Evidence that the *cis* preference of the Tn5 transposase is caused by nonproductive multimerization

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The transposase (Tnp) of the bacterial transposon Tn5 acts 50- to 100-fold more efficiently on elements located *cis* to the site of its synthesis compared with those located in *trans*. In an effort to understand the basis for this *cis* preference, we have screened for Tnp mutants that exhibit increased transposition activity in a *trans* assay. Two mutations in the carboxyl terminus were isolated repeatedly. The EK345 mutation characterized previously increases Tnp activity eightfold both in *cis* and in *trans*. The novel LP372 mutation, however, increases Tnp activity 10-fold specifically in *trans*. Combining both mutations increases Tnp activity 80-fold. Interestingly, the LP372 mutation maps to a region shown previously to be critical for interaction with Inh, an inhibitor of Tn5 transposition, and results in reduced inhibition activity by both Tnp and Inh. Tnp also inhibits Tn5 transposition in *trans*, and this has been suggested to occur by the formation of inactive Tnp multimers. Because Inh and (presumably) Tnp inhibit Tn5 transposition by forming defective multimers with Tnp, the inhibition defect of the *trans*-active LP372 mutant suggests that the *cis* preference of Tnp may also be attributable to nonproductive Tnp–Tnp multimerization. In addition, we show that increasing the synthesis of EK345/LP372 Tnp, but not wild-type Tnp, leads to very high levels of transposition, presumably because this altered Tnp is defective in the inhibitory activity of the wild type protein.

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Tn5 is a composite bacterial transposon consisting of two IS50 elements in inverted orientation with respect to a unique DNA sequence encoding resistances to several antibiotics (for review, see Berg 1989) (Fig. 1). The proteins that are involved in the transposition reaction are the 476-amino-acid transposase (Tnp), which is required for transposition (Isberg et al. 1982; Johnson et al. 1982), and the 421-amino-acid inhibitor (Inh), which is a truncated version of Tnp lacking its amino-terminal 55 amino acids. Inh is defective in promoting Tn5 transposition and, instead, inhibits the reaction in a dose-dependent manner (Biek and Roth 1980; Johnson and Reznikoff 1984; Yin and Reznikoff 1988). The genes encoding Tnp and Inh are under independent transcriptional and translational control (Krebs and Reznikoff 1986). In addition, Tn5 transposition requires identical 19-bp sequences at the ends of the element, termed outside ends (OEs), which contain the recognition site for Tnp binding (Johnson and Reznikoff 1983; Sasakawa et al. 1983; de la Cruz et al. 1993).

It was shown previously that Inh does not repress Tnp

expression (Isberg et al. 1982; Johnson et al. 1982) and, subsequently, that it is not capable of binding to the OE of Tn5 (de la Cruz et al. 1993), ruling out competitive binding to the ends as a mechanism of inhibition. Instead, Inh forms heteromultimers with Tnp that can still bind to the Tn5 ends but presumably represent a non-productive bound complex (de la Cruz et al. 1993). In addition to the inhibition activity of Inh, it has been found recently that Tnp itself (DeLong and Syvanen 1991; Wiegand and Reznikoff 1992) also inhibits the transposition reaction in *trans*. The precise mechanism by which Tnp inhibits the reaction is unknown, but this activity was proposed to be mediated through a Tnp–Tnp interaction leading to an inactive species (Wiegand and Reznikoff 1992).

Many bacterial transposases are preferentially *cis* active, and several mechanisms have been proposed to explain this phenomenon (McFall 1986; Kleckner 1990). For example, the transposase may be either physically or functionally unstable, which would effectively limit the range for transposase action to near the site of its synthesis. Severe physical instability (i.e., rapid turnover) has been shown to be responsible for the *cis* preference of the Tn903 transposase (Derbyshire et al. 1990). Alternatively, the transposase may be made in very limiting

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amounts. If more than one transposase protein were required for a single transposition event, this would limit action at *trans* sites simply because multiple molecules would rarely reach those sites. The basis for the *cis* preference of Tn5 Tnp is unknown, but it is known that it is not physically unstable (Johnson 1983). Furthermore, its abundance is not limiting the transposition reaction (this study) because increasing the level of Tnp synthesis by at least two orders of magnitude does not increase the frequency of transposition in *trans* or in *cis*.

In this study, we have investigated the basis for the *cis* preference of Tnp by isolating and characterizing *tnp* mutations with increased *trans* activity. Two mutations were recovered repeatedly after generalized mutagenesis of *tnp*: a glutamate to lysine at position 345, EK345; and a leucine to proline at position 372, LP372. The EK345 mutation has been characterized previously (Wiegand and Reznikoff 1992, 1994) and increases Tnp activity both in *cis* and in *trans*. The novel LP372 mutation increases Tnp activity exclusively in *trans*. Both mutations are additive when combined, suggesting that they affect different aspects of the transposition process. Because the LP372 mutation maps to a region required for Tnp-Inh multimerization (Weinreich et al. 1994) and it also reduces the inhibition activity of both Tnp and Inh, this provides evidence that both the *cis* preference and the *trans* inhibition properties of Tnp are mediated through a nonproductive multimerization event. We also demonstrate that increasing the level of the LP372 and EK345/LP372 Tnp derivatives, which are partially defective in *trans* inhibition, leads to a large overall increase in the transposition frequency. Because increasing the synthesis of the wild-type Tnp does not significantly alter the Tn5 transposition rate, this suggests that host factors are not limiting the transposition reaction but that wild-type Tnp itself limits the efficiency of the reaction.

Results

Isolation of trans-active *Tnp* mutations

A papillation screen was used to identify mutations in Tnp that exhibited greater activity in *trans* (depicted in Fig. 2). This screen depends on a defective (*Tnp*⁻) Tn5*lac* transposon inserted into an F episome, which is maintained in a *recA* Δ *lacZYA* strain. If a source of transposase is introduced into this indicator strain, it can catalyze the movement in *trans* of the defective Tn5*lac* element to random sites in the chromosome. Insertions

Figure 3. Activities of the *trans*-active mutants in IS50. (A) Location of mutations within IS50R. (B) The transposition activity of the wild-type and mutant Tnp derivatives measured by the *trans*-papillation assay. Twelve colonies on three separate plates were followed for papillae accumulation over the course of several days. For the LP372/EK345 derivative, it was impossible to count the papillae after 3.5 days. The papillae first appeared in the middle of the colony and obscured subsequent transposition events as they overgrew the rest of the Lac⁻ colony.

of the Tn5lac element into actively transcribed genes, in the correct frame and orientation, create hybrid β -galactosidase proteins. These are scored as red papillae in the white (Lac⁻) colonies on lactose MacConkey plates and the appearance of papillae over time is a measure of the *trans* activity of the Tnp protein being analyzed. The plasmid used for the source of Tnp harbors an IS50R element containing the "-3C" promoter mutation that abolishes the Inh transcript and results in undetectable levels of Inh protein (Schulz and Reznikoff 1991). This plasmid also contains a small deletion at the inside end (IE) that renders the IS50R element incapable of movement. The *recA* mutation in the host strain ensures that a recombination event cannot place the active Tnp gene *cis* to the Tn5lac element, which would be scored incorrectly as a *trans*-active mutant in the screen.

The Tnp gene was mutagenized randomly using PCR mutagenesis (Zhou et al. 1991) and segments of the mutagenized gene were cloned into an unmutagenized plasmid, transformed into the indicator strain, and the resulting colonies were examined over the course of 4–5 days for those showing increased levels of papillation over the wild type. Five independent candidates (i.e., derived from five independent PCR reactions) mutagenized

in the carboxy-terminal region of Tnp of ~20,000 colonies were chosen and analyzed. These candidates contained either an EK345 or LP372 substitution. The positions of these changes within IS50R and their effects on the transposition frequency are indicated in Figure 3. No candidates were obtained in the central third of Tnp of an additional 20,000 colonies screened, and an independent screen of the amino-terminal third of Tnp has also not resulted in mutants with a *trans*-active phenotype (H. Zhou, pers. comm.).

The EK345 mutation has been isolated and characterized previously (Wiegand and Reznikoff 1992, 1994). We present our characterization of this Tnp derivative for purposes of providing a direct comparison to the double mutant EK345/LP372 that was constructed. In addition, we have examined the effect of the EK345 mutation on the inhibitory activity of Inh.

Transposition activities of the mutants are increased in trans

The papillation data suggested that both mutations resulted in an ~10-fold increase in activity. When the two mutations were combined, a ~100-fold increase in activity was observed (Fig. 3B). The *trans* activity of these Tnp mutants was then measured using the more quantitative mating-out assay by following the movement of a marked Tn5 element on a second compatible plasmid, which does not encode either Tnp or Inh. The data from the mating-out assay indicate that the EK345 mutation results in an eightfold more active Tnp molecule (Table 1), in exact agreement with the result reported previously (Wiegand and Reznikoff 1992). The LP372 mutation results in a 10-fold increase in *trans* activity, and when both mutations were combined the activity increases to 80-fold. Because the effects of the two mutations are additive, this suggests that they are increasing the activity of Tnp through different mechanisms. This hypothesis is supported by additional characterization of these mutants (below).

Transposition frequencies in cis indicate that the LP372 Tnp is specifically more active in trans

The LP372 and EK345 mutations, together with the Inh "-3C" promoter mutation, were moved into an otherwise wild-type Tn5 background, and the *cis* transposition rates were measured using the mating-out assay (Ta-

Table 1. Trans activities of Tnp mutants

Transposase source	Transposition frequency	Relative frequency
pRZ4725 WT	4.4×10^{-6}	1.0
pRZ4725 EK345	3.4×10^{-5}	7.7
pRZ4725 LP372	4.4×10^{-5}	10.0
pRZ4725 EK345/LP372	3.5×10^{-4}	79.6

Transposition frequencies were determined by the mating-out assay and represent the average of at least 12 measurements. The standard error was $\leq 20\%$.

ble 2). The EK345 mutation leads to an eightfold increase in the transposition frequency. The LP372 change, however, results in no increase over the wild-type *cis* transposition rate. Also, the LP372 substitution results in no increase over the EK345 transposition rate when it is combined with that mutation. These data indicate that the LP372 mutation does not lead to increased transposition rates in *cis*.

A very good approximation of the *cis/trans* ratio for the Tnp mutants can be obtained after dividing the *cis* frequencies (Table 2) by the *trans* frequencies reported in Table 1. These calculations indicate that wild-type Tnp has a 72-fold preference for *cis* versus *trans* Tn5 ends (Table 2), in agreement with previous estimations (Isberg and Syvanen 1981; Johnson et al. 1982) and the EK345 Tnp exhibits essentially the same *cis* preference as the wild-type protein. The LP372 Tnp, however, is significantly (~10-fold) more active on *trans* ends than the wild type, and this accounts entirely for its increased activity in the *trans* assays above. The EK345/LP372 derivative is approximately sevenfold more active at *trans* sites than the EK345 derivative alone, indicating that the LP372 mutation results in a *trans*-active phenotype in the EK345 Tnp as well.

Effect of mutations on the inhibition properties of both Tnp and Inh

Inhibition by the Tn5 Inh protein is caused by mixed multimer formation with Tnp that leads to an inactive Tnp–Inh complex (de la Cruz et al. 1993). The transposase also has the capacity to inhibit the transposition reaction in *trans* by an unknown mechanism (DeLong and Syvanen 1991; Wiegand and Reznikoff 1992). The effect of the EK345 and LP372 mutations on this inhibitory activity was measured using IS50R derivatives on both medium- and high-copy plasmids. Because Tnp and Inh are not autoregulated, increased copy number leads to increased protein levels and a concomitant increase in the inhibition activity (Yin and Reznikoff 1988). The λ infection assay was used to quantitate the level of inhibition and measures inhibition of wild-type Tn5 transposition from the λ vehicle to the *Escherichia coli* chromosome in the presence of resident IS50 derivatives (Johnson et al. 1982).

The wild-type and EK345 Tnp proteins inhibit trans-

position approximately threefold from the medium copy pBR322 plasmid (Fig. 4A). In contrast, the LP372 protein is completely defective in this inhibition. Moreover, the EK345/LP372 Tnp actually increases the transposition rate of the wild-type Tn5 to the chromosome by 50% (Fig. 4A). Therefore, the LP372 mutation results in decreased inhibitory activity for Tnp.

A much greater inhibitory effect is seen if IS50 is present on a high-copy plasmid; this affords a more sensitive measure of the relative inhibition activities among the various mutant proteins. The trends in inhibitory activity in high copy closely parallel the medium copy situation (Fig. 4B,C). The wild-type Tnp and Inh proteins have a 200- and 100-fold inhibitory effect, respectively, on Tn5 transposition from the λ . The EK345 Tnp has a similar inhibitory activity (50–100% of the wild-type level). The EK345 Inh protein is about fivefold less active than the wild-type Inh but still exhibits substantial inhibition. The presence of the LP372 mutation, however, results in 120-fold less inhibition for Tnp and 33-fold less for Inh, regardless of the presence of the EK345 mutation. A Western blot analysis of the steady-state levels of Tnp and Inh revealed that the wild-type and EK345 derivatives were equally abundant (not shown). The LP372 mutation, however, results in a four- to fivefold decrease in both Tnp and Inh levels, as does the EK345/LP372 double mutation (not shown). First, this suggests that the *trans*-active LP372 mutation destabilizes the structure of Tnp and Inh. Second, it presents a caveat to the inhibition data, in that a portion of the decreased inhibition by the LP372 derivatives probably results from lower expression levels. However, as Tnp LP372 is only four- to fivefold less abundant than the wild type, it must have substantially less inhibitory activity (~30-fold) because it is 120-fold less active as an inhibitor in this assay. Similarly, Inh LP372 is six- to eightfold less effective as an inhibitor. This assumes that inhibition varies linearly over this range with the protein concentration. This is probably a reasonable assumption for the wild-type Tnp, however, as a plasmid copy number increase of ~30-fold leads to a 60-fold increase in the inhibition activity (Fig. 4, cf. A with B).

Increasing the amount of wild-type Tnp does not lead to coordinate increases in the transposition rate

One explanation for *cis* preference is that Tnp is made in

Table 2. *Cis* transposition activities of Tnp mutants

Tnp source	Transposition frequency	<i>Cis</i> transposition frequency ^a	Relative <i>cis</i> frequency	<i>Cis/trans</i> ratio ^b
pRZ4870 WT	$3.2 \pm 0.6 \times 10^{-4}$	3.2×10^{-4}	1.0	72
pRZ4870 EK345	$2.5 \pm 0.5 \times 10^{-3}$	2.5×10^{-3}	7.8	73
pRZ4870 LP372	$3.5 \pm 0.8 \times 10^{-4}$	3.1×10^{-4}	1.0	7.1
pRZ4870 EK345/LP372	$4.0 \pm 0.5 \times 10^{-3}$	3.7×10^{-3}	12	11

^aBecause the Tn5 element in this assay is at about six copies per cell, there will be a minor *trans* contribution to the measured frequencies. The (corrected) *cis* transposition frequencies were calculated by subtracting the corresponding *trans* frequencies in Table 1 from the transposition frequencies in column 2.

^b*Cis/trans* ratios were calculated by dividing the number in column three by the corresponding *trans* frequencies in Table 1.

Figure 4. *Trans* inhibition by Tnp derivatives in medium and high copy. (A) The inhibitory activity of Tnp and mutant derivatives on the medium-copy plasmid pRZ4725 was determined using the λ infection assay as described in Materials and methods. Note that the double mutant EK345/LP372 actually acts *in trans* to catalyze the transposition of the wild-type Tn5 to the chromosome. The Tnp[−] Inh[−] control was the parental vector pBR322. (B) Inhibition activity of IS50R derivatives present on high-copy plasmids. The plasmids used were pRZ7016 for the Tnp (MA56)-only inhibition assays and pRZ3271(+56A) for the Inh-only assays (C). The Tnp[−] Inh[−] control plasmid was pRZ7038 (Wiegand and Reznikoff 1992). The transposition frequency of Tn5 from λ in the absence of Tnp and Inh was between 1.5×10^{-3} and 4.0×10^{-3} in the different experiments. Note also that the inhibition defect of LP372 Tnp is not merely attributable to its increased transposition activity in *trans*, as EK345 Tnp has a similar increase in its *trans* activity and yet retains wild-type inhibitory activity.

limiting amounts. Therefore, we tested the effect of increasing Tnp synthesis on Tn5 transposition in *cis* and in *trans*. The Tnp gene containing strong translational initiation signals and the MA56 mutation, which eliminates the start codon for Inh, was placed under the control of the strong λ P_R promoter to regulate and increase its level of synthesis. The MA56 mutation results in a 10-fold increase in the Tn5 transposition rate caused by the loss of Inh expression (Wiegand and Reznikoff 1992). Exactly the same increase in transposition is observed when Inh expression is eliminated with a promoter mu-

tation, indicating that the MA56 mutation has no effect on Tnp activity. In this construction, Tnp synthesis is thermoinducible because of the presence of the heat-labile λ repressor encoded by the plasmid. No increase in the transposition frequency is seen in *cis* or in *trans* after increasing Tnp synthesis 100-fold (Weinreich et al. 1994) using a temperature shift from 32°C to 37°C (Table 3).

Similar results were obtained with an independent plasmid containing Tnp (MA56) under the control of the *lacUV5* promoter. This plasmid also contains the *lacI^q* gene and a reporter mini-Tn5 element downstream of

Table 3. Induction of Tnp derivatives vs. transposition rate

Plasmid	Induction	Transposition frequency ^a	Relative frequency ^b
pRZ4871 WT (<i>cis</i>)	—	2.1×10^{-5}	1.0
pRZ4871 WT (<i>cis</i>)	37°C	1.1×10^{-5}	0.5
pRZ4775 WT (<i>trans</i>)	—	6.1×10^{-6}	1.0
pRZ4775 WT (<i>trans</i>)	37°C	5.0×10^{-6}	0.8
pRZ4870 WT	N.A.	3.2×10^{-4}	1.0
pRZ4834 WT	—	4.1×10^{-5}	0.13
pRZ4834 WT	IPTG	8.2×10^{-5}	0.26
pRZ4870 LP372	N.A.	3.5×10^{-4}	1.0
pRZ4856 LP372	—	9.7×10^{-4}	2.8
pRZ4856 LP372	IPTG	3.3×10^{-3}	9.4
pRZ4870 EK345/LP372	N.A.	4.0×10^{-3}	1.0
pRZ4857 EK345/LP372	—	8.3×10^{-3}	2.1
pRZ4857 EK345/LP372	IPTG	1.2×10^{-2}	3.0

^aThe transposition frequencies were determined by the mating-out assay and represent the average of at least eight independent measurements. For induction of λ P_R Tnp constructs pRZ4871 and pRZ4775, cultures were grown and mated at 37°C (induced) or 32°C (uninduced). The mini-Tn5 reporter element for the *trans* measurements was contained on pRZ4729. A temperature shift from 32°C to 37°C resulted in a 50% increase in the transposition frequency for wild type (Tnp⁺ Inh[−]) Tn5, and the transposition frequencies are corrected for this contribution. IPTG (0.2 mM final) was added during the mating only and not during the overnight growth for measurements with P_{lacUV5} Tnp constructs pRZ4834, pRZ4856, and pRZ4857. The standard error was $\leq 20\%$.

^bThe relative frequencies are calculated to the uninduced cultures for the first set of data and to the pRZ4870 derivatives (which contain the natural Tnp promoter) for the second set. The transposition frequencies for the pRZ4870 plasmids are from Table 2.

the Tnp gene. In the uninduced state, the basal level of Tnp expression from this plasmid (pRZ4834) was significantly higher than from pRZ4870, which encodes Tnp from its natural promoter. Yet, the transposition frequency was actually eightfold lower in this background (Table 3). Induction of Tnp synthesis with IPTG resulted in at least a further 10-fold increase in Tnp levels; however, the transposition frequency increased only twofold (Table 3). Intermediate levels of IPTG induction also did not significantly alter the transposition rate. These results indicate that the amount of Tnp is not limiting the transposition rate even in the absence of *Inh*. Therefore, there must be some mechanism distinct from low-level Tnp synthesis that limits the frequency of Tn5 transposition. Furthermore, low abundance of Tnp cannot be invoked to explain its *cis* preference.

When Tnp derivatives containing the LP372 and EK345/LP372 mutations are placed under the control of the *lacUV5* promoter, however, higher levels of transposition are observed. In contrast to the wild type, the uninduced levels of transposition were about threefold higher than from the analogous wild-type promoter constructs (Table 3). This is what is expected for a Tnp protein with increased *trans* activity and decreased inhibitory activity (i.e., higher expression levels lead to higher rates of transposition). Inducing Tnp synthesis leads to another three- to fourfold increase in the transposition frequency (Table 3). It should be noted, however, that the amount of Tnp induced for the LP372 and EK345/LP372 mutants was significantly less than for the wild-type protein. If we could increase the amount of Tnp EK345/LP372 to that of the wild-type protein, perhaps even greater transposition rates would be observed. The transposition rate for the induced EK345/LP372 Tnp was 1.2×10^{-2} over the course of the mating-out experiment compared with 8.2×10^{-5} for the wild type (Table 3). Because the size of the target for transposition in this assay is 38 kb (pOX38-Gen) and the *E. coli* genome is 4700 kb, we are detecting only 0.8% of the transposition events. On the basis of this value and that ~10 generations occurred over the course of the experiment, the absolute transposition frequency is 0.07 event per cell per generation. This suggests that host factors are not limiting the transposition rate, at least to ~0.1 event per cell, and that the limitation to the wild-type Tn5 transposition frequency is probably intrinsic to Tnp itself.

DNA-binding characteristics of Tnp mutants

The major retarded complex formed between wild-type Tnp and the OE contains a Tnp–*Inh* heteromultimer representing a nonproductive bound complex (de la Cruz et al. 1993; Wiegand and Reznikoff 1994). Therefore, we examined the DNA-binding characteristics of the increased activity mutants to see whether they had altered the formation of this complex. The Tnp derivatives were first placed under the control of the T7 promoter. Tnp expression was induced and then crude extracts were

examined for their DNA-binding activity to the Tn5 OE. The wild-type Tnp binds to the OE to give the inhibitory complex I (Fig. 5B). The EK345 Tnp results in complex I formation plus a group of faster migrating complexes (II) that have been shown to contain Tnp degradation products missing ~100 amino acids at the carboxyl terminus (Wiegand and Reznikoff 1994). The LP372 Tnp results in the formation of slightly slower mobility complex than the wild-type complex I, a small amount of complex II species, and also results in a significant amount of a complex that does not enter the gel. The EK345/LP372 Tnp also results in a complex that does not enter the gel, the complex (II) products indicative of the EK345 mutation and a smear near the wild-type complex I position (Fig. 5B). The complexes formed with the double mutant protein approximate a summation of the complexes formed with each mutant protein individually with the exception that complex I now forms a smear. All of these complexes are specific for the OE, as they are not formed with an identical DNA fragment bearing a mutation in the OE sequence (not shown).

The most significant correlation for the increased activity LP372 and EK345/LP372 Tnp proteins is that both either form less of complex I or alter its mobility, and both proteins form specific complexes that do not enter the gel. The alteration in complex I formation by the increased activity mutants strengthens the proposal that this is an inhibitory complex. An SDS gel showing the total cell extracts (Fig. 5A) also suggests that the LP372 Tnp has increased DNA-binding activity as it is less abundant than the wild-type protein but gives a similar amount of complex I retardation. This is supported by experiments with purified proteins that indicate that Tnp LP372 has a fivefold greater DNA-binding activity than the wild-type protein (data not shown).

The pattern of Tnp–OE complex formation with crude extracts is similar to that obtained with the purified proteins (Fig. 5D); however, purification has removed essentially all of the carboxy-terminal truncated proteins giving rise to the complex II species. (The small difference in mobility between complex I species formed with wild-type and LP372 Tnp is only evident when a small amount of complex is generated.) Note that *Inh* and a degradation product of Tnp (Tnp α), which is missing ~35 amino-terminal amino acids (Weinreich et al. 1994), cofractionate with Tnp (Fig. 5C). These products have been shown to be present together in nearly stoichiometric levels with wild-type Tnp in complex I (Wiegand and Reznikoff 1994) and may be necessary for its formation. The fact that the LP372 and EK345/LP372 Tnp preparations exhibit greater DNA-binding activity to the OE and yet contain relatively less Tnp α and *Inh* suggests that the oligomerization properties of these mutant proteins might be altered.

Tnp LP372 is defective in its interaction with *Inh*

Because both Tnp and *Inh* derivatives containing the

Figure 5. SDS gel and DNA-binding activity of wild-type Tnp and mutants. A 3–10% SDS gel of total cell extracts after induction of Tnp (A) or after purification of Tnp (C) is shown. The numbers at the left are protein size standards in kilodaltons. The arrowhead indicates the position of Tnp; some amino-terminal proteolysis of Tnp occurs during purification, giving rise to Tnp α , and this migrates ahead of Tnp. The additional contaminant in the wild-type and EK345 preparations is Inh. One microliter of each extract (B) or 450 ng of purified protein (D) was examined for DNA binding to a 60-bp fragment containing the Tn5 OE. The position of the wild type complex I is indicated as well as the faster migrating complex II species. Complex II has been shown to arise from the binding of Tnp proteolytic products lacking a complete carboxyl terminus to the OE (Wiegand and Reznikoff 1994). Note that the LP372 and EK345/LP372 extracts give rise to a significant amount of complexes that do not enter the gel.

LP372 mutation are partially defective in inhibition, these proteins may be defective in multimer formation. To examine this possibility, we used the fact that Inh forms multimers with Tnp *in vitro*, which are capable of binding to the OE. Although Inh alone does not bind to the OE, it has been shown that the addition of purified Inh to limiting amounts of Tnp significantly increases the observed DNA-binding activity, presumably by forming more Tnp–Inh multimers that do bind to DNA (de la Cruz et al. 1993). To examine this interaction between Tnp and Inh *in vitro*, a constant amount of purified wild-type and LP372 Tnp resulting in <5% complex formation was incubated with OE DNA and increasing amounts of wild-type Inh. The stimulation of complex I formation was quantitated and is presented in Figure 6. Inh stimulates wild-type binding by 14.3-fold at the highest amount of Inh. In contrast, Inh only stimulates

Tnp LP372 binding 5.3-fold and does not stimulate the formation of the LP372 complex not entering the gel. Because the wild-type complex I contains Tnp–Inh multimers, this suggests either that Tnp LP372 is defective in interacting with Inh or that the Tnp LP372–Inh multimers that do form do not have the same affinity for the OE as the wild-type multimers.

Discussion

Mechanisms to explain cis preference

A number of bacterial DNA-binding proteins have been described that are *cis* active. In a strict genetic sense, this designation simply reflects that these proteins exhibit inefficient complementation in *trans*. Although a num-

Figure 6. Inh stimulation of Tnp wild type and LP372 binding to the OE. A constant amount (50 ng) of purified Tnp proteins and OE DNA were incubated with 0, 125, 250, 500, and 1000 ng of purified Inh in binding buffer. After a 30-min incubation at 30°C, the free and complexed DNA were separated on an 8% polyacrylamide gel (A) and the amount of complex I formation was quantitated using a Betagen. (B) The ratio of complex I formation in the presence of Inh to that with no added Inh. The defect in stimulating the LP372 protein is not simply attributable to a saturation of active protein, as increasing the OE DNA concentration two- and fourfold at the end of the titration results in a corresponding increase in complex I formation and free DNA (data not shown). The retarded complex formed with 1000 ng of Inh only is not specific to the OE (de la Cruz et al. 1993) and results from a contaminant in the Inh protein preparation.

ber of mechanisms have been proposed to explain this phenomenon, there is little evidence available to test which mechanisms are actually used, as very few mutants have been described that alter the *cis*-active phenotype of these proteins.

At least five mechanisms have been proposed to explain preferential *cis* activity (McFall 1986; Kleckner 1990); however, these are not mutually exclusive. One explanation proposes that the protein has a relatively high affinity for nonspecific DNA compared with that

for its specific DNA-binding site. This activity would favor association with DNA close to the site of the synthesis, and then one-dimensional diffusion along the DNA would result in preferential contact with the specific DNA-binding site located in *cis*. This mechanism has been proposed to explain the *cis* action of the λ Q protein (Echols et al. 1976; Burt and Brammar 1982). Alternatively, the protein could be sequestered during or shortly after translation, preventing efficient interaction with *trans* DNA sites. The *cis* preference of the Φ X174 gene A replication protein has been suggested to result from such a rapid channeling to membrane sites (van der Mei et al. 1972; McFall 1986). Another mechanism proposes that the activity of the protein is mechanistically coupled to its expression; for example, the nascent protein has an activity that the mature protein does not. Very low protein expression could also limit action to *cis* sites. This is an especially attractive explanation for transposition reactions, as protein multimers are likely required for activity. If only a few molecules are present per cell, the protein concentration at *trans* sites would rarely be sufficient to catalyze a transposition event. Finally, *cis* preference could be caused by functional or physical protein instability. It has been demonstrated that the *cis* preference of the Tn903 Tnp is correlated with its very short half-life (Derbyshire et al. 1990).

In an effort to understand the mechanistic basis for the *cis* preference of the Tn5 Tnp we sought Tnp mutants with increased *trans* activity. We identified a novel mutation, LP372, that specifically increases the *trans* activity of Tnp 10-fold. The inhibitory activity of both Tnp and Inh derivatives bearing the LP372 mutation is also decreased substantially, and this has important implications for models attempting to explain the *cis* preference of Tnp. This importance follows from an understanding of the mechanisms by which Inh and Tnp inhibit Tn5 transposition (below) and has led us to propose that Tnp multimerization before contacting an end sequence is nonproductive and contributes to the *cis* preference of this protein.

Dominant-negative interference can explain the inhibition phenotypes of both Tnp and Inh

Inhibition of transposition by Inh, which is a dimer in solution, is caused by heteromultimer formation with Tnp (de la Cruz et al. 1993). These heteromultimers still bind to the OE of Tn5 and presumably also block the site of action of free Tnp in the cell. Because Tnp contains all of the coding sequence of Inh, presumably it has the ability not only to multimerize with Inh but also with itself. Tnp multimer formation is most certainly a requisite step in the transposition reaction (Baker and Mizuuchi 1992), and the temporal order of binding to end sequences and multimerization may be critical for a successful transposition event.

Tnp has also been shown to inhibit in *trans* the reaction it promotes in *cis* (DeLong and Syvanen 1991; Wiegand and Reznikoff 1992). This was proposed to be caused by the interaction of a functionally inactive Tnp

monomer (or breakdown product of Tnp) with an active Tnp monomer, forming a nonproductive dimer (Wiegand and Reznikoff 1992). However, other explanations are equally possible. For instance, premature dimerization of two active Tnp proteins may mask the DNA-binding domain. This model is attractive because it does not invoke a conformational change in Tnp and is consistent with our inability to detect DNA complexes containing only Tnp (i.e., our purified Tnp consists of mostly multimeric Tnp species inactive for DNA binding). Another possibility requires that Tnp interacts with a host protein that is essential for the transposition reaction. If this factor is limiting, then free Tnp and complexed Tnp would compete for occupancy of the end sequences leading to inhibition. In distinction with the previous explanations, this model does not require Tnp multimer formation to explain the inhibition.

Trans inhibition by Tnp is likely caused by nonproductive multimerization

The LP372 mutation decreases the inhibitory activity of both Tnp and Inh. A deletion analysis has shown that an 18-amino-acid region that contains this L372 residue is critical for Tnp–Inh multimer formation (Weinreich et al. 1994). A carboxy-terminal deletion in Tnp from residue 388 to 476 results in a protein that is still capable of forming multimeric Tnp–Inh OE complexes as measured by gel retardation. A deletion of residues 370–476, however, results in the formation of only monomer Tnp–OE complexes (Weinreich et al. 1994; Wiegand and Reznikoff 1994). For this reason we propose that the LP372 change disrupts Tnp–Inh and Tnp–Tnp interactions. The most logical explanation for the inhibition defect of Inh LP372 is weakened interactions between Inh and Tnp. Biochemical analysis of Tnp LP372 also supports the contention that this mutation disrupts interactions between the two proteins, as we have shown that Tnp LP372 is defective in its functional interaction with wild type Inh *in vitro*. Because Tnp and Inh are 88% identical, differing only in their amino-terminal domains, it seems very likely that the LP372 mutation also disrupts Tnp–Tnp interactions. Although we cannot dismiss other explanations completely, we suggest that the LP372 mutation causes an inhibition defect for both Inh and Tnp by the same mechanism: disrupting nonproductive Inh–Tnp or Tnp–Tnp multimer formation.

The cis activity of Tnp can also be explained by nonproductive multimerization

Our analysis of the LP372 *trans*-active mutant suggests that functional instability, at least in part, can explain the *cis* preference of Tnp. The finding that the LP372 mutation increases the *trans* activity of Tnp and decreases the inhibitory activity of both Tnp and Inh simultaneously provides a link between the two properties. We suggest that a defect in formation of nonproductive Tnp multimers not only explains the inhibition defect of this mutant but can also explain its *trans*-active

phenotype. This model does not invoke a conformational change in Tnp and only proposes that dimerization before contacting an OE is nonproductive and functions to limit Tnp action to *cis* sites.

This model suggests that after Tnp is synthesized it can follow one of two fates, excluding the possible interaction with Inh. Either it contacts a Tn5 end sequence that stabilizes it in a form that can interact productively with a second Tnp molecule at the opposite Tn5 end or it diffuses through the cytoplasm in a metastable state. If it encounters a *trans* Tn5 end, it is stabilized. However, if it dimerizes with another Tnp monomer it is also stabilized but becomes inactive for transposition either because it cannot interact with an end sequence or such an interaction is now nonproductive. The defect in inactive dimer formation caused by LP372 would allow Tnp to diffuse more readily through the cell in an active monomeric state (i.e., increase *trans* activity). This model allows both productive and nonproductive Tnp multimerization to occur using the same or different protein interfaces. Other factors may certainly contribute to *cis* preference. For instance, the relative affinities of Tnp for the end sequences versus another Tnp molecule may change in progression from the nascent to the mature protein.

A temperature-sensitive *trans*-active mutation in IS50 was isolated previously (DeLong and Syvanen 1991) and an analysis of its properties lends further support to this model. Although this mutation decreases the *cis* activity of Tnp, it increases the relative *trans* activity substantially to 12–100% of the *cis* value. This phenotype results from a leucine to phenylalanine change at position 449, near the carboxyl terminus. Interestingly, although the significance was not appreciated at that time, this mutation also results in a Tnp protein that has only 20% of *trans*-inhibition activity of the wild type. Furthermore, when the inhibitory activity of Tnp LF449 was measured against Tn5 also containing Tnp LF449, almost no inhibition was seen. Because both LP372 and LF449 mutations result in increased *trans* activity and decreased inhibition, this lends support to the contention that *cis* preference is caused by the formation of nonproductive multimers, which can explain both phenotypes.

Host factors are not limiting the Tn5 transposition frequency

Increasing the level of Tnp by two orders of magnitude (in the absence of Inh) was found not to have a significant effect on the transposition frequency. This is the expected result as Tnp both promotes and inhibits Tn5 transposition. When we measured the effect of increasing Tnp synthesis using the *trans*-papillation assay, transposition rates actually declined (not shown). This is in contrast to the Mu, Tn7, and Tn10 bacterial elements that can transpose at a frequency equal to or greater than one event per generation if Tnp is not limiting (Morisato et al. 1983; Craig 1989; Mizuuchi 1992). Recently, the Tnp of the maize transposable element Ac was found

also to have inhibitory activity. Increasing Tnp dosage eventually leads to an inhibition of Ds element excision (Scofield et al. 1993).

We were, however, able to achieve high rates of transposition, to ~0.1 event per cell per generation, by increasing the synthesis of the EK345/LP372 Tnp derivative. Therefore, the inability to achieve higher transposition rates with increasing amounts of wild-type protein is probably not attributable to some limiting host factor but, rather, reflects an intrinsic property of Tnp itself. This is likely caused by the inhibitory activity of Tnp that the double mutant overcomes to a large extent.

DNA-binding characteristics of Tnp mutants

In examining the binding of Tnp LP372 and EK345/LP372 proteins to the OE it was found that they both formed complexes that did not enter the gel. These complexes may very well reflect a more active species in the transposition reaction, perhaps containing more than one Tn5 OE DNA. Tnp LP372 formed another complex with a slightly slower mobility than the wild-type complex I. Because the wild-type complex I contains a heteromultimer of Tnp and Inh, this might indicate that this new complex formed with LP372 Tnp contains a multimer of Tnp alone. Alternatively, LP372 Tnp may have an altered conformation that affects the mobility of this complex. We did not detect an LP372 Tnp–OE complex with a faster mobility than the wild type, which might have been expected if this mutant protein is significantly monomeric. However, this mutation only gives a partial *trans*-active phenotype in vivo that may not result in significant population of monomers in the in vitro preparations. Alternatively, if such a monomeric complex were formed in vitro, it might be unstable and rapidly pair with another Tnp LP372–OE to form the slower mobility complexes that we observe.

This double mutant Tnp will prove very useful for future functional studies of Tnp, for detecting transposition intermediates in vivo, and for developing a cell-free system to study Tn5 transposition. It might also be instructive to perform another round of mutagenesis on this mutant to achieve even higher rates of Tn5 transposition that might result from stabilizing the active structure of the double mutant Tnp.

Materials and methods

Bacterial strains and reagents

All strains were derivatives of *E. coli* K-12, except for the B-strain BL21(DE3) pLysS (Studier et al. 1990) used for Tnp overproduction. MDW320 was the indicator strain used in the *trans*-papillation assay (Weinreich et al. 1994). It is CSH26 *recA*/pOX386. RZ212 *recA* (Wiegand and Reznikoff 1992) and RZ47.01 (Johnson and Reznikoff 1984) were the donor strains for all mating-out experiments. RZ224 *polA* Nal^R was the recipient strain for all mating-out experiments. MC1061 (Sambrook et al. 1989) was the host strain for the inhibition assays. Bacteria were cultured in Luria broth (LB) medium. Antibiotics were purchased from Sigma, and the final concentrations were

100 µg/ml of ampicillin, 15 µg/ml of tetracycline, 40 µg/ml of kanamycin, 20 µg/ml of naladixic acid, and 5 µg/ml of gentamycin. Restriction enzymes were purchased from New England Biolabs and Promega. Reverse transcriptase was from Molecular Genetic Resources, and radionucleotides were purchased through Amersham.

Plasmid constructions

pOX386 is pOX38-Gen (Johnson and Reznikoff 1984) containing an insertion of a transposition defective Tn5*lac* element and has been described previously (Weinreich et al. 1994). pRZ4725 was constructed by replacing the 375-bp *EcoRI*–*Bam*HI fragment from pBR322 with the *EcoRI*–*Bcl*II fragment containing IS50R from pRZ3271(–3C) (Schulz and Reznikoff 1991). This IS50R element harbors the Inh promoter mutation –3C, which eliminates the Inh transcript.

The pRZ4725 EK345 and LP372 derivatives were isolated as described below. pRZ4725 EK345/LP372 was constructed by replacing the *Not*I–*Hind*III fragment of pRZ4725 LP372 with the analogous IS50R fragment carrying the EK345 mutation.

The pRZ4870 wild-type and mutant derivatives were constructed by replacing the *EcoRI*–*Sal*I wild-type IS50R fragment of pRZ143 (Rothstein et al. 1980) with the *EcoRI*–*Sal*I fragment of pRZ4725 and mutant derivatives. This places the –3C mutation and the various *trans*-active mutations in an otherwise wild-type Tn5 background for measuring *cis* transposition rates.

pRZ7016 and pRZ7016 EK345 contain IS50R with the MA56 mutation on the high-copy plasmid pBKS[–] (Wiegand and Reznikoff 1992). These plasmids encode Tnp but not Inh. The LP372 and EK345/LP372 mutations were introduced into this background by replacing the *Nhe*I–*Bgl*II IS50R fragment of pRZ7016 with the analogous fragments from pRZ4725 LP372 and pRZ4725 EK345/LP372. pRZ7038 is a IS50R deletion derivative of pRZ7016 that does not encode Tnp or Inh (Wiegand and Reznikoff 1992).

pRZ3271(+56A) contains IS50R on the high-copy plasmid pUC18 (Schulz and Reznikoff 1991). The +56A mutation creates an amber mutation in the unique portion of the Tnp-coding sequence and, hence, produces only Inh. Mutant derivatives were constructed by replacing the *Not*I–*Bgl*II fragment of pRZ3271(+56A) with the analogous IS50R fragments from pRZ4725 EK345, pRZ4725 LP372, and pRZ4725 EK345/LP372.

pRZ4793 has been described (Weinreich et al. 1994) and contains the Tnp gene on the T7 overexpression plasmid pET21d⁺ (Studier et al. 1990). The plasmids for overproducing the various Tnp mutants were constructed by cloning the *Bsp*HI–*Sal*I IS50R fragments containing the entire Tnp gene from pRZ4725 mutant derivatives into the *Nco*I–*Sal*I sites of pET21d⁺. The *Bsp*HI–*Nco*I junction was sequenced to verify the presence of the wild-type Tnp amino terminus.

pRZ4795 was constructed in several steps to encode the Tnp gene of IS50R fused to the efficient Shine–Dalgarno sequence of pET21d⁺. This gene was then placed under the control of the *lacUV5* promoter on pB10a (M. Imae, unpubl.), which also encodes *lacI^q* and *amp*. The MA56 mutation was introduced to eliminate Inh synthesis, and then a mini-Tn5 element was placed downstream of the Tnp (MA56) gene to yield the final plasmid, pRZ4834. This mini-Tn5 element has two copies of the outer 56 bp of Tn5 surrounding the kanamycin resistance gene of Tn903. pRZ4856 is identical to pRZ4834, except that it contains the LP372 mutation. pRZ4857 is pRZ4834 containing the EK345 and LP372 mutations.

pRZ4711 was constructed by inserting wild-type Tn5 [obtained on an *Eco*RI fragment from pRZ7060 (T. Wiegand, unpubl.)] into the *Eco*RI site of pACYC184 to give pRZ4704.

pRZ4704 was then digested to completion with *Pst*I, and a *Pst*I fragment containing the kanamycin resistance gene from Tn903 was inserted between the Tn5 ends. This plasmid does not encode functional Tnp but can be complemented to transpose if Tnp is supplied in *trans*.

PCR mutagenesis and isolation of trans-active mutants

Two primers terminating at the *Hpa*I and *Bgl*II sites of IS50R were constructed to amplify the Tnp gene in pRZ4725. The PCR reaction conditions were essentially as described (Zhou et al. 1991) except that they were performed in the presence of 0.5 mM MnCl₂ and with 35 cycles of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C and *Taq* polymerase from Promega. The DNA was extracted once with phenol–chloroform and precipitated with ethanol using glycogen (Boehringer Mannheim) as a carrier. The amplified DNA was then digested with *Nhe*I and *Bgl*II or with *Not*I and *Nhe*I. These ~500-bp mutagenized IS50R fragments were gel purified and cloned into the *Nhe*I–*Bgl*II or *Not*I–*Nhe*I sites of pRZ4725. DNA from at least six independent PCR reactions was subcloned into pRZ4725 and then screened for *trans*-active Tnp candidates as described below.

After ligation, the mutagenized plasmids were electroporated into MDW320 and plated onto lactose MacConkey plates containing ampicillin and tetracycline to give from 50 to 200 colonies per plate. These plates were incubated at 37°C for ≤8 days and candidates showing increased levels of papillation were restreaked and then saved. The number of colonies showing no papillae after 8 days ranged from 20–80%. After verifying that the plasmid was responsible for the increased papillation phenotype by retransformation, the entire sequence of the mutagenized region was determined. Six independent candidates were obtained: two of these had an EK345 mutation, three had an LP372 mutation, and the remaining candidate, which only showed a twofold increase, had multiple mutations and was not studied further. The candidates containing the LP372 and EK345 mutations also had silent and additional mutations. We confirmed that the EK345 and LP372 mutations were responsible for the increased transposition rates by separating these mutations from all other mutations. These plasmids were used subsequently for the analyses described below.

Mating-out assays

The mating-out assay measures the transposition frequency of a Tn5 element onto the F-factor, pOX38-Gen, after bacterial conjugation (Johnson and Reznikoff 1984). For the *trans* mating-out assay the strain MDW537 was used. This is RZ212 containing the pACYC184-derived plasmid, pRZ4711. pRZ4711 contains an internally deleted Tn5 element that does encode functional Tnp but can be complemented to transpose if Tnp is supplied in *trans*. MDW537 was transformed with pRZ4725 or mutant derivatives, and independent cultures were grown overnight in LB containing ampicillin, kanamycin, and gentamycin. A 1:100 dilution of the donor was mixed with a 1:50 dilution of the recipient, RZ224, in LB and incubated on a rotary drum at 37°C for 6–8 hr. Exconjugants were selected by plating dilutions onto LB plates containing gentamycin and naladixic acid. Exconjugants that had received a Tn5 insertion were selected on the same plates plus kanamycin. The transposition frequency is obtained by dividing the number of exconjugants containing Tn5 insertions by the total number of exconjugants.

The *cis* mating-out procedure used RZ212 as the donor. Selection during the overnight growth was for the presence of the Tn5-containing plasmid (kanamycin) and the episome (gentamycin). For induction of Tnp synthesis from the *lacUV5* pro-

motor plasmids, IPTG (0.2 mM final) was included only during the mating and RZ47.01 was the donor strain. Otherwise the procedure was identical to that above.

Trans-inhibition assay

In this assay, MC1061, containing a resident IS50R element, is infected with the replication and integration-defective phage λNK467 (N. Kleckner, Harvard University, Cambridge, MA) at a low multiplicity of infection (m.o.i.). Transposition of the Tn5 element on λNK467 to the MC1061 chromosome is inhibited if IS50R encodes either Tnp or Inh (Wiegand and Reznikoff 1992). Transposition events are scored by plating dilutions of infected cells onto LB plates containing kanamycin.

Four independent cultures were grown overnight in LB containing 0.2% maltose and the appropriate antibiotic to select for the IS50R plasmid. The cultures were adjusted to contain 10 mM MgSO₄, 100 μl of cells was mixed with λNK467 at an m.o.i. of 0.2 and incubated for 15 min at 32°C. LB was added to 1 ml and the incubation was continued for an additional 20 min. Dilutions were plated onto LB plates containing kanamycin and incubated overnight at 37°C. The transposition frequency was calculated as the total number of kanamycin-resistant colonies per milliliter divided by the number of input phage.

Overproduction of Tnp and preparation of crude extracts

The 40-ml cultures of BL21(DE3) pLysS containing the Tnp overproducer plasmid pRZ4793 or mutant derivatives were grown at 37°C to an OD₅₅₀ of 0.3 and induced with 0.2 mM IPTG for 60 min. The medium was LB containing ampicillin and chloramphenicol. Cultures were collected by centrifugation at 10,000g and 4°C. The pellet was resuspended in 400 μl of 0.1 M NaCl (TEGX) buffer (Weinreich et al. 1994) and sonicated on ice with four 5-sec bursts using a Branson Sonifier (model W185). The debris was removed by centrifugation for 5 min in an Eppendorf centrifuge (12,000g), and the supernatant was frozen in liquid nitrogen. Extracts were added directly to the binding reactions above and examined on 3–10% SDS gels after staining with Coomassie blue.

Purification of Tnp and Inh was performed essentially as described (de la Cruz et al. 1993).

Gel retardation

The gel retardation was performed exactly as described (de la Cruz et al. 1993), except that the binding buffer contained 2 mM EDTA for reactions with crude extracts. Assays to measure the enhancement of binding by Inh were also performed as described (de la Cruz et al. 1993).

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References

- Baker, T.A. and K. Mizuuchi. 1992. DNA-promoted assembly of the active tetramer of the Mu transposase. *Genes & Dev.* **6**: 2221–2232.
- Berg, D.E. 1989. Transposon Tn5. In *Mobile DNA* (ed. D.E. Berg, and M.M. Howe), pp. 185–210. American Society for Microbiology, Washington, D.C.
- Biek, D. and J.R. Roth. 1980. Regulation of Tn5 transposition in *Salmonella typhimurium*. *Proc. Natl. Acad. Sci.* **77**: 6047–6051.
- Burt, D.W. and W.J. Brammar. 1982. The *cis*-specificity of the Q-gene product of bacteriophage lambda. *Mol. & Gen. Genet.* **185**: 468–472.
- Craig, N.L. 1989. Transposon Tn7. In *Mobile DNA* (ed. D.E. Berg and M.M. Howe), pp. 211–225, American Society for Microbiology, Washington, D.C.
- de la Cruz, N.B., M.D. Weinreich, T.W. Wiegand, M.P. Krebs, and W.S. Reznikoff. 1993. Characterization of the Tn5 transposase and inhibitor proteins: A model for the inhibition of transposition. *J. Bacteriol.* **175**: 6932–6938.
- DeLong, A. and M. Syvanen. 1991. Trans-acting transposase mutant from Tn5. *Proc. Natl. Acad. Sci.* **88**: 6072–6076.
- Derbyshire, K., M. Kramer, and N.D.F. Grindley. 1990. Role of instability in the *cis* action of the insertion sequence IS903 transposase. *Proc. Natl. Acad. Sci.* **87**: 4048–4052.
- Echols, H., D. Court, and L. Green. 1976. On the nature of *cis*-acting regulatory proteins and genetic organization in bacteriophage: the example of gene Q of bacteriophage λ . *Genetics* **83**: 5–10.
- Isberg, R.R. and M. Syvanen. 1981. Replicon fusions promoted by the inverted repeats of Tn5. *J. Mol. Biol.* **150**: 15–32.
- Isberg, R.R., A.L. Lazaar, and M. Syvanen. 1982. Regulation of Tn5 by the right repeat proteins: Control at the level of the transposition reactions? *Cell* **30**: 883–892.
- Johnson, R.C. 1983. "Transposon Tn5: Proteins and DNA sequences required for its transposition and control." Ph.D. thesis, University of Wisconsin-Madison, Madison, WI.
- Johnson, R.C. and W.S. Reznikoff. 1983. DNA sequences at the ends of transposon Tn5 required for transposition. *Nature* **204**: 280–282.
- . 1984. Copy number control of Tn5 transposition. *Genetics* **107**: 9–18.
- Johnson, R.C., J.C. Yin, and W.S. Reznikoff. 1982. Control of Tn5 transposition in *Escherichia coli* is mediated by protein from the right repeat. *Cell* **30**: 873–882.
- Kleckner, N. 1990. Regulation of transposition in bacteria. *Annu. Rev. Cell Biol.* **6**: 297–327.
- Krebs, M.P. and W.S. Reznikoff. 1986. Transcriptional and translational initiation sites of IS50. Control of transposase and inhibitor expression. *J. Mol. Biol.* **192**: 781–792.
- McFall, E. 1986. *cis*-Acting proteins. *J. Bacteriol.* **167**: 429–432.
- Mizuuchi, K. 1992. Transpositional recombination: Mechanistic insights from studies of Mu and other elements. *Annu. Rev. Biochem.* **61**: 1011–1051.
- Morisato, D., J.C. Way, H.-J. Kim, and N. Kleckner. 1983. Tn10 transposase acts preferentially on nearby transposon ends *in vivo*. *Cell* **32**: 799–807.
- Rothstein, S.J., R.A. Jorgensen, K. Postle, and W.S. Reznikoff. 1980. The inverted repeats of Tn5 are functionally different. *Cell* **19**: 795–805.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sasakawa, C., G.F. Carle, and D.E. Berg. 1983. Sequences essential for transposition at the termini of IS50. *Proc. Natl. Acad. Sci.* **80**: 7293–7297.
- Schulz, V.P. and W.S. Reznikoff. 1991. Translation initiation of IS50R read-through transcripts. *J. Mol. Biol.* **221**: 65–80.
- Scofield, S.R., J.J. English, and J.D.G. Jones. 1993. High level expression of the *Activator* transposase gene inhibits the excision of *Dissociation* in tobacco cotyledons. *Cell* **75**: 507–517.
- Studier, F.W., A.H. Rosenberg, J.J. Dunn, and J.W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**: 60–89.
- van der Mei, D., J. Zandberg and H.S. Jansz. 1972. The effect of chloramphenicol on synthesis of Φ X174-specific proteins and the detection of cistron A proteins. *Biochim. Biophys. Acta* **287**: 312–321.
- Weinreich, M.D., L. Mahnke, and W.S. Reznikoff. 1994. A functional analysis of the Tn5 transposase: Identification of domains required for DNA binding and dimerization. *J. Mol. Biol.* **241**: 166–177.
- Wiegand, T.W. and W.S. Reznikoff. 1992. Characterization of two hypertransposing Tn5 mutants. *J. Bacteriol.* **174**: 1229–1239.
- . 1994. Interaction of Tn5 transposase with the transposon termini. *J. Mol. Biol.* **235**: 486–495.
- Yin, J.C. and W.S. Reznikoff. 1988. p2 and inhibition of Tn5 transposition. *J. Bacteriol.* **170**: 3008–3015.
- Zhou, Y., X. Zhang, and R.H. Ebright. 1991. Random mutagenesis of gene-sized DNA molecules by use of PCR with Taq polymerase. *Nucleic Acids Res.* **19**: 6052.

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