (86 μg); 6, pCW12 (17 μg).

least part of the recognition sequence for the *tnsB*-dependent binding activity.

Although binding was detected to the 22-bp repeat with pCW12 extracts, we were unable to detect binding with extracts derived from $attTn7_{84}$::Tn*7* (Fig. 5, lanes 4 and 5). One explanation for this result is that pCW12 extracts have considerably more *tnsB*-dependent binding activity (at least 30-fold, Fig. 4) than do $attTn7_{84}$::Tn*7* extracts. We also note that a large amount of pCW12 extract was required to detect even modest binding to the L1 sequence compared to intact Tn*7L* or Tn*7R* (compare Figs. 4 and 5), suggesting that the affinity of the *tnsB*-dependent activity for a single sequence element is much less than its affinity for intact Tn*7L* and Tn*7R*.

DISCUSSION

We have used a highly sensitive *in vitro* gel retardation assay (31, 32) to identify a specific DNA binding activity that recognizes the termini of Tn*7* and is dependent on the Tn*7* transposition gene *tnsB*. This activity does not require the other transposition genes (*tnsA*, *tnsC*, *tnsD*, or *tnsE*) of Tn*7*. Although it is attractive to suggest that the TnsB protein interacts directly with the ends of Tn*7*, our binding analyses, performed with crude cell extracts, do not establish this point. The TnsB protein may regulate the expression of or modify an *E. coli*-encoded protein that binds directly to the ends of Tn*7*. Whether or not the TnsB protein itself and/or a host protein binds to the ends of Tn*7*, we have biochemically identified a specific DNA binding activity likely to be an integral component of the Tn*7* transposition machinery.

We have demonstrated that the *tnsB*-dependent binding activity specifically recognizes both Tn*7L* and Tn*7R* segments that contain the necessary cis-acting sites for Tn*7* transposition. We have also shown that binding to either end is inhibited by the other end, indicating that the *tnsB*-dependent activity recognizes similar sequences in Tn*7L* and Tn*7R*. The ends of Tn*7* are structurally related: each end contains several copies of a closely homologous 22-bp sequence and the termini are highly related inverted repeats (refs. 13 and 14; Fig. 1*b*). We have shown directly that the *tnsB*-dependent activity can recognize a single copy of one of these 22-bp repeats, L1. The finding that a gene essential to Tn*7* transposition provides an activity that recognizes the 22-bp repeat sequence suggests that these sequences are indeed critical cis-acting sites in transposition, a view supported by analysis of the extents of Tn*7L* and Tn*7R* required for transposition (L.K.A. and N.L.C., unpublished data). One explanation for the several different *tnsB*-dependent complexes observed with Tn*7L* and Tn*7R* is that different complexes are formed between the *tnsB*-dependent binding activity and the various repeated 22-bp elements in these segments. Multiple protein–DNA complexes have been observed with other DNA-binding proteins and DNA segments containing repeated recognition sites (33, 34). Alternatively, a single *tnsB*-dependent complex may be altered by interactions (specific or nonspecific) with host-encoded proteins to produce multiple species.

Our data also suggest that the *tnsB*-dependent binding activity recognizes a single copy of the L1 22-bp repeat much less efficiently than it recognizes intact Tn*7L* and Tn*7R*. One possible explanation for this result is that the L1 sequence contains only part of (or a poor version of) the authentic recognition sequence for the *tnsB*-dependent binding activity. Perhaps the *tnsB*-dependent activity most efficiently recognizes Tn*7*'s terminal inverted repeats, which contain 22-bp repeat sequences, or one of the other 22-bp repeats. Alternatively, the apparently higher affinity of *tnsB*-dependent activity for intact Tn*7L* and Tn*7R* may reflect cooperative interactions (35) between the multiple repeated copies of the 22-bp sequence in these DNA segments. We also observed that the affinity of the *tnsB*-dependent activity is greater for Tn*7R* than for Tn*7L*. Similarly, these differences may reflect differences between the *tnsB* recognition sequence(s) *per se* in these DNA segments or may reflect different cooperative interactions provided by the variously positioned 22-bp repeats in each end (Fig. 1*b*). It is not unreasonable to suggest that differential interactions between the *tnsB*-dependent binding activity and the variously spaced 22-bp repeats in Tn*7L* and Tn*7R* may contribute to functional differences between these ends.

The Role of *tnsB* in Tn*7* Transposition. Genetic analyses have established that *tnsB* is required for Tn*7* transposition (ref. 12; C.S.W. and N.L.C., unpublished data), and we have shown here that the *tnsB* gene product contributes to specific end-recognition. Many activities in addition to end-recognition are required to promote transposition, including DNA breakage and reunion and target-site recognition. The bacteriophage Mu transposition protein MuA specifically recognizes the ends of Mu (3) and also is involved in site-specific DNA cleavage (R. Craigie and K. Mizuuchi, personal communication). The complex array of *tns* genes suggests that other element-encoded proteins may also contribute to end activity in Tn*7* transposition. Host-encoded proteins may also participate directly in Tn*7* transposition. Although we have observed host-dependent interactions with the ends of Tn*7*, further work is required to establish their functional significance.

The *tnsB*-dependent end-binding activity may also play a regulatory role. One of the 22-bp repeats in Tn*7R* is located at the proposed *tnsA* promoter (ref. 15 and Fig. 1*b*). Rogers *et al.* (12) observed decreased expression from the *tnsA* promoter in the presence of *tnsB*. Although *tnsB* may contribute to the regulatory circuitry of Tn*7*, our demonstration that the *tnsB*-dependent binding activity recognizes Tn*7L* in addition to Tn*7R* strongly suggests that this activity also participates directly in Tn*7* transposition. Other proteins that participate directly in genetic recombination and in gene regulation have been identified (2, 36, 37).

In summary, we have identified a specific DNA-binding activity that is dependent on a Tn*7*-encoded transposition gene and that recognizes the ends of Tn*7*. Our analyses have also directly identified sequences likely to be critical cis-acting sites in Tn*7* transposition.

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