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Genetics

Regulation of Tn*5* transposition in *Salmonella typhimurium*

(transposition inhibition/transposon/drug-resistance element/translocation)

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ABSTRACT **The drug-resistance element Tn*5* transposes with high frequency immediately after entry into a cell. Establishment of Tn*5* within a cell results in a decrease in this transposition frequency. This phenomenon resembles "zygotic induction" of repressible operons and prophages. Evidence is presented that Tn*5* transposition is under negative control by a factor encoded within the element itself. Established Tn*5* elements (that contain point mutations inactivating the resistance gene) are able to inhibit transposition of an incoming Tn*5* element by a factor of 12- to 70-fold. Several deletion derivatives of Tn*5* lack the ability to inhibit transposition.**

Transposable drug-resistance elements are short segments of DNA that code for resistance to one or more antibiotics and have the property of being able to insert in a *rec*-independent manner into new sites of the DNA. (See refs. 1–4 for reviews and collections of papers on this topic.) The ability of such elements to be transferred from cell to cell and to transpose into the chromosome of the recipient allows them to be added to the genome of new individuals, even individuals of a different species. These genes have attained a degree of autonomy; they can spread horizontally in populations and can cross species barriers. The molecular mechanism by which transposons move to new sites in the DNA remains unclear.

It seems reasonable that this transposition process might be regulated. Heffron and coworkers (5–7), and Chou *et al.* (8), have presented detailed evidence that transposon Tn3 possesses a mechanism for regulating its transposition activity. However, the biological role of this mechanism is uncertain because the mechanism has only been shown to operate in mutant transposons; the *in vivo* signals to which the mechanism responds are not known. We have recently found that transposition of Tn*5*, a transposable element that confers resistance to kanamycin (9–11), is under negative control of a *trans*-acting product encoded by Tn*5*. This control can be demonstrated for nonmutant elements, and the selective value of the mechanism is easily rationalized. It is not known whether this regulation is by repression of transposition functions or whether it occurs by direct interaction of an inhibitory factor with DNA sites or proteins necessary for transposition. Because of this uncertainty we refer to the activity as "transposition inhibition." Results similar to ours have recently been reported for transposon Tn*10* by Beck *et al.* (12).

MATERIALS AND METHODS

Media. Nutrient broth (Difco) containing 0.5% NaCl was used as rich medium. Solid medium contained 1.5% Difco agar. When necessary, this was supplemented with kanamycin sulfate (Sigma) at 75 μg/ml or tetracycline hydrochloride (Sigma) at 10 μg/ml. Minimal medium was the E medium of Vogel and Bonner (13), which when necessary was supplemented with kanamycin sulfate at 125 μg/ml. Histidine was used at a concentration of 0.1 mM in minimal plates. Minimal lactose plates contained the NCE medium described by Berkowitz *et al.* (14) with 0.2% lactose.

Phage Growth. Transductional crosses were performed by using a mutant of phage P22 (HT105/2) that transduces with high frequency (15) and that contains an *int*$^-$ mutation (isolated by G. Roberts). Phage were grown according to the procedure of Hoppe *et al.* (16).

Bacterial Strains. All strains used are derivatives of *Salmonella typhimurium* LT2. *Escherichia coli* strains carrying F′ *lac*::Tn*5* were kindly donated by Doug Berg. Table 1 lists strains used.

Isolation of Mutants of Tn*5*. (*i*) Kanamycin-sensitive (Kan^S) point mutants were isolated by localized hydroxylamine mutagenesis of P22 generalized transducing phage grown on TT2713 (*hisC9642*::Tn*5*) according to the methods of Hong and Ames (17). Mutagenized phage was used to transduce deletion strain *his-644* to growth on histidinol (requiring inheritance of the donor *hisD* gene), and the transductants were replica-printed to plates containing kanamycin in order to identify Kan^S clones. (*ii*) Deletion mutants were isolated by selection for derivatives of a *hisG*::Tn*5* strain that had regained expression of the *hisD* gene. The *hisD* gene is located promoter-distal to *hisG*; its product catalyzes the conversion of histidinol (Hol) to histidine. Deletion mutations could allow *hisD* expression either by fusion of the *hisD* gene to Tn*5* transcription units or by removing the polar effect that Tn*5* exerts on histidine operon expression. Cultures of TT2616 (*hisG9648*::Tn*5*) were spread on histidinol plates, and Hol$^+$ (His$^+$) revertants were picked after 2–3 days. In addition, Hol$^+$ revertants were selected by using a strain containing a Tn*10* element promter-proximal to Tn*5* (TT3230). With this strain, most Hol$^+$ derivatives result from fusion of the *hisD* gene to Tn*5* transcription units.

All of the Tn*5* mutations used in further experiments were placed in a common genetic background by transduction into deletion *his-3050* (selecting Hol$^+$). These strains were then made *rec*$^-$ by transduction to tetracycline resistance with phage grown on TT520 (*srl-202*::Tn*10*) followed by transduction to sorbitol utilization with phage grown on TR2951 (*recA1*). Exceptions are strains TT4150 and TT4151, which were first transduced to tetracycline resistance with phage grown on TT521 (*srl-202*::Tn*10 recA1*) followed by transduction to sorbitol utilization with phage grown on LT2. (*rec* function is transiently provided by the donor fragment.)

Mapping and Characterization of the *neo* Mutations. The Tn*5* *neo* gene specifies resistance to the aminoglycoside antibiotics neomycin and kanamycin. To construct a map of the *neo* gene, Hol$^+$ revertants of *hisG9648*::Tn*5* that had become

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Abbreviations: Kan^S and Kan^R, kanamycin-sensitive and -resistant, respectively; Hol$^+$, histidinol-utilizing; Lac$^+$, lactose-utilizing; His$^+$ histidine-independent.

Table 1. Multiply marked strains

Strain	Genotype	Source
TT3230	*hisG8543*::Tn*10 hisG9648*::Tn*5*	This laboratory
TT2677	*hisG9635*::Tn*5 hisT1504* (*hisG1102*?)	This laboratory
TT2715	*hisD9644*::Tn*5 hisT1504* (*hisG1102*?)	This laboratory
TT4580	*his-646 hisC*9901::(Tn*5 lac*$^+$ Tn*5*)	This laboratory
TT4582	*his-646 hisC9901*::(Tn*5 lac*$^+$ Tn*5*)	This laboratory
TT4632	*proAB47 recA1*	This paper
TT4154	*hisG9648*::Tn*5 recA1*	This paper
TT4634	*hisG9648*::Tn*5* (*neo-17*) *proAB47 recA1*	This paper
TT4155	*hisC9642*::Tn*5* (*hisG1102*?) *recA1*	This paper

TT4149–4153 (not listed above) are kanamycin-sensitive (Kan^S) derivatives of *hisG9648*::Tn*5* and are isogenic with TT4154 (above), while TT4085–4096 are Kan^S derivatives of *hisC9642*::Tn*5* and are isogenic with TT4155 (above). Refer to Table 2 for corresponding *neo* allele numbers. All strains containing the *recA1* mutation have genetic backgrounds isogenic with TT4630 (*recA1*).

sensitive to kanamycin were used as recipients in transductional crosses with P22 phage grown on *neo* point mutants isolated as described above. For these crosses, 0.1 ml of phage lysate ($\approx 10^9$ plaque-forming units) and 0.1 ml of fully grown recipient culture ($\approx 10^8$ cells) were plated nonselectively on nutrient broth plates, incubated overnight, and then replica-printed to kanamycin-containing plates. In addition, Hol$^+$ deletions that extend outside the Tn*5* element were mapped by transductional crosses with *hisG* point mutants [described by Hoppe *et al.* (16)]. The Tn*5* elements in TT2713 (*hisC9642*::Tn*5*) and TT2616 (*hisG9648*::Tn*5*) are in the same orientation in closely linked *his* genes. Thus, for genetic mapping, transductional crosses can be performed between deletion mutants of one Tn*5* and point mutations in the other. These crosses are possible even for deletions extending outside of the Tn*5* element.

All *neo* mutations were characterized as to reversion frequency (data not shown), and hydroxylamine-induced *neo* mutations were characterized as to suppressibility by nonsense suppressors (*neo-5* and *neo-6* are ambers). Complementation tests indicated that all point mutations affect a single complementation group (data not shown).

Determination of Tn*5* Transposition Frequency. (*1a*) *Transduction of Tn5 into a* rec$^+$ *recipient.* P22 phage grown on auxotrophic Tn*5* insertion mutants was used to transduce LT2 to kanamycin resistance. Phage ($\approx 10^9$ plaque-forming units) and recipient cells ($\approx 10^8$) were plated together nonselectively on nutrient broth plates and then replica-printed to nutrient plates containing kanamycin after overnight growth. Transductants were patched on the same medium and then replica-printed to minimal kanamycin plates in order to identify prototrophs. Prototrophic, kanamycin-resistant (Kan^R) transductants are assumed to have arisen by transposition of Tn*5* because they did not inherit the auxotrophic requirement of the donor. (See *Results* for support of this assumption.) (*1b*) *Transduction of* rec$^+$ *recipients containing Tn5 insertions to prototrophy.* P22 phage grown on LT2 was used to transduce Tn*5* insertion auxotrophs to prototrophy on minimal medium. Transductants were patched on the same medium, grown overnight, and then replica-printed to plates containing kanamycin in order to identify the transductants that retained resistance to kanamycin. Prototrophic Kan^R transductants are assumed to be the result of transposition of Tn*5* to a new site. Such Kan^R transductants are fairly rare ($\approx 0.1\%$), and when observed they have been checked for stability of their prototrophic phenotype. Those that were unstable (i.e., segregated auxotrophs of the parental type) were scored as duplications rather than transpositions (18). (*2*) *Transposition of Tn5 into* rec$^-$ *recipients.* Transductions were performed by preincubating 0.1 ml of fully grown *recA*$^-$ recipient culture and 0.1 ml of P22 phage lysate (that had been suitably diluted to give 100–300 Kan^R transductants) for 1½ hr (at room temperature) and then plating 0.1 ml on nutrient plates containing kanamycin. The number of Kan^R transductants (transposition events) was scored after 36 hr. In some crosses the recipients carried a Tn*5* element with a *neo* (Kan^S) mutation. Kan^R transductants are assumed to have arisen by Tn*5* transposition, because homologous recombination is eliminated by the *recA* mutation. (See *Results* for evidence supporting this contention.) (*3*) *Transduction of a transposable* lac *region into* rec$^-$ *recipients.* A transposable *lac* operon was utilized that consists of the *E. coli lac* genes flanked by Tn*5* insertions, the entire unit being capable of transposition (unpublished results). Such structures have been studied previously by Guarente *et al.* (19). The construction and characterization of compound *lac* transposons will be described elsewhere. The transductions were performed by plating $\approx 10^9$ phage and $\approx 10^8$ recipient cells directly on lactose plates. The number of lactose-utilizing clones was scored after 3 days. Because the recipients in these crosses are *recA*$^-$, transductants cannot arise by homologous recombination. They are assumed to arise by Tn*5*-mediated transposition. This is supported by the fact that all such lactose-utilizing (Lac$^+$) transductants are Kan^R, and Kan^R Lac$^+$ insertion auxotrophs have been obtained in such crosses (unpublished data).

RESULTS

High-Frequency Transposition of Tn*5*. The first evidence suggesting that Tn*5* transposition might be regulated was the observation that Tn*5* transposes at very high frequency when newly introduced into a recipient by transduction (unpublished results). By analogy with zygotic induction of temperate phages, this behavior suggested that when Tn*5* enters a cell lacking the putative repressor, transposition functions might be derepressed. Once Tn*5* is established in a strain, the transposition frequency is much lower, suggesting some reduction in expression of transposition functions.

The basic experimental situation for these initial observations is diagrammed in Fig. 1. At the left in Fig. 1, Kan^R transductants were selected and scored for auxotrophic requirements; those that inherited Tn*5* by homologous recombination inherited the donor's auxotrophic requirement. However, approximately 10% of these Kan^R transductants do not inherit the donor's auxotrophic requirement. This surprisingly frequent class of transductants arises when the donor Tn*5* transposes to a new site in the recipient chromosome. This conclusion is supported by the fact that $\approx 0.5\%$ of the "transposition transductants" contain insertions into genes that result in new auxotrophic requirements. It is also supported by the fact that such transductants arise in *rec*$^-$ recipient strains.

In contrast to this high frequency of transposition, an established Tn*5* element seems to be quite stable. This reduced transposition frequency was estimated by transducing a Tn*5* insertion auxotroph to prototrophy as diagrammed at the right in Fig. 1. Any recipient cell in which Tn*5* has transposed to a secondary site may be transduced to prototrophy and still retain its Kan^R phenotype. Recipients that have only the original Tn*5* element would become Kan^S when transduced to prototrophy, because the mutant site (including Tn*5*) would be replaced by donor material. This cross estimates the frequency within the

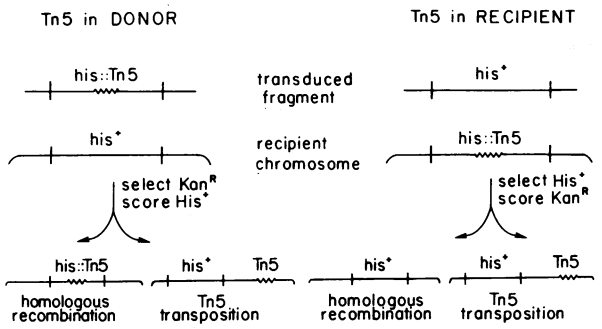

FIG. 1. Scoring transposition frequency of Tn*5*. (*Left*) P22 generalized transducing phage grown on a Tn*5* insertion auxotroph (e.g., *his*::Tn*5*) is used to transduce a prototrophic *rec*$^+$ strain to kanamycin resistance. The Kan^R transductants are then scored for the presence of the donor's auxotrophic requirement. If the incoming Tn*5* transposes, a histidine-independent (His$^+$) Kan^R transductant will arise. (*Right*) P22 phage grown on a prototrophic strain not containing Tn*5* is used to transduce a Tn*5* insertion auxotroph (e.g., *his*::Tn*5*) to prototrophy. The prototrophic transductants are scored for retention of their Kan^R phenotype. If the recipient cell carries a transposed Tn*5* element, it can give rise to a His$^+$ Kan^R transductant.

recipient population of cells that have acquired a secondary copy of Tn*5* by transposition.

Typical data from such crosses are presented in Table 2. It can be seen that the transposition frequency is much higher when Tn*5* is transferred from the donor into a new recipient strain. These crosses probably underestimate the actual differences in transposition frequency, because when Tn*5* is used as a donor only a short time is allowed for transposition to occur; when Tn*5* is in the recipient, the transposition event could occur at any time in the many generations since the strain was last cloned.

These results, although consistent with the hypothesis that Tn*5* transposition is regulated, could be interpreted in other ways because the transductions in which Tn*5* was used as a donor or as a recipient are not equivalent. When used as a donor, Tn*5* transposes from a linear transduced fragment; but when used as a recipient, Tn*5* probably transposes from a supercoiled molecule. The states of the DNA and the selection conditions are different in the two situations. The following experiments were performed to test the regulation hypothesis more stringently.

Table 2. Frequency of Tn*5* transposition in transductional crosses

Genotype (strain)	Fraction of recombinants undergoing transposition: Tn*5* in donor	Tn*5* in recipient
pyr::Tn*5* (TT4699)	0.12 ± 0.06	0.0020
trp::Tn*5* (TT4700)	0.09 ± 0.07	<0.0017
met::Tn*5* (TT4701)	0.04 ± 0.02	<0.0025
hisG::Tn*5* (TT2677)	0.09 ± 0.05	<0.0025
hisD::Tn*5* (TT2715)	0.12 ± 0.06	0.0023*
cys::Tn*5* (TT2726)	0.09 ± 0.04	<0.0020

With Tn*5* in the donor strain, P22 phage was used to transduce LT2 to kanamycin resistance. Transductants were scored for inheritance of the donor's auxotrophic requirement. Transposition frequency is expressed as the fraction of the Kan^R transductants that are prototrophic. With Tn*5* in the recipient strain, P22 phage grown on LT2 was used to transduce various *rec*$^+$ insertion auxotrophs to prototrophy. Transductants were scored for sensitivity to kanamycin. Transposition frequency is expressed as the fraction of the prototrophic transductants that remain Kan^R. At least 100 transductants were scored for each cross; the results represent mean and standard deviation for four or more crosses.

* Of 440 His$^+$ transductants, one remained Kan^R; this one was not checked for being a duplication.

Negative Effect of Tn*5* on its own Transposition. The basic experiment consists of transducing Tn*5* into recipient cells that already contain a Tn*5* element. If Tn*5* produces an inhibitor of its own transposition, the Tn*5*-carrying recipient should contain this inhibitor and the donor Tn*5* element should transpose at much lower frequency than it does in recipients containing no Tn*5* element. To permit selection for Kan^R transductants, the recipient cells carry Tn*5* elements with mutations that inactivate the resistance determinant (*neo*). To ensure that such mutations do not also affect genes involved in transposition, point mutations were selected after hydroxylamine mutagenesis. Spontaneously arising *neo* mutations would be expected to often result from deletion or rearrangement of extensive regions of Tn*5* [unpublished results, and by analogy to the findings of Kleckner and Ross for Tn*10* (20)]. A map of these *neo* mutations is shown in Fig. 2. Isolation and genetic mapping of these Tn*5* mutations is discussed in *Materials and Methods*.

The transposition frequency of the donor Tn*5* was measured by using recipient strains carrying the *recA1* mutation and a Tn*5* element with one of the *neo* mutations shown on the map in Fig. 2; donor phage was grown on Kan^R Tn*5* insertion auxotrophs. Selection was made for kanamycin resistance. The results of these crosses are presented in Table 3.

Because all recipients are *rec*$^-$, Kan^R transductants cannot arise by homologous recombination events. Kan^R transductants can be obtained only by transposition of the donor Tn*5* to a chromosomal location. This contention is supported by the fact that 0.3–1% of the Kan^R transductants are auxotrophic for a new requirement as a result of Tn*5* insertion into a gene. (Use of a *rec*$^-$ recipient also prevents detection of transpositions that preexist in the donor population prior to growing phage; these could be inherited by homologous recombination.) *rec*-independent formation of specialized P22 lysogens does not contribute to the number of Kan^R transductants observed as evidenced by the fact that the frequency of Kan^R transductants isolated does not decrease in an isogenic strain containing an

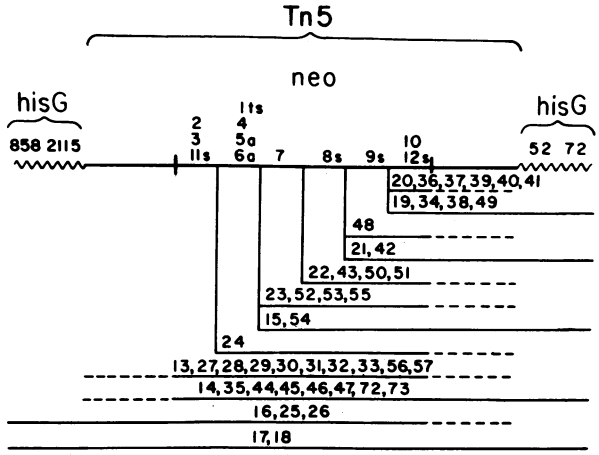

FIG. 2. Deletion map of the kanamycin resistance gene (*neo*) of Tn*5*. Tn*5* is shown as a solid line, while adjacent chromosomal *his* sequences are represented by wavy lines. The *neo* gene is not drawn to scale with respect to the entire element. Dashed lines delineate the possible extent of deletions. Several *hisG* point mutations adjacent to the site of *hisG9648*::Tn*5* that were used in mapping deletions extending outside the element are indicated. Mutations *neo-35* through *neo-47* and *neo-52* through *neo-55* were isolated in a strain (TT3230) that has a Tn*10* insertion within the *hisG* gene, promoter proximal to Tn*5*. The type of point mutation, where known, is indicated (a, amber; ts, temperature sensitive; s, stable).

Table 3. Frequency of Tn5 transposition in Tn5(*neo*^{S}) recipients

Recipient strain	*neo* allele	Relevant genotype	Transposition frequency						
			pyr::Tn5	*trp*::Tn5	*cys*::Tn5	*hisD*::Tn5	*hisG*::Tn5	Mean†	EOP*
TT4630	—	No Tn5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TT4085	2	Tn5 *neo* p	0.04 (5)	0.08 (4)	0.07 (4)	0.05 (8)	0.06 (9)	0.06 ± 0.05	1.0
TT4086	11	Tn5 *neo* p	0.06 (4)	0.04 (3)	0.03 (3)	0.04 (6)	0.08 (6)	0.05 ± 0.04	1.0
TT4087	3	Tn5 *neo* p	0.06 (5)	0.08 (4)	0.09 (4)	0.04 (8)	0.02 (8)	0.05 ± 0.04	1.1
TT4089	4	Tn5 *neo* p	0.06 (3)	0.08 (3)	0.08 (3)	0.06 (7)	0.08 (8)	0.07 ± 0.04	1.1
TT4090	5	Tn5 *neo* a	0.01 (4)	0.01 (4)	0.02 (4)	0.01 (8)	0.01 (8)	0.01 ± 0.01	1.2
TT4091	6	Tn5 *neo* a	0.04 (3)	0.03 (1)	0.02 (3)	0.04 (4)	0.06 (4)	0.04 ± 0.02	1.1
TT4092	7	Tn5 *neo* p	0.04 (4)	0.08 (3)	0.09 (4)	0.05 (8)	0.05 (9)	0.06 ± 0.05	1.2
TT4093	8	Tn5 *neo* p	0.07 (4)	0.05 (3)	0.06 (5)	0.05 (8)	0.06 (10)	0.06 ± 0.04	1.1
TT4094	9	Tn*t neo* p	0.06 (4)	0.07 (4)	0.05 (4)	0.06 (8)	0.06 (9)	0.06 ± 0.03	1.1
TT4095	10	Tn5 *neo* p	0.01 (3)	0.03 (1)	0.02 (3)	0.02 (3)	0.02 (3)	0.02 ± 0.01	1.1
TT4096	12	Tn5 *neo* p	0.04 (4)	0.06 (4)	0.06 (4)	0.05 (8)	0.07 (9)	0.06 ± 0.03	1.1
TT4149	14	Tn5 Δ	0.29 (4)	0.39 (3)	0.19 (3)	0.41 (7)	0.37 (7)	0.35 ± 0.29	1.0
TT4150	15	Tn5 Δ	0.45 (3)	1.65 (4)	0.99 (4)	0.94 (4)	0.93 (7)	1.00 ± 0.68	1.1
TT4151	16	Tn5 Δ	0.24 (3)	0.53 (2)	0.18 (3)	0.34 (4)	0.37 (4)	0.32 ± 0.17	1.1
TT4153	18	Tn5 Δ	0.31 (4)	0.90 (4)	0.62 (4)	0.51 (7)	0.63 (8)	0.59 ± 0.31	1.0
TT4152	17	Tn5 Δ	0.36 (4)	0.86 (4)	0.53 (5)	0.54 (7)	0.47 (8)	0.53 ± 0.30	0.9
TT4634	17	Tn5 Δ *ataA* Δ	0.44 (3)	0.49 (2)	0.37 (2)	0.41 (4)	0.42 (5)	0.42 ± 0.20	0.9
TT4632	—	No Tn5 *ataA* Δ	0.56 (3)	0.63 (3)	0.43 (3)	0.68 (5)	0.82 (6)	0.66 ± 0.32	1.0

P22 phage grown on the indicated five Tn5 insertion auxotrophs was used to transduce *recA1* recipients containing various Tn5(*neo*^{S}) mutations (p, point; a, amber; Δ, deletion) to kanamycin resistance. Donor strains are TT4699 (*pyr-2380*::Tn5), TT4700 (*trp-2475*::Tn5), TT2726 (*cys-1556*::Tn5), TT2715 (*hisD9644*::Tn5), and TT2677 (*hisG9653*::Tn5). Transposition frequency is expressed as the number of Kan^R transductants (transposition events) for a given recipient relative to the number obtained with TT4630 (an isogenic recipient strain that does not contain Tn5). Each result is expressed as the mean of a number of determinations; the number of times a given cross was performed is indicated in parentheses. (In each experiment the recipient control without Tn5 gave 100–300 Kan^R transductants.) The frequencies listed for TT4085 and TT4089 may be slightly higher than actual values, because the *neo* mutations in these strains revert at frequencies detectable in these crosses.

* The frequencies have not been corrected for slight differences of recipients as phage hosts; efficiency of P22 plating (EOP) relative to TT4630 for each recipient is presented.

† Effect of a given Tn5(*neo*^{S}) recipient on transposition of Tn5; expressed as the mean transposition frequency ± SD for the five Tn5 donors in a given recipient relative to TT4630. Standard deviations were calculated by using all the individual experimental values (not the averaged values as reported for each donor).

ataA deletion, which removes the P22 attachment site. The frequency of auxotrophs recovered is also unaffected by the *ataA* deletion (≈1.0% of the Kan^R transductants of TT4630 are auxotrophic, while ≈0.67% of the Kan^R transductants of TT4632, an isogenic *ataA* deletion derivative, are auxotrophic). The most direct evidence that these transductants are due to Tn5 transposition was obtained in an experiment in which 30 such transductants were analyzed by Southern blot hybridization of *Eco*RI endonuclease digests with a Tn5 probe; these transductants were found to have Tn5 homology on fragments of many different sizes (data not shown).

It is clear from the results in Table 3 that the frequency of Tn5 transposition is decreased in recipient strains that contain a Tn5 element with a resistance gene point mutation. This effect is seen to range from 12- to 70-fold, depending on the particular Tn5 *neo* mutant used (when expressed as the average value for 5 Tn5 insertion donors). The reason for this variability is not known.

To rule out the possibility that differences in expression of kanamycin resistance somehow produce artifactual decreases in transposition frequency in certain strains, an experiment was devised that does not depend on this selection. Strains containing a transposable *lac* operon, consisting of the *E. coli lac* genes flanked by Tn5 elements, were used as donors in transductions with the same recipients used in Table 3 above. In addition, isogenic strains containing the wild-type parent Tn5 insertions were used as recipients. In these crosses selection was made for lactose utilization rather than kanamycin resistance. The results of this experiment are listed in Table 4; recipient Tn5 elements inhibit transposition of this compound *lac* transposon.

Location of the Putative Inhibitor Gene Within Tn5. Unlike point mutations in the *neo* gene, the deletion mutations tested destroyed the inhibitory effect Tn5 normally exerts on transposition of an incoming Tn5 element (see, for example, TT4149–4151 in Table 3). The reason for the small decrease in transposition frequency (2- to 3-fold) in some of these crosses is not clear, but a similar decrease is observed with two deletion strains (*neo-17* in TT4152, *neo-18* in TT4153) that remove all of Tn5 (see Fig. 2 and Table 3). Deletion *neo-15* has its left endpoint within the *neo* gene of Tn5 and extends out of Tn5 into adjacent chromosomal material; this deletion removes the inhibitory effect of Tn5 on transposition of Tn5 from transduced fragments (see Table 3). It is likely that the region of Tn5 to the right of the *neo* gene (in Fig. 2) is essential for the production of the transposition inhibitor.

DISCUSSION

We have shown that the transposition of Tn5 in transductional crosses is negatively affected by the presence of a Tn5 element in the recipient. This behavior is analogous to the zygotic induction phenomenon seen for negatively controlled systems such as phage λ and the *lac* operon (21, 22). The observation suggests that Tn5 encodes a factor that inhibits its transposition. The regulatory factor might affect the synthesis of a protein needed for transposition, or it could interact directly with sites involved in transposition to prevent their function. Results of others (6, 7, 11, 23) indicate that transposition of at least some transposable drug-resistance elements, including Tn5, involves functions encoded within the transposable element. If the inhibitor of Tn5 transposition acts by repression, these genes would be likely candidates for regulation. Alternatively, the

Table 4. Frequency of Tn5-mediated *lac* transposition

Recipient strain	*neo* allele	Relevant genotype	Transposition frequency TT4580	Transposition frequency TT4582
TT4630	—	No Tn5	1.0	1.0
TT4155	—	*hisC*::Tn5 (Kan^R)	0.17 ± 0.04 (4)	0.06 ± 0.02 (5)
TT4085	2	Tn5 *neo* p	0.15 ± 0.04 (5)	0.15 ± 0.04 (4)
TT4086	11	Tn5 *neo* p	0.19 ± 0.07 (4)	0.12 ± 0.04 (5)
TT4087	3	Tn5 *neo* p	0.10 ± 0.04 (4)	0.07 ± 0.04 (3)
TT4089	4	Tn5 *neo* p	0.16 ± 0.05 (5)	0.11 ± 0.04 (4)
TT4090	5	Tn5 *neo* a	0.03 ± 0.02 (5)	0.04 ± 0.04 (4)
TT4091	6	Tn5 *neo* a	0.15 ± 0.02 (3)	0.10 ± 0.06 (4)
TT4092	7	Tn5 *neo* p	0.19 ± 0.12 (5)	0.15 ± 0.08 (4)
TT4093	8	Tn5 *neo* p	0.12 ± 0.04 (5)	0.12 ± 0.08 (4)
TT4094	9	Tn5 *neo* p	0.12 ± 0.02 (5)	0.11 ± 0.01 (4)
TT4095	10	Tn5 *neo* p	0.06 ± 0.05 (3)	0.07 ± 0.01 (4)
TT4096	12	Tn5 *neo* p	0.09 ± 0.03 (5)	0.09 ± 0.05 (4)
TT4154	—	*hisG*::Tn5 (Kan^R)	0.10 ± 0.02 (3)	0.09 ± 0.01 (3)
TT4149	14	Tn5 Δ	1.08 ± 0.51 (4)	1.10 ± 0.32 (5)
TT4150	15	Tn5 Δ	0.69 ± 0.13 (4)	0.83 ± 0.17 (3)
TT4151	16	Tn5 Δ	1.10 ± 0.64 (3)	0.71 ± 0.30 (4)
TT4152	17	Tn5 Δ	0.76 ± 0.14 (4)	0.74 ± 0.19 (4)
TT4153	18	Tn5 Δ	0.85 ± 0.23 (4)	0.89 ± 0.23 (4)

P22 phage grown on the indicated two donor strains carrying Tn5-*lac*-Tn5 transposons was used to transduce the same recipients listed in Table 3, as well as recipients carrying a wild-type Tn5 (Kan^R) to Lac^+. Transposition frequency is expressed as the frequency of Lac^+ transductants for a given cross relative to the control strain lacking Tn5 (TT4630). The frequencies were not corrected for the minor differences of P22 plating efficiency (see Table 3). The relative efficiency of P22 plaquing of strains TT4154 and TT4155 (not listed in Table 3) is 1.1 for each. Each result is the mean and SD of a number of experiments (the number of experiments being listed in parentheses) in which 100–400 Lac^+ transductants were obtained with TT4630 as recipient.

inhibitor could act directly on the transposition protein (transposase) or on DNA sites critical to transposition.

The possibility was considered that the inhibition effect of Tn5 might be exerted by long-range *cis* effects rather than in *trans*. There is precedent for this in that only one copy of TnA can be inserted in a single plasmid (24). We think this possibility is unlikely because (*i*) strains carrying multiple Tn5 elements are easily constructed and appear to be perfectly viable, and (*ii*) the inhibitory effect on transposition (into the chromosome) is exerted by Tn5 elements present on an F′ plasmid in the recipient cell.

It should be noted that the inhibited level of transposition of Tn5 when brought into a cell containing a resident Tn5 appears higher than the level seen for the resident chromosomal Tn5 insertion itself (as reported in Table 2). This could be due to preferential *cis* action of the inhibitor. It may also be the result of lifting of transposition inhibition by phage infection, or by an increased transposition frequency of Tn5 from transduced fragments relative to frequency of transposition from the chromosome (perhaps due to effects of supercoiling).

The possible evolutionary significance of a negatively regulated transposition mechanism is easily rationalized. Such a mechanism would allow a transposon to leave unstable DNAs (e.g., transduced fragments or plasmids subject to exclusion) and become stably associated with the cell genome at high frequency. Once the transposon is stably integrated into the chromosome (or into a compatible plasmid) transposition activity would be reduced. This could be advantageous to the transposon, because it would reduce the possibility of killing the host cell by gene inactivation or chromosome instability.

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1. Starlinger, P. (1980) *Plasmid* **3**, 241–259.
2. Kleckner, N. (1977) *Cell* **11**, 11–23.
3. Bukhari, A. I., Shapiro, J. A. & Adhya, S. L., eds. (1977) *DNA Insertion Elements, Plasmids and Episomes* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
4. Ford, N., ed. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43.**
5. Heffron, F., So, M. & McCarthy, B. J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6012–6016.
6. Gill, R., Heffron, F., Dougan, G. & Falkow, S. (1978) *J. Bacteriol.* **136**, 742–756.
7. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18**, 1153–1163.
8. Chou, J., Casadaban, M. J., Lemaux, P. J. & Cohen, S. N. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4020–4024.
9. Berg, D. E., Davies, J., Allet, B. & Rochaix, J. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3628–3632.
10. Berg, D. E. (1977) in *DNA Insertion Elements, Plasmids and Episomes*, eds. Bukhari, A. I., Shapiro, J. A. & Adhya, S. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 205–212.
11. Rothstein, S. J., Jorgensen, R. A., Postle, K. & Reznikoff, W. S. (1980) *Cell* **19**, 795–805.
12. Beck, C. F., Moyed, H. & Ingraham, J. L. (1980) *Mol. Gen. Genet.*, in press.
13. Vogel, H. & Bonner, D. (1956) *J. Biol. Chem.* **218**, 97–106.
14. Berkowitz, D., Hushon, J. M., Whitfield, H. J., Roth, J. R. & Ames, B. N. (1968) *J. Bacteriol.* **96**, 215–220.
15. Schmieger, H. (1972) *Mol. Gen. Genet.* **119**, 75–88.
16. Hoppe, I., Johnston, H. M., Biek, D. & Roth, J. R. (1979) *Genetics* **92**, 17–26.
17. Hong, J. S. & Ames, B. N. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 3158–3162.
18. Anderson, R. P. & Roth, J. R. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1083–1087.
19. Guarente, L. P., Isberg, R. R., Syvanen, M. & Silhavy, T. J. (1980) *J. Mol. Biol.* **141**, in press.
20. Kleckner, N. & Ross, D. G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1233–1246.
21. Jacob, F. & Wollman, E. (1954) *C. R. Hebd. Seances Acad. Sci.* **239**, 317.
22. Pardee, A. B., Jacob, F. & Monod, J. (1958) *C. R. Hebd. Seances Acad. Sci.* **246**, 3125.
23. Meyer, R., Boch, G. & Shapiro, J. A. (1979) *Mol. Gen. Genet.* **171**, 7–13.
24. Robinson, M. K., Bennett, P. M. & Richmond, M. H. (1977) *J. Bacteriol.* **129**, 407–414.