Gene, 104 (1991) 125–131
© 1991 Elsevier Science Publishers B.V. All rights reserved 0378-1119/91/$03.50

GENE 04085

Erratum

Gene, 96 (1990) 1–7
Elsevier

GENE 03811

Identification of transposition proteins encoded by the bacterial transposon Tn*7*

(Recombination; immunoblotting; nucleotide sequence; physical map)

Karina A. Orle and Nancy L. Craig

Department of Microbiology and Immunology, Department of Biochemistry and Biophysics, George W. Hooper Foundation, University of California, San Francisco, CA 94143 (U.S.A.) Tel. (415)576-5157

In the process of proofreading/revising of the above paper a number of errors have been introduced. We apologise to the authors for this mishap and republish a correct version of the entire article.

SUMMARY

The bacterial transposon, Tn*7*, encodes an elaborate array of transposition genes, *tnsABCDE*. We report here the direct identification of the TnsA, TnsB, TnsC and TnsD polypeptides by immunoblotting. Our results demonstrate that the complexity of the protein information devoted to Tn*7* transposition is considerable: the aggregate molecular size of the five Tns polypeptides is about 300 kDa. We also report the sequence of the *tnsA* gene and of the 5′ ends of *tnsB* and *tnsD*. This analysis reveals that all five *tns* genes are oriented in the same direction within Tn*7*.

INTRODUCTION

Mobile DNA segments encode proteins that mediate their translocation (Berg and Howe, 1989). The bacterial transposon Tn*7* (Barth et al., 1976; Craig, 1989) is distinguished in that it encodes five genes, *tnsABCDE*, that mediate two distinct but overlapping transposition pathways differing in their target sites (Fig. 1A; Hauer and Shapiro, 1984; Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). *tnsABC* + *tnsD* promote insertion into sites sharing considerable sequence similarity including *attTn7*, Tn*7*'s preferred chromosomal insertion site, and pseudo-*att*Tn*7* sites. *tnsABC* + *tnsE* promote insertion into many different target sites not obviously related to each other or to *tnsD* sites.

The *tns* gene positions were approximately defined in previous analyses of insertion and deletion mutants of Tn*7* (Fig. 1A; Hauer and Shapiro, 1984; Smith and Jones, 1984; Ouartsi et al., 1985; Rogers et al., 1986; Waddell and Craig, 1988). Sequencing has identified *tnsE* (Smith and Jones, 1986) and provided information about parts of *tnsA* (Gay et al., 1986) and *tnsC* (Smith and Jones, 1986). We report here the complete *tnsA* sequence and the sequences of the 5′-regions of *tnsB* and *tnsD*. After this work was completed, the sequence of the entire *tnsABCD* region was determined with results in good agreement with our own

Correspondence to: Ms. K.A. Orle, HSW 1542, University of California, San Francisco, CA 94143 (U.S.A.) Tel. (415)476-1493; Fax (415) 476-6185.

Abbreviations: aa, amino acid(s); bp, base pair(s); Δ, deletion; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); ORF, open reading frame; p, plasmid; PAGE, polyacrylamide-gel electrophoresis; pp, polypeptide; RBS, ribosome-binding site; SDS, sodium dodecyl sulfate; Tn, transposon; Tns, Tn7 transposition protein(s); *tns*, gene(s) encoding Tns; ::, novel joint (fusion).

0378-1119/90/$03.50 © 1990 Elsevier Science Publishers B.V. (Biomedical Division)

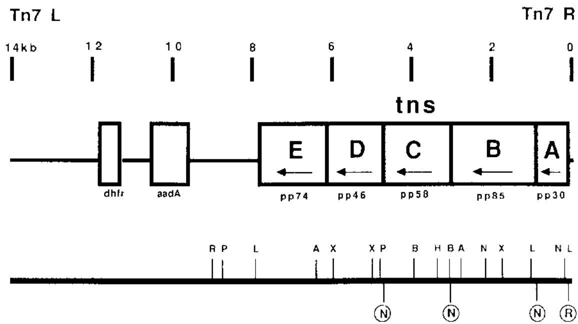

Fig. 1. Physical map of Tn*7*. At the top of the figure, the ends of Tn*7* are designated Tn*7*R for the right end and Tn*7*L for the left end. Positions and orientations of the known Tn*7* genes are shown: the *tns* genes (Gay et al., 1984; Smith and Jones, 1986; Rogers et al., 1986; Waddell and Craig, 1988; this work), the *dhfr* gene (encoding dihydrofolate reductase = trimethoprim resistance) (Fling and Richards, 1983; Simonsen et al., 1983) and the *aadA* gene (encoding adenylyltransferase = streptomycin and spectinomycin resistance) (Fling et al., 1985). The *tns* genes express polypeptides (pp) of the given observed sizes (kDa) (Smith and Jones, 1986; this work). Restriction sites rightwards of Tn*7*'s *Eco*RI site are shown; those above the line occur naturally within Tn*7* and the circled sites below the line we introduced (see Fig. 2 and Table I). A, *Hpa*I; H, *Hin*dIII; L, *Bal*I; N, *Nco*I; P, *Pvu*II; R, *Eco*RI; X, *Xba*I.

(Flores et al., 1990). Previous studies identified candidates for some Tns proteins (Brevet et al., 1985; Waddell, 1989). We also report here the direct identification of TnsA, TnsB, TnsC and TnsD.

In Tn*7*, considerable information is devoted to transposition: five *tns* genes spanning about 8 kb which, as we demonstrate here, encode five Tns polypeptides, that total about 300 kDa. Biochemical roles for the Tns proteins are beginning to be identified: TnsB binds specifically to the ends of Tn*7* (McKown et al., 1987; L. Arciszewska, R. McKown and N.L.C., in preparation), TnsC is an ATP-binding protein (P. Gamas and N.L.C., unpublished results), and TnsD binds specifically to *att*Tn*7* (Waddell and Craig, 1989; K. Kubo and N.L.C., unpublished results).

The reagents we describe here, plasmids in which *tns* expression is under regulatable, heterologous control, and Tns-specific antibodies, will be exceedingly useful tools in dissecting the mechanism and control of Tn*7* transposition.

RESULTS AND DISCUSSION

(a) Characterization of *tnsA*

The nt sequence of the rightmost 1200 bp of Tn*7* is shown in Fig. 2A; the first 500 bp of this sequence were determined by Lichtenstein and Brenner (1982) and by Gay et al. (1986) and we have determined the remainder. Inspection of this sequence reveals an 819-bp ORF with its 5′ end towards the right end of Tn*7* whose position agrees well with the genetic and physical mapping of *tnsA* (Waddell and Craig, 1988). A DNA segment containing this region provides *tnsA* function as evaluated with an in vitro Tn*7* transposition system (P. Gamas, R. Bainton and N.L.C., in preparation). The indicated ATG which is 134 bp from the right terminus of Tn*7* (Tn*7*R) is a good candidate for TnsA translation initiation but the TnsA N-terminal sequence has not yet been directly determined. The M_r of TnsA predicted from its nt sequence is 30 989. Examination of the predicted TnsA protein sequence has not revealed any striking similarities to other known proteins. *tnsA* and *tnsB* likely form an operon whose transcription can be repressed by the binding of TnsB to its promoter region (Fig. 1; Gay et al., 1986; Rogers et al., 1986; Waddell and Craig, 1988; McKown et al., 1987; L. Arciszewska, R. McKown and N.L.C., in preparation; see next paragraph).

We directly identified TnsA through examination by immunoblotting of extracts derived from cells containing various *tnsA* plasmids. pKAO52 contains a *tnsA* segment downstream from a LacI-regulated promoter. When cells containing pKAO52 are treated with the *lac* inducer IPTG, anti-TnsA antibodies detect a 30-kDa polypeptide (Fig. 3, lane 3) whose amount is much reduced in the absence of IPTG (lane 2) and is not detectable in cells lacking *tnsA* (lane 1). The 30-kDa polypeptide is also evident in cells containing the entire *tns* region on a plasmid (lane 6). The low level of TnsA from the *tnsABCDE* plasmid likely reflects both lower plasmid copy number and repression of the *tnsA* promoter by TnsB: increased TnsA is observed when *tnsB* is disrupted (lane 8). Disruption of *tnsA* also results in the appearance of an increased amount of truncated TnsA (lane 7). We were unable to detect TnsA from a *tnsABCDE* plasmid when *tnsC* is disrupted (lane 9); the significance of this observation is unclear because TnsA is difficult to detect with intact *tnsABCDE* (lane 6). Low levels of TnsA are also detected from *tnsABC* plasmid (lane 10). No TnsA was detected with single-copy chromosomal Tn*7* (lane 11).

(b) Characterization of *tnsB*

Several types of evidence including analysis of fusions (Rogers et al., 1986) and the observation of putative truncated TnsB polypeptides from *tnsB* insertion mutations (Waddell, 1989) suggested that the 5′ end of *tnsB* is adjacent to *tnsA*. Our nt sequence analysis of the region downstream from *tnsA* (Fig. 2A) revealed a candidate ORF for the TnsB N terminus. Chemical analysis of the N terminus of TnsB, purified by its ability to bind specifically to the ends of Tn*7* (McKown et al., 1987; L. Arciszewska, R. McKown and N.L.C., manuscript in preparation), demonstrates that the indicated ATG is the *tnsB* translation start codon. The slight overlap of the *tnsA* and *tnsB* ORFs may reflect translational coupling (Rogers et al., 1986; Normark et al., 1983).

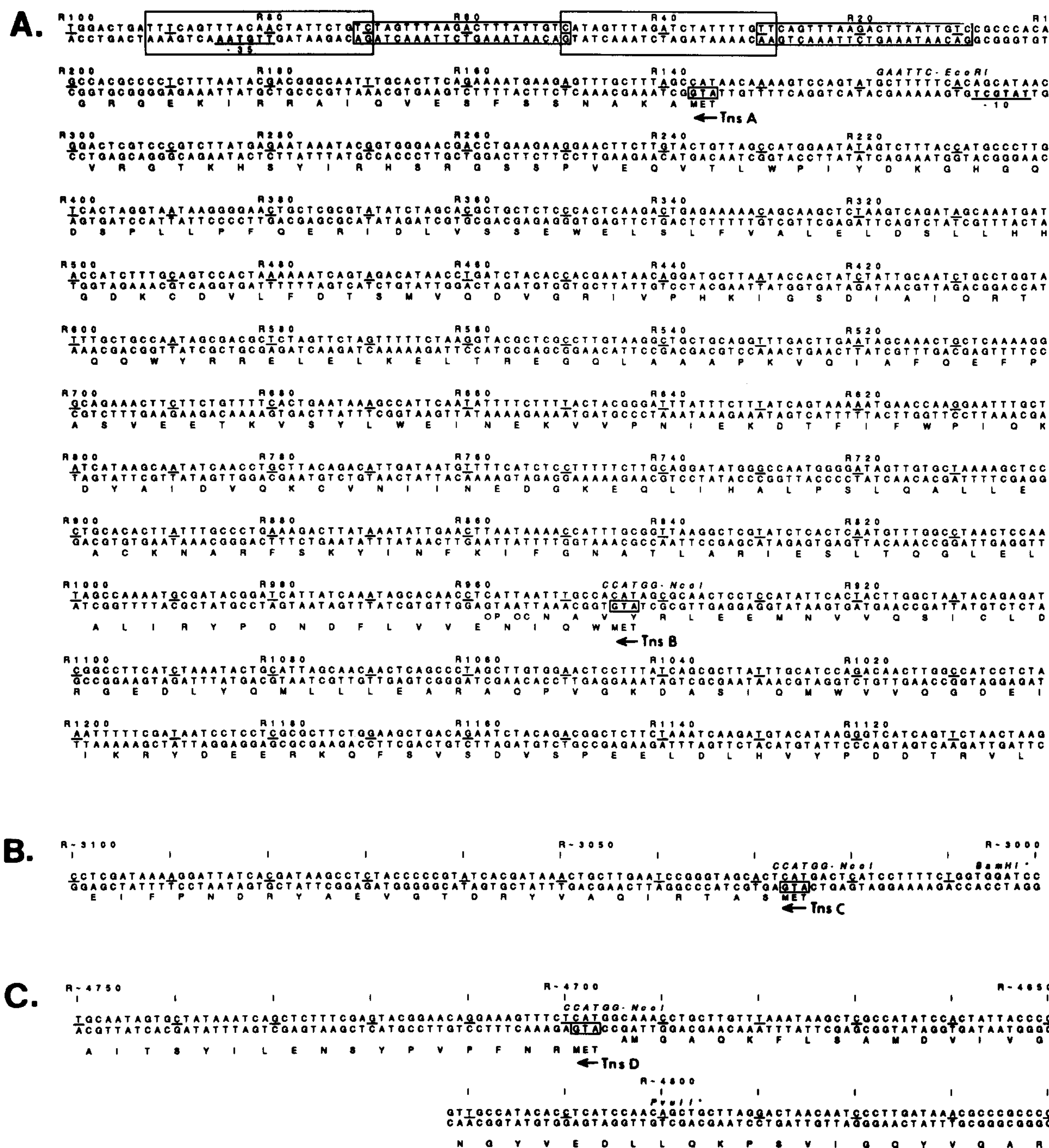

Fig. 2. Nucleotide sequence of Tn7R. The nt sequence of some regions of Tn7 is shown. The terminal bp is numbered R1 and numbers increase towards the center of Tn7 (right to left); ten bp intervals are denoted by underlined nt. The arrows show the direction of the indicated genes, and the boxed ATGs the proposed start codons. These sequences have been deposited with GenBank, Accession Numbers M37391, M37392, and M37393. (A) *tnsA* and the N terminus of *tnsB*. The nt sequence from R1 to about R500 was initially determined by Gay et al. (1986) and Lichtenstein and Brenner (1982); our sequence is identical with that of Gay et al. (1986). We determined the remainder of Tn7R sequence shown. The boxed areas between R1 and R90 are highly similar repeats of a 22-bp sequence (Lichtenstein and Brenner, 1982) which are parts of TnsB binding sites (McKown et al., 1987; L. Arciszewska, R. McKown and N.L.C., manuscript in preparation). Likely −10 and −35 regions of the proposed *tnsAB* promoter (Gay et al., 1986) are shown. (B) *tnsC*. The nt sequence of the proposed N terminus of *tnsC* as determined by Smith and Jones (1986) is shown. (C) *tnsD*. The sequence of the proposed N terminus of *tnsD* we have determined is shown.

Anti-TnsB antibodies detect an 85-kDa polypeptide in extracts from cells with pKAO55 (Fig. 3B, lanes 2,3), which contains a translational fusion of *tnsB* to a *lacZ* RBS, that is absent from cells lacking *tnsB* (lane 1). This polypeptide is present in the absence of IPTG (lane 2); IPTG addition (lane 3) results in a modest increase in 85-kDa TnsB and the concomitant appearance of many smaller TnsB species which result from proteolytic degradation of TnsB (L.

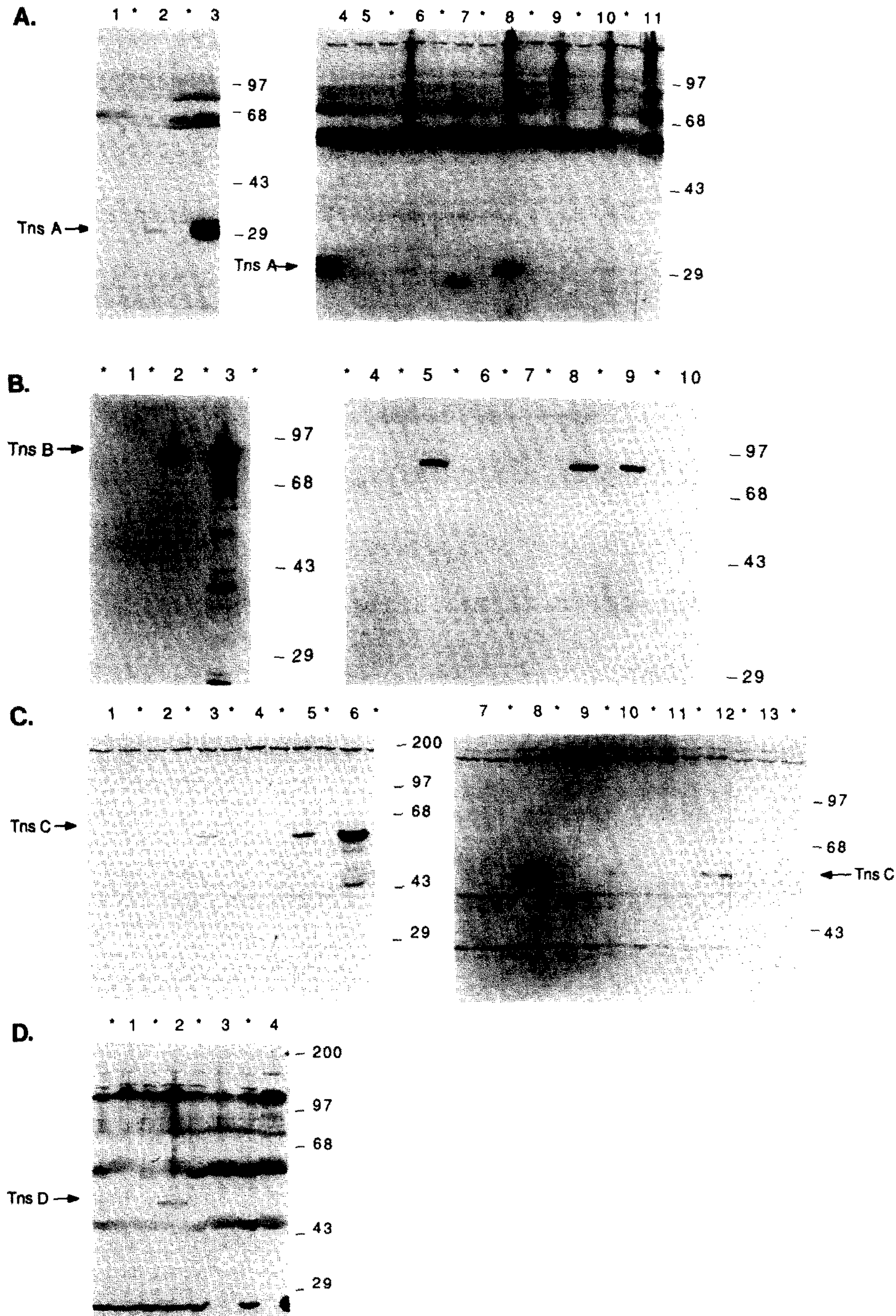

Fig. 3. Identification of the Tns proteins by gel electrophoresis and immunoblotting. Strain NLC51 containing the indicated plasmids or strain LA3 were grown in LB broth supplemented with appropriate antibiotics; 1 mM IPTG was added 2–3 h prior to harvest as indicated. Whole-cell lysates were prepared from mid-log cells by boiling in 125 mM Tris · HCl pH 6.8/4% SDS/10% 2-mercaptoethanol/20% glycerol, separated by 0.1% SDS–8% PAGE, transferred to nitrocellulose, incubated with appropriate anti-Tns antibodies (below) and immune complexes were detected with goat anti-rabbit alkaline phosphatase conjugates (BioRad, Richmond, CA). We generated anti-Tns antibodies by constructing *lacZ-tns* fusion genes, isolating the LacZ-Tns fusion proteins by preparative gel electrophoresis and subsequent electroelution and then used this material as an immunogen in rabbits (Caltag, San Francisco, CA); anti-Tns (and anti-LacZ) antibodies were affinity purified from serum by hybridization to and elution from nitrocellulose strips to which LacZ-Tns fusion proteins, fractionated by preparative gel electrophoresis, were affixed (Olmsted, 1981). Lanes marked with asterisks contained extracts of plasmid-free NLC51. (Panel **A**) Identification of TnsA. Lanes: **1**, pKK223-3; **2**, pKAO52; **3** and **4**, pKAO52 + IPTG; **5**, pACYC184; **6**, pCW4

Arciszewska, R. McKown, and N.L.C., in preparation). Similar TnsB instability can be observed when TnsB is produced from transcriptional fusions (data not shown); thus instability does not reflect the aa change in the translational fusion (Trp^2 → Gly).

An 85-kDa TnsB is also detected with a *tnsABCDE* plasmid (lane 5) but no TnsB was detected when a polar insertion mutation disrupted either *tnsA* (lane 6) or *tnsB* (lane 7), supporting the view that *tnsA* and *tnsB* form an operon. The TnsB level was not detectably changed by inactivation of *tnsC* (lane 8), *tnsD* or *tnsE* (lane 9). A much lower level of TnsB is detectable in cells containing chromosomal Tn*7* (lane 10). The relative amounts of TnsB observed using anti-TnsB antibodies is in good agreement with the relative amounts of *tnsB*-dependent DNA-binding activity (McKown et al., 1987).

(c) Characterization of *tnsC*

Previous nt sequence analysis (Smith and Jones, 1986) revealed a candidate TnsC ORF in the same orientation as *tnsA* and *tnsB*, i.e., with its 5′ end towards Tn*7*R (Fig. 2C). The indicated ATG is a good candidate for TnsC initiation: a *tnsC* polypeptide that begins at this codon can provide *tnsC* activity as evaluated by an in vitro Tn*7* transposition assay (P. Gamas, R. Bainton and N.L.C., in preparation) and also provides a TnsC polypeptide of appropriate size (see next paragraph). Chemical analysis of purified TnsC is also consistent with this hypothesis (P. Gamas and N.L.C., unpublished results).

We directly identified TnsC by immunoblotting of extracts derived from cells containing various *tnsC* plasmids. Plasmid pKAO54 contains a *tnsC* DNA segment transcriptionally fused to a *lac* promoter (Table I). In the presence of IPTG, anti-TnsC antibodies detect a 58-kDa protein (Fig. 3C, lane 3) not detectable in cells which are lacking *tnsC* (lane 1) or have not been exposed to IPTG (lane 2). An indistinguishable TnsC polypeptide is produced with pKAO53 in which *tnsC* is fused to a *lac* RBS (lanes 5, 6); considerable TnsC is evident without addition of IPTG (lane 5); exposure to IPTG (lane 6) results in substantial increase in the amount of 58-kDa TnsC and also in the appearance of smaller TnsC species, likely resulting from TnsC proteolysis. Similar proteolytic degradation can also be observed with a transcriptional fusion (data not shown; P. Gamas and N.L.C., unpublished results); thus alteration of the second aa in the translational fusion (Ser^2 → Gly) does not prompt degradation.

TnsC is also produced by a plasmid containing *tnsABCDE* (lane 8), the low level likely reflecting, in part, low plasmid copy number; TnsC is not detectable when *tnsC* is disrupted by an insertion mutation (lane 11). Some TnsC is detectable when *tnsB* is disrupted by a polar insertion mutation (lane B), consistent with the proposal (Waddell and Craig, 1988) that *tnsC* can be transcribed from a second (*tnsC*) promoter, not only from the *tnsAB* promoter. However, the amount of TnsC observed when *tnsB* is disrupted is considerably reduced (perhaps tenfold; compare lanes 8 and 10), indicating that the *tnsAB* promoter and/or TnsB likely play some role in *tnsC* expression.

We note that similar levels of TnsC (and of TnsA and TnsB) were present with *tnsABCDE* and *tnsABC* plasmids (Fig. 3); this argues against the hypothesis that TnsE is a positive regulator of *tnsC* transcription (Smith and Jones, 1986).

(d) Characterization of *tnsD*

Analysis of several *tnsD*-dependent activities (Waddell and Craig, 1988; 1989) and of *lacZ-tnsD* fusion products (data not shown) suggested that the 5′ end of *tnsD* is oriented towards Tn*7*R. The nt sequencing of the R4700 region (Fig. 2C) revealed a candidate *tnsD* ORF crossing a *Pvu*II site required for *tnsD* function (Rogers et al., 1986; Waddell and Craig, 1988).

We directly identified TnsD by immunoblotting of extracts from cells containing *tnsD* plasmids. pKAO41 contains a fusion of the proposed *tnsD* start codon to a *lac* RBS. In cells containing this plasmid (Fig. 3, lane 2), anti-TnsD antibodies specifically recognize a 46-kDa polypeptide absent from cells lacking this plasmid (lane 1). A polypeptide of identical size was sometimes detected in cells containing a transcriptional fusion of *tnsD* to a *lac* promoter but only at exceedingly low levels (data not shown). The *tnsD* translational fusion changes the second TnsD aa from Arg^2 → Gly (Fig. 2C); this alteration does not inactivate TnsD as evaluated by several *tnsD*-dependent reactions including transposition to *att*Tn*7* in vivo (data not shown), specific binding to *att*Tn*7* (K. Kubo and N.L.C., unpublished results) and transposition to *att*Tn*7* in vitro (P. Gamas, R. Bainton and N.L.C., manuscript in preparation). TnsD was undetectable with either a *tnsABCDE*

(*tnsABCDE*$^+$); **7**, pCW4::miniMuΩ74 (*tnsA*::miniMu); **8**, pCW4::miniMuΩ57 (*tnsB*::miniMu); **9**, pCW4::miniMuΩ34 (*tnsC*::miniMu); **10**, pCW15 (*tnsABC*$^+$); **11**, LA3. (Panel **B**) Identification of TnsB. Lanes: **1**, pKK223-3; **2**, pKAO55; **3**, pKAO55 + IPTG; **4**, pACYC184; **5**, pCW4 (*tnsABCDE*$^+$); **6**, pCW4::miniMuΩ74 (*tnsA*::miniMu); **7**, pCW4::miniMuΩ57 (*tnsB*::miniMu); **8**, pCW4::miniMuΩ34 (*tnsC*::miniMu); **9**, pCW15 (*tnsABC*$^+$); **10**, LA3. (Panel **C**) Identification of TnsC. Lanes: **1**, pKK223-3; **2**, pKAO54; **3**, pKAO54 + IPTG; **4**, pKK233-3; **5**, pKAO53; **6**, pKAO53 + IPTG; **7**, pACYC184; **8**, pCW4 (*tnsABCDE*$^+$); **9**, pCW4::miniMuΩ74 (*tnsA*::miniMu); **10**, pCW4::miniMuΩ57 (*tnsB*::miniMu); **11**, pCW4::miniMuΩ34 (*tnsC*::miniMu); **12**, pCW15 (*tnsABC*$^+$); **13**, LA3. (Panel **D**) Identification of TnsD. Lanes: **1**, pKK233-3; **2**, pKAO41; **3**, pCW4 (*tnsABCDE*$^+$); **4**, LA3.

TABLE I

Bacterial strains and plasmids

Strain or plasmid	Relevant feature	Construction[a]	Source
E. coli			
NLC51	*E. coli* F⁻ *araD*139 *Δ*(*argF-lac*)U169 *rpsL*150 *relA*1 *flbB*5301 *deoC*I *ptsF*25 *rbsR* *val*^{R} *recA*56	—	McKown et al. (1987)
LA3	NLC51 *att*Tn*784*::Tn7	—	McKown et al. (1987)
Plasmids			
pT7-11	Vector with polylinker	—	Tabor and Richardson (1985)
pKK223-3	pBR322-based expression vector with *Ptac*	—	Brosius and Holy (1984)
pKK233-2	pBR322-based expression vector with *Ptrc* and *lacZ* RBS	—	Amann and Brosius (1985)
pMJ1560	*lacI*^{Q} source	—	Stark (1987)
pFSIQ	*lacI*^{Q} source	—	Stephenson and Kuhn (1988)
pCW4	*tnsABCDE* in pACYC184	—	Waddell and Craig (1988)
pCW4::miniMuΩ74	miniMu in *tnsA*	—	Waddell and Craig (1988)
pCW4::miniMuΩ57	miniMu in *tnsB*	—	Waddell and Craig (1988)
pCW4::miniMuΩ34	miniMu in *tnsC*	—	Waddell and Craig (1988)
pCW15	*tnsABC* in pACYC184	—	Waddell and Craig (1988)
pKAO52	*Ptac – tnsA* transcriptional fusion; *lacI*^{Q} in vector	The *tnsA* fragment extending from the *Eco*RI * site at R113 to the *Bal*I site at R1008 (flanked by *Sma*I to *Hin*dIII of the pT7-11 polylinker) was inserted between the *Eco*RI and *Hin*dIII sites of pKK223-3; the *Eco*RI-*Hin*dIII *lacI*^{Q} fragment from pMJ1560 was inserted into the vector *Pvu*II site by blunt-end ligation	This study
pKAO55	*Ptrc-lacZ* RBS-*tnsB* translational fusion; *lacI*^{Q} in vector	The *tnsB* fragment extending from the *Nco*I * site at R941 to the *Hin*dIII site at about R3400 was inserted between the *Nco*I and *Hin*dIII sites of pKK233-2; the *Eco*RI *lacI*^{Q} fragment of pFSIQ was inserted into the vector *Eco*RI site	This study
pKAO54	*Ptac-tnsC* transcriptional fusion; *lacI*^{Q} in vector	The *tnsC* fragment extending from the *Bam*HI site at about R3100 (flanked by *Bam*HI to *Eco*RI of the pUC18 polylinker) to the *Pvu*II site at about R4800 (flanked by *Hin*cII to *Hin*dIII of the pUC18 polylinker) was inserted between the *Eco*RI and *Hin*dIII sites of pKK223-3; a *lacI*^{Q} fragment was added as for pKAO52.	This study
pKAO53	*Ptrc-lacZ* RBS-*tnsC* translational fusion; *lacI*^{Q} in vector	The *tnsC* fragment extending from the *Nco*I * site at about R3100 to the *Pvu*II site at about R4700 (flanked by *Hin*cII to *Pst*I of the pUC18 polylinker) was inserted between the *Nco*I and *Pst*I sites of pKK233-2; a *lacI*^{Q} fragment was added as for pKAO52.	This study
pKAO41	*Ptrc-lacZ* RBS-*tnsD* translational fusion	The *tnsD* fragment extending from the *Nco*I * site at about R4700 to the *Hpa*I site at about R6400 (flanked by *Hin*cII to *Pst*I of the pUC18 polylinker) was inserted between the *Nco*I and *Pst*I sites of pKK233-2	This study

[a] Restriction sites marked with * were introduced into various *tns* plasmids by site-directed mutagenesis (Kunkel et al., 1987). Positions of these sites and other *tns* restriction sites are shown in Fig. 1 and the sequence of these regions is shown in Fig. 2.

plasmid (lane 3) or single-copy Tn7 (lane 4), a finding in good agreement with the low levels of *att*Tn7 binding activity under these conditions (Waddell and Craig, 1989). Also, more *att*Tn7 binding activity is present in extracts from cells containing the *tnsD* translational fusion (pKAO41) than from cells containing a *tnsD* transcriptional fusion (K. Kubo and N.L.C., unpublished results).

We have recently purified the *tnsD*-dependent *att*Tn7 binding activity (K. Kubo and N.L.C., unpublished results); this purification selects for TnsD, i.e., the same 46-kDa protein detected by the anti-TnsD antibodies. These experiments revealed that very little TnsD is present even in cells containing pKAO41 so that the apparently 'dirty' nature of the TnsD immunoblots probably reflects the low level of TnsD.

ACKNOWLEDGEMENTS

We thank other members of the laboratory for useful advice and discussion, Mary Betlach for her assistance with the site-directed mutagenesis and Karyl Nakamura for her assistance with the manuscript. This work was supported by a grant to N.L.C. from the National Institutes of Health.

REFERENCES

Amann, E. and Brosius, J.: 'ATG vectors' for regulated high-level expression of cloned genes in *Escherichia coli*. Gene 40 (1985) 183–190.

Barth, P.T., Datta, N., Hedges, R.W. and Grinter, N.J.: Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. J. Bacteriol. 125 (1976) 800–810.

Berg, D.E. and Howe, M.M. (Eds.): Mobile DNA. American Society for Microbiology, Washington, DC, 1989.

Brevet, J., Faure, F. and Borowski, D.: Tn7-encoded proteins. Mol. Gen. Genet. 201 (1985) 258–264.

Brosius. J. and Holy, A.: Regulation of ribosomal RNA promoters with a synthetic *lac* operator. Proc. Natl. Acad. Sci. USA 81 (1984) 6929–6933.

Craig, N.L.: Transposon Tn7. In: Berg, D.E. and Howe, M.M. (Eds.), Mobile DNA. American Society for Microbiology, Washington, DC, 1989, pp. 211–215.

Fling, M. and Richards, C.: The nucleotide sequence of the trimethoprim-resistant dihydrofolate reductase gene harbored by Tn7. Nucleic Acids Res. 11 (1983) 5147–5158.

Fling, M., Kopf, J. and Richards, C.: Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3″(9)-*O*-nucleotidyltransferase. Nucleic Acids Res. 13 (1985) 7095–7106.

Flores, C., Qadri, M.I. and Lichtenstein, C.: DNA sequence analysis of five genes: *tnsA*, *B*, *C*, *D* and *E*, required for Tn7 transposition. Nucleic Acids Res. 18 (1990) 901–911.

Gay, N., Tybulewicz, V. and Walker, J.: Insertion of transposon Tn7 into the *Escherichia coli glmS* transcriptional terminator. Biochem. J. 234 (1986) 111–117.

Hauer, B. and Shapiro, J.A.: Control of Tn7 transposition. Mol. Gen. Genet. 194 (1984) 149–158.

Kubo, K. and Craig, N.L.: Bacterial transposon Tn7 utilizes two different classes of target sites. J. Bacteriol. 172 (1990) 2774–2778.

Kunkel, T.A., Roberts, J.D. and Zakour, R.A.: Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. 154 (1987) 367–382.

Lichtenstein, C. and Brenner, S.: Unique insertion site of Tn7 in the *E. coli* chromosome. Nature 297 (1982) 601–603.

McKown, R., Waddell, C., Arciszewska, L. and Craig, N.L.: Identification of a transposon Tn7-dependent DNA binding activity that recognizes the ends of Tn7. Proc. Natl. Acad. Sci. USA 84 (1987) 7807–7811.

Normark, S., Bergstrom, S., Edlund, T., Grundstrom, T., Jaurin, B., Lindberg, F. and Olsson, O.: Overlapping genes. Annu. Rev. Genet. 17 (1983) 499–525.

Olmsted, J.: Affinity purification of antibodies from diazotized paper blots of heterogeneous protein samples. J. Biol. Chem. 256 (1981) 11955–11957.

Ouartsi, A., Borowski, D. and Brevet, J.: Genetic analysis of Tn7 transposition. Mol. Gen. Genet. 198 (1985) 221–227.

Rogers, M., Ekaterinaki, N., Nimmo, E. and Sherratt, D.: Analysis of Tn7 transposition. Mol. Gen. Genet. 205 (1986) 550–556.

Simonsen, C.C., Chen, E.Y. and Levinson, A.D.: Identification of the type I trimethoprim-resistant dihydrofolate reductase specified by the *Escherichia coli* R-plasmid 483: comparison with procaryotic and eucaryotic dihydrofolate reductases. J. Bacteriol. 155 (1983) 1001–1008.

Smith, G. and Jones, P.: Effects of deletions in transposon Tn7 on its frequency of transposition. J. Bacteriol. 157 (1984) 962–964.

Smith, G. and Jones, P.: Tn7 transposition: a multigene process. Identification of a regulatory gene product. Nucleic Acids Res. 14 (1986) 7915–7927.

Stark, M.J.: Multicopy expression vectors carrying the *lac* repressor gene for regulated high-level expression of genes in *Escherichia coli*. Gene 51 (1987) 255–267.

Stephenson, F.J. and Kuhn, I.: Plasmid positive selection vectors. In: Rodriguez, R.L. and Denhardt, D.T. (Eds.), Vectors: A Survey of Molecular Cloning Vectors and Their Uses. Butterworth, Boston, 1988, pp. 131–152.

Tabor, S. and Richardson, C.C.: A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA 82 (1985) 1074–1078.

Waddell, C.: Tn7 Transposition: Identification and Characterization of the Tn7-Encoded Transposition Genes. Ph.D. Thesis, University of California, San Francisco, CA, 1989.

Waddell, C. and Craig, N.L.: Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Develop. 2 (1988) 137–149.

Waddell, C. and Craig, N.L.: Tn7 transposition: recognition of the *att*Tn7 target sequence. Proc. Natl. Acad. Sci. USA 86 (1989) 3958–3962.