Transposition of Tn1000: In Vivo Properties

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Transposition mediated by the Tn1000 transposase was investigated by using transposon variants carrying synthetic or wild-type termini but no intact Tn1000 genes. Transposon Tn1001, whose only homologies to Tn1000 are in its 38-base-pair terminal inverted repeats, transposed at the same rate as Tn1005, an artificial construct carrying wild-type Tn1000 termini and approximately 1 kilobase of flanking Tn1000 DNA at each end, when transposase was supplied in *trans*. The majority of the transpositions into pOX38 gave rise to cointegrates, but approximately 10% of the products expressed phenotypes of direct transpositions. The expression and temperature dependence of the *tnpA* gene product were examined by studying transposition of Tn1001 to bacteriophage lambda. The temperature optimum for transposition was 37°C, and the transposase was stable for up to 2 h at this temperature.

Transposon Tn1000 ($\gamma\delta$) was identified on the F plasmid of *Escherichia coli* K-12 (11, 15). Like other members of the Tn3 family, Tn1000 encodes a large transposase (M_r , 114,000), and it forms cointegrate intermediates which are later resolved by the *tnpR* gene product. The transposition properties of Tn3 and Tn1000 elements are expected to be similar, but there may be differences. The Tn1000 terminal inverted repeats, *res* site, and resolvase gene region are closely related to the corresponding elements of Tn3 (14, 23, 34). However, the inverted repeats of Tn1000 are only 35 base pairs (bp) long, and the Tn3 and Tn1000 *tnpA* gene products do not substitute for one another (20).

The leftward end of Tn1000 (includes IRL) displays imperfect sequence homology to the inverted repeats of Tn3 over a 38-bp interval. In this paper we demonstrate that 38-bp terminal inverted repeats corresponding to the left end of Tn1000 were the only sequences required in *cis* for transposition sponsored by the Tn1000 transposase. We found a significant proportion of direct transpositions, and we describe the in vivo properties of the Tn1000 transposase.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. The bacterial strains (derivatives of *E. coli* K-12) and plasmids are listed and described in Table 1. Bacteriophage λ b2 is a deletion mutant of phage λ with 12% of the parent phage genome, in the *b* region, deleted (31). Plasmid pMS421 (M. Susskind, unpublished results) is a derivative of pSC101 harboring the streptomycin and spectinomycin resistance genes, with the *lacI^r* gene inserted on an *EcoRI* fragment. It was used to control the *tnpA* gene in pXRD4043 (Fig. 1). Plasmid pOX38 is composed of largest *HindIII* fragment of F (17), which contains the entire *tra* operon.

Small plasmid constructs are shown in Fig. 1. Plasmid pXRD3705 [*tnpA* Δ (*tnpR-res*)] was constructed from pOX16 (15) by replacing the interior *XhoI* fragment of Tn1000 with the *kan* gene derived from pUC4K (Pharmacia). The altered Tn1000 element in this plasmid is called Tn1005. Plasmid

pXRD4061 is a derivative of pUC8 (37) containing Tn1001. Tn1001 contains synthetic 38-bp inverted repeats homologous to the left end of Tn1000 and flanking the *kan* gene from pUC4K. The Tn1001 element on pXRD4061 is bounded by inverted segments of a portion of the multiple cloning sequence from M13mp18. Use of the designations Tn1001 and Tn1005 for these artificially constructed elements has been cleared by the Plasmid Reference Center (E. M. Lederberg, director).

Plasmid pXRD4043 is a pACYC184 derivative that contains all of the Tn1000 *tnpA* sequence from pOX13 (15) distal to the *SalI* site spliced to the *tac* promoter from pDR540 (Pharmacia [12]) by a synthetic 16-bp DNA adapter (Fig. 1D, region II). The adapter contains an artificial Shine-Dalgarno sequence and the first 7 bp at the amino-terminal end of the *tnpA* gene. In this construction, pACYC184 was digested with *HindIII* and *HincII*, and the outer 12 bp of IRL (adjacent to *tnpA*) were removed at the *HaeIII* site. The sequence of the region shown in Fig. 1D was verified by the dideoxy method (38).

Media and chemicals. Bacteria for mating were grown in L broth (26). For matings with donor cultures at very low concentration, L-broth was preconditioned by growing AB2463 in it to 5×10^7 bacteria per ml. The medium was sterilized by filtration twice through Acrodisc filters (0.45 μ m pore size). For transfection, NZY broth (0.2% maltose, 10 mM CaCl_2 , 20 mM MgCl_2 , 1% NaCl, 1% N-Z-amine (Kraft, Inc.), and 0.5% yeast extract [27]) was used. Iso-propyl- β -D-thiogalactopyranoside (IPTG) was purchased from Sigma Chemical Co. Antibiotics (Sigma) were used at the following concentrations: ampicillin, 50 μ g/ml; kanamycin, 50 μ g/ml; tetracycline, 12.5 μ g/ml; spectinomycin, 50 μ g/ml; streptomycin, 50 μ g/ml; and chloramphenicol, 30 μ g/ml.

DNA preparation, digestion, and synthesis. For electrophoretic detection of cointegrates and small plasmids, a sodium dodecyl sulfate (SDS) minilysis procedure was used (2). Large quantities of small plasmids and large cointegrate plasmids were prepared by lysis with lysozyme and Sarkosyl and banding in CsCl as described previously (18). Restriction enzymes (New England Biolabs) and T4 DNA ligase (Pharmacia Molecular Biologicals) were used in Tris acetate buffer (32), except for *SalI*, for which high-salt buffer (8 mM

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description	Source
Strains		
AB2463	<i>thr-1 leu-6 thi-1 argE3 his-4 proA2 lacY1 galK2 ara-14 mtl-1 xyl-5 rpsL31 tsx-33 λ⁻ supE44 recA13</i>	M. Guyer (21)
C600	<i>F⁻ lacY1 tonA21 λ⁻ thr-1 leu-6 thi-1 supE44</i>	H. Shizuya (1)
C600 λ ⁺	C600 lysogenized with λpapa	This research
DH5	<i>F⁻ endA1 hsdR17 supE44 thi-1 λ⁻ recA1 gyrA96 relA1</i>	Bethesda Research Laboratories (19)
RD17	<i>thi-1 rel-1? Δ(pro-lac)_{XIII} λ⁻ supE44 recA56</i>	This laboratory (13)
RD6005	DH5(pXRD4061 pMS421 pXRD4043)	This research
RD6006	DH5(pXRD4061 pMS421)	This research
RD6782	RD17(pXRD4043 pXRD4061 pOX38)	This research
RD6783	RD17(pXRD4043 pXRD3705 pOX38)	This research
Plasmids		
pXRD3705	Tet ^r Amp ^r ; pOX16 derivative carrying Tn1005	This research
pXRD4043	pACYC184 variant carrying <i>tnpA</i> of Tn1000 expressed from the <i>tac</i> promoter; Cam ^r	This research
pXRD4061	pUC8 derivative carrying Tn1001; Amp ^r	This research
pMS421	pSC101 derivative; Str ^r Spc ^r <i>lacI^q</i>	M. Susskind
pOX38	Deleted F; Tra ⁺	M. Guyer (17)
pXRD4001-4004; pXRD4007; pXRD4011-4016; pXRD4020-4021	Transposition products resulting from mobilization experiments with RD6782; direct transpositions or cointegrates mediated by Tn1001	This research

Tris hydrochloride, pH 7.6, 150 mM NaCl, 6 mM MgCl₂, 0.2 mM EDTA) was used.

Oligodeoxynucleotides were synthesized by the phosphoramidite method (3) with an automated DNA synthesizer (Applied Biosystems model 380A) and purified on 20% polyacrylamide gels. DNA sequencing was done by a modification of the dideoxy method (38) with reverse transcriptase (Boehringer Mannheim) and double-stranded plasmid DNA. Deoxy- and dideoxynucleotides were purchased from Pharmacia.

Transposition assays. (i) **Rate measurements of Tn1000 and Tn1005 transposition.** Fluctuation tests were used to avoid preformed cointegrates resulting from the *res* and *tnpR* mutant character of Tn1001 and Tn1005. Donor cultures (RD6782 or RD6783) were grown to a concentration of 2×10^8 cells per ml in L-broth containing chloramphenicol and kanamycin and were serially diluted (factor of 0.5 at each step) with preconditioned L-broth to a concentration of 10 bacteria per ml. Identical 0.1-ml samples from these dilutions were distributed into 20 wells for mating and into 10 additional wells for donor cell counts. Incubation was continued at 37°C for 10 generations (about 7.5 h). The entire contents of the wells to be used for donor cell counts were plated on L plates containing kanamycin. Recipient culture (0.1 ml, 2×10^8 bacteria per ml) was added to each of the remaining wells to be used for mating, and incubation was continued for an additional hour at 37°C. Mating was interrupted by addition of 10^8 PFU of phage T6 per well of mated culture. The interrupted mating cultures were incubated at 37°C for another 15 min and then stored on ice overnight.

In a second microtiter tray, 0.1-ml portions of the same dilutions were also dispensed into 20 wells, and the tray was incubated at 37°C for 15 h to check for the fraction of samples showing growth at each dilution. This fraction indicated which series of diluted and mated samples had received donor inocula of 1 to 2 bacteria per sample. From the mated cultures which had been stored on ice, the entire contents of each well for which the average donor inoculum was 1 cell was plated on L-plates containing kanamycin and

streptomycin to determine the number of Kan^r transconjugants per well. Transposition rates were calculated by the equation $r = m \ln 2 / \bar{N}$, where m is the average number of Kan^r transconjugants per well and $\bar{N}$ is the average number of donors per well, corrected to account for wells that received no bacteria because of the low initial concentration.

(ii) **Transposition of Tn1001 into bacteriophage λb2.** Overnight cultures of RD6005 or RD6006 were grown from single colonies in L-broth containing appropriate antibiotics. After dilution into NZY broth, cultures were grown at the desired temperature (27, 32, 37, or 42°C) with vigorous shaking to a concentration of 5×10^7 /ml. IPTG was added to 1 mM, and the cultures were incubated for an additional 90 min to allow induction of the *tnpA* gene. Cultures were then infected at a multiplicity of 5 with bacteriophage λb2, and after incubation for 15 min at the desired temperature without agitation, they were shaken vigorously for 90 min. The lysate was treated with CHCl₃, and the debris was removed by centrifugation. Phage outputs of the lysates were measured by titering on strain C600.

Transposition products were detected by transfection of C600 λ⁺ with the output bacteriophages at a multiplicity of 0.1. After 15 min of preadsorption at 37°C, the transfection mixture was diluted with an equal volume of prewarmed NZY broth, incubated at 37°C with vigorous shaking for 1 h to allow expression of the *kan* gene, and plated on L-plates containing kanamycin. Transposition frequencies are expressed as the number of Kan^r transfectants per output phage. The transfection efficiency (measured on C600 λ⁺ with purified λ::kan cointegrate phages) was ≈35%.

For temperature shift experiments, induction was for 90 min at a given temperature (27, 32, or 37°C). The cells were pelleted and then washed and suspended in NZY broth at room temperature. The resulting culture was infected with λb2 and incubated at the desired temperature for transposition measurements as described above.

Labeling of protein in maxicells. Pulse-chase experiments were done in maxicells. The maxicell procedure was similar to that of Calhoun and Gray (7) with minor alterations.

FIG. 1. Plasmid constructs. Plasmids are drawn to scale, except for the inverted repeats. Tn1000 inverted repeats (boxes with solid arrowheads), the Tn3 inverted repeat (box with hatched arrowhead), and synthetic 38-bp inverted repeats (boxes with open arrowheads) are enlarged for clarity. Restriction sites are represented as B (*Bam*HI), E (*Eco*RI), H (*Hind*III), P (*Pst*I), Pv (*Pvu*II), Sa (*Sal*I), Sm (*Sma*I), Ss (*Sst*I), and X (*Xho*I). (A) Map of pXRD3705, which contains Tn1005. This artificial transposon is flanked by the inverted repeats from wild-type Tn1000. (B) Map of pXRD4061, which is a pUC8 derivative containing Tn1001. The synthetic 38-bp inverted repeats flanking the *kan* gene in Tn1001 are homologous to the δ end of Tn1000, including IRL. MCS represents part of the multiple cloning sequence in M13mp18 (contains restriction sites [reading inward toward the center of the element] for *Hind*III, *Pst*I, *Sal*I, *Bam*HI, and *Sma*I. *Sma*I cuts between the last base pair of the inverted repeat and the MCS. (C) Map of pXRD4043, which contains the *tnpA* gene of Tn1000 under the control of the *tac* promoter (12). The vector portion of this plasmid is from pACYC184. (D) DNA sequence of the promoter region and immediately adjacent portion of *tnpA* gene in pXRD4043. The *tac* promoter (region I) was obtained from pDR540 as a *Hind*III-*Bam*HI fragment. The 16-bp adapter (bracketed region II) has a *Bam*HI cohesive end (GATC) at one end and a *Sal*I cohesive end (TCGA) at the other end. After ligation, the *Hind*III site and the *Sal*I site are preserved, while the *Bam*HI site is not reconstituted. Region III represents a portion of *tnpA* gene sequence obtained from pOX13 (15).

Plasmid-carrying derivatives of the *recA56* strain RH68 (the *Spc*^r allele of RD17; see Table 1) were used. Because of its *Phr*⁺ phenotype, the experiment was conducted in the dark. The UV irradiation of cells was achieved with a UVS-54 Mineralight for 40 s at 10 erg/s per mm². IPTG was added to 1 mM (if required) at the same time that [³⁵S]methionine (1,130 Ci/mmol; New England Nuclear Corp.) was added (to 50 μ Ci/ml). After 60 min, nonradioactive methionine was added to 200 μ g/ml, and incubation was continued.

Samples (0.45 ml) were withdrawn at various times after addition of unlabeled methionine, and cells were washed twice with M9 medium (29). The cells were lysed with SDS lysis buffer, and the lysates were analyzed by electrophoresis with 10% SDS-polyacrylamide gels. Labeled proteins were visualized by autoradiography.

RESULTS

When Tn1000 transposase was expressed from the *tac* promoter on pXRD4043 (Fig. 1) in RD17, from which the entire *lac* operon (including *lacI*) was deleted, the new transposase operon was fully induced. This strain was viable and displayed a normal growth rate, in contrast to the observations for overproduced Tn3 transposase reported by Casadaban et al. (8). It increased the frequency of Tn1000 transposition by 50- to 60-fold, and the transposition frequencies were unaffected by the donor or target plasmid copy number (data not shown).

In the studies described below, plasmids pXRD3705 (contains Tn1005; Fig. 1) and pXRD4061 (contains Tn1001; Fig. 1) were used. Tn1001 is Δ (*tnpA-res-tnpR*), and because it is flanked by inverted repeats of the multiple cloning sequence in pXRD4061, it can be removed and reinserted in vitro into other desired locations. The orientation of Tn1001 was determined by the unique asymmetrically placed *Kpn*I site and *Eco*RI sites immediately interior to the 38-bp inverted repeats. Tn1005 is *tnpA* Δ (*res-tnpR*), and it retains the wild-type Tn1000 inverted repeats IRR and IRL, together with approximately 1 kilobase (kb) of the DNA interior to IRR and IRL.

Mobilization of Tn1000 variants Tn1001 and Tn1005. Experiments conducted with Tn1001 and Tn1005 established an upper bound on the sequence requirements for full transposition proficiency. Transposase was supplied in *trans* from pXRD4043. Because both elements were deleted for *tnpR*, and *res*, cointegrates were expected to be the predominant products. Use of a conjugative plasmid as the transposition target caused accumulation of transposition products in batch cultures, with apparent transposition frequencies of up to 0.1 per donor (data not shown). This high frequency was partly attributable to mating between donors in which transposition had occurred soon after subculture (6, 36). The contribution from preformed cointegrates was avoided by using fluctuation tests to measure rates.

Transposition rates were measured by transmission of *kan* from donor RD6782 or RD6783 (Table 1) to the *recA* strain AB2463 by pOX38. The transposition rate for Tn1005 in RD6783 was 3×10^{-4} Kan^r transconjugants per pOX38⁺ transconjugant (average of three experiments; standard deviation, 2×10^{-4}). The mobilization frequency for Tn1000 in batch cultures (1.5×10^{-4} transconjugants per pOX38⁺ transconjugant) was similar, as expected, since mobilization frequencies of *tnpR*⁺*res*⁺ plasmids reflect the time interval extending from the cointegration to the resolution events, and in this sense they correspond to a rate (15).

The transposition rate for Tn1001 in RD6782 was 3×10^{-4} Kan^r transconjugants per pOX38⁺ transconjugant (average of four experiments; standard deviation, 1.6×10^{-4}). The similarity of this rate to the rate for Tn1005 indicates that no Tn1000 sequences in addition to the 38-bp termini were required for transposition catalyzed by the *tnpA* gene product. In the absence of pXRD4043, no transposition was detected ($<10^{-8}$ Kan^r transconjugants per pOX38⁺ transconjugants; 4×10^8 transconjugants were screened).

Genetic analysis, sizes, and stability of transposition products. Previous reports had indicated that 3 to 5% of the Tn3 transposition events were direct transpositions (4). Up to 10% of the Tn1001 transposition products from batch matings displayed phenotypes expected for direct transposition (data not shown), but they might have arisen by recombination between direct repetitions in the cointegrates, followed by mating among cells in the donor culture.

FIG. 2. *Eco*RI restriction analysis of some of the transposition products resulting from mobilization experiments with RD6782. Digested DNAs of the pXRD4061 and pOX38 starting plasmids are in the central lanes. Plasmids to the left conferred resistance only to kanamycin, and plasmids to the right (cointegrates) conferred resistance to kanamycin and ampicillin. Fragments containing novel joints (white triangles) and the fragment generated from pXRD4061 (black triangle, right margin) are marked. The variable amounts of pXRD4061 DNA visible in cointegrate samples correspond to variable amounts of a small plasmid evident in undigested DNA (data not shown).

Among Tn1001 transposition products derived from rate experiments, 13% (178 of 1,324 Kan^r colonies tested; derived from 8 of 17 sample cultures) displayed the Kan^r Amp^s phenotype associated with direct transpositions. For Tn1005, 8% of the products (132 of 1,692 colonies tested; all derived from 1 of 21 sample cultures) displayed the phenotype of direct transposition. The remaining products had the phenotype of cointegrates (Amp^r, Kan^r).

Representative Tn1001 transposition products conferring resistance to both kanamycin and ampicillin or to kanamycin alone were characterized by analysis of SDS minilysates (not shown). Of eight independent Kan^r Amp^r examples, five contained variable but abundant amounts of a plasmid that comigrated with pXRD4061, together with a plasmid that migrated more slowly than pOX38. The amounts of the small plasmid varied among strains and with the growth protocol. Four of five independent Kan^r Amp^s clones produced no small plasmids during growth, but one did in small amounts.

To provide a further check on the genetic results, transposition product plasmids were digested with *Eco*RI, *Pst*I, and either *Cl*aI or *Xho*I (depending on which *Eco*RI fragment of pOX38 contained the insertion) (Fig. 2). The size of each insertion was calculated by subtraction of the length of the missing pOX38 *Eco*RI fragment from the sum of the lengths of the new *Eco*RI fragments. Three stable plasmids conferring resistance to kanamycin and ampicillin (pXRD4004, pXRD4020, and pXRD4016) each contained insertions of 5.3 ± 0.3 kb, which corresponds to the insertion size expected for cointegration with pXRD4061 (4.0 kb plus 1.4 kb). Of five plasmids conferring resistance to kanamycin

only, three (pXRD4002, pXRD4021, and pXRD4014) contained insertions of 1.3 ± 0.3 kb, which is the size expected from direct transposition of Tn1001. One of these plasmids (pXRD4011), which displayed slight instability during growth, contained 5.4 ± 0.2 kb of additional DNA, which would have been expected for a cointegrate. After subculture, strains carrying pXRD4011 reverted to ampicillin resistance, with the production of small plasmids. The remaining Kan^r Amp^s plasmid (pXRD4013) received an additional 2.0 ± 0.25 kb of DNA (see below).

Mapping of insertions in pOX38. The insertion or cointegration sites on pOX38 were mapped in eight product plasmids. Four of the plasmids carried insertions in the *Eco*RI fragment *f*_{E3} of pOX38, and the location and orientation of the inserts were determined by digestion with *Cl*aI and with *Cl*aI and *Eco*RI in combination, as well as by the *Eco*RI data. The other four plasmids carried insertions in the composite *Eco*RI fragment *f*_{E2}/*f*_{E5} of pOX38. The position and orientation of these insertions were determined by digestion with *Eco*RI, *Xho*I, and *Eco*RI and *Xho*I in combination.

The results of the mapping data are summarized in Fig. 3. The three putative cointegrates were flanked by direct repeats of Tn1001, as expected. Three of the putative products of direct transposition (pXRD4002, pXRD4021, and pXRD4014) displayed the expected insertion of a single copy of Tn1001. However, pXRD4013 contained two copies of Tn1001 in inverted orientation, and pXRD4011 was a cointegrate despite its failure to confer resistance to ampicillin. Some clustering of insertion sites was evident, but this may not reflect transpositional target specificity (see Discussion).

Temperature dependence and properties of transposase in vivo. In vivo characterization of transposase was performed

FIG. 3. Insertion sites of Tn1001 transposition products in pOX38. (A) Coordinates shown, proceeding clockwise from 0 to 100, are derived from our own data from the F map (9) (corrected to 100 kb) with the *Eco*RI site at 54 kb as the reference point. The 100/0-kb joint is set at the left end of IS3. The *Hind*III site at coordinate 0.2/44.33 kb is the fusion site of F *Eco*RI fragments *f*_{E2} and *f*_{E5}. The known restriction sites have been corrected accordingly. One of the *Cl*aI sites (C) in *f*_{E3} and the two *Xho*I sites in *f*_{E5} have been previously mapped (24, 30). The remaining sites shown in the figure were derived from our own data. Other restriction sites are designated as in Fig. 1. Open and solid triangles represent locations of direct transpositions with the *kan* gene in the counter-clockwise or clockwise orientation, respectively. Open and solid circles indicate cointegrates with the *kan* gene in counter-clockwise or clockwise orientation, respectively. (B) Structure of direct transpositions (Δ, Δ) and cointegrates (●, ●). Straight lines represent pOX38 DNA, and wavy lines represent pXRD4061 DNA. Other symbols are described in the legend to Fig. 1.

by using transposition of *Tn1001* in RD6005 or RD6006 (Table 1) to λ b2 as an assay (see Materials and Methods). The time course of *tnpA* expression was explored by infecting RD6005 induced with IPTG at various times at 37°C. After an initial linear increase, the amount of transposition leveled off after 60 min of induction at 2×10^{-4} transfectants per PFU. The amount of transposition did not increase after overnight induction with antibiotic selection for retention of plasmids (data not shown). Although these measurements were made with batch cultures and hence reflect the subsequent replication of phage genomes that had received transposed material, the order-of-magnitude agreement with the rate data was expected, since the time interval in the phage experiment (55 min) was comparable to the generation time in the rate experiments (44 min).

To examine possible causes for the limitation in transposition frequency, labeling with pulses at different times after induction or by pulse-chase experiments with [35 S]methionine were conducted with maxicells (7, 35). Addition of small amounts of radioisotope at different times after induction indicated that a protein with a molecular weight of 103,000 was produced at an undiminished rate during a 15-min to 4-h period after induction. (The approximate molecular weight predicted from the *tnpA* sequence was 114,000.) This was seen only in induced bacteria carrying pXRD4043 (data not shown). Another factor that might limit transposition frequency is instability of the *tnpA* gene product. This possibility was examined by pulse-chase experiments (Fig. 4). Strains carrying pXRD4043 produced a 102,000-molecular-weight polypeptide and an 87,000-molecular-weight polypeptide in increased amounts after induction with IPTG, but the 102,000-molecular-weight species did not appear in the absence of pXRD4043. Over a 2-h period following the addition of unlabeled methionine, there was only a slight decrease in the intensity of the band on the autoradiogram, indicating that the *tnpA* polypeptide from *Tn1000* was not extensively degraded under these condi-

FIG. 4. Stability of *Tn1000* transposase in maxicells. All strains used in this experiment had the RH68 background (*Spc^r* allele of RD17; Table 1). The molecular sizes of size standard polypeptides are indicated. Plasmids in the various samples were: lane 1, F128 (*lacI^q*) (5); lanes 2 to 8, F128 (*lacI^q*) and pXRD4043. Except for lane 2 (uninduced), induction and 35 S labeling were done at the same time (see Materials and Methods). For the induced samples (lanes 3 to 8), further incubation after addition of nonradioactive methionine was continued for 0, 15, 30, 60, 90, and 120 min, respectively. Band I is β -galactosidase, and band II is *Tn1000* transposase.

FIG. 5. Temperature dependence of *Tn1001* transposition onto λ b2. Transposition frequencies were expressed as number of Kan^r transfectants per 10^5 PFU. Results are for experiments in which induction and infection were performed at the same temperature. The + signs indicate averages of data from individual experiments (solid dots). Phage outputs at different temperatures are shown in the inset.

tions. The 87,000-molecular-weight species has not been identified.

This point was examined further by transposition experiments with cells that had been induced with IPTG for 90 min, pelleted, washed with NZY broth, and then suspended in medium lacking IPTG. The ability of such bacteria to support transposition was examined over a 2-h period following removal of the inducer (33). There was no significant decrease in the transposition frequency, indicating that transposase retained biological activity *in vivo* for up to 2 h (data not shown).

All experiments reported up to this point were conducted at 37°C. However, transposition of *Tn3* is greatly depressed at elevated temperatures (20, 25). The temperature dependence of *Tn1001* transposition was examined, again with transposition to λ b2 as an assay. Unlike *Tn3*, transposition mediated by *Tn1000* transposase was maximal at 37°C (Fig. 5). The bacteriophage outputs varied as a function of temperature (inset, Fig. 5), but the conclusion was the same after the data were corrected to transpositions per cell. The reduced *Tn3* transposition frequency observed at 37°C was attributed to the instability of transposase (20, 25). However, we have shown here that *Tn1000* transposase is stable at 37°C.

To determine whether the transposase expression or the transposition process itself was temperature dependent, induction was conducted at 27, 32, or 37°C, the cells were washed to remove IPTG, and the temperature was shifted for the transposition assay. Shifting temperatures from 37 to

32 or 27°C and shifting temperatures from 32 to 27°C reduced the transposition frequencies from the values observed at the higher temperature (data not shown). Induction at 27°C followed by transposition assay at 32°C and induction at 32°C followed by transposition assay at 37°C yielded increased transposition levels relative to the values obtained at the lower temperatures (data not shown). We conclude that the temperature dependence of transposition (Fig. 5) is not attributable to *tnpA* expression alone. The activity of Tn1000 transposase itself, or of some required host function, is reversibly temperature dependent.

DISCUSSION

The only Tn1000 sequences required in *cis* for transposition mediated by Tn1000 transposase are the inverted termini: two synthetic 38-bp inverted termini homologous to the left end of Tn1000 are no less transposition proficient than are the normal IRR and IRL joined to 1,000 bp of flanking Tn1000 DNA. This latter finding is consistent with the results for Tn3 (22) and with our previous observation with Tn1000 transposition from a single terminus (8a). A previous study with Tn3 had indicated that direct transpositions can be detected in batch cultures (4). The possibility that the results were confounded by mating among donors and different growth rates for clones with resolved plasmids was not stringently ruled out. With cultures started from single-cell inocula and maintained at low concentration, we observed for Tn1001 apparent direct transpositions among ~10% of the transposition products. This was similar to the proportion seen with Tn3; however, we found that cointegrates displayed varying levels of instability, in contrast to the findings of Bennett et al. (4). Direct transpositions were less frequent with Tn1005, but we consider this result to reflect the relatively small number of sample cultures that were analyzed. In vitro studies by Craigie and Mizuuchi (10) indicate that direct transpositions can occur as a result of premature strand scission in the replicative transposition pathway, and we therefore did not consider direct transpositions unusual.

Some clustering of target sites in pOX38 was evident: three insertions occurred in a 300-bp region between *ssf* and *oriT*, and three others were grouped in a 900-bp target region near *repA*. Only one of eight plasmids mapped contained an insertion in the *tra* region, even though this region constitutes more than half of the pOX38 target replicon. If regions required for replication, partition, control of cell division, and transfer are excluded as potential targets, the distribution of insertions in the remaining regions does not suggest a high level of target specificity or preferred orientation for Tn1001. This is one of the hallmarks of Tn1000 transposition (16). The structure of pXRD4013 is unusual, because it appears to have resulted from two direct transpositions. Such structures, which contain inverted repeats of the Tn element, have been implicated as intermediates in transposition by Tn3 variants (28).

Some properties of the Tn1000 transposase differed from those reported for the Tn3 counterpart. Overproduction of *tnpA* did not affect the cell growth rate. In maxicells, the transposase polypeptide was not rapidly degraded, and in vivo, transposition proficiency remained nearly unchanged after removal of the inducer. Thus, the protein appears to be stable and biologically active at 37°C. The temperature optimum for transposition mediated by the Tn1000 transposase was significantly higher than has been observed for

Tn3 (25). Our findings are consistent with the hypothesis that both the expression and the transposase activity itself are temperature dependent. The temperature optimum of 37°C for Tn1000 transposition may be correlated with its presence on the F plasmid of *E. coli* K-12. Tn1000 may have evolved so that its transposition is most efficient at the temperature optimum for F conjugation.

The transposition frequency of a *tnpR* Tn3 mutant is 4.6-fold higher and the transposition frequency of a *tnpR* translation-up *tnpA* Tn3 mutant is 27-fold higher than that of the wild-type Tn3 (8). Overproduction of the *tnpA* gene of Tn1000 from the *tac* promoter led to no more than a 50-fold increase above wild-type transposition levels. The reason for this limitation is not known.

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