COPY NUMBER CONTROL OF Tn*5* TRANSPOSITION

REID C. JOHNSON[1] AND WILLIAM S. REZNIKOFF[2]

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Manuscript received October 15, 1983
Revised copy accepted January 11, 1984

ABSTRACT

Transposition of Tn*5* in *Escherichia coli* strains containing one or multiple copies of the transposable element was investigated. It was found that the overall frequency of transposition within a cell remained constant regardless of the number of copies of Tn*5* present in that cell. Experiments measuring the transposition frequency of differentially marked Tn*5*s confirmed that the frequency of transposition of an individual Tn*5* decreased proportionally with the total number of copies of the element present in a cell. The IS*50R*-encoded function, protein 2, which has previously been shown to be an inhibitor of transposition, is sufficient to mediate this inhibitory effect. The concentration of protein 2 in a cell appears to modulate the transposition of individual Tn*5* elements in such a way that the overall transposition of Tn*5* in a cell remains constant.

TRANSPOSONS are segments of DNA that have evolved the capacity to translocate from one location in a cell's genome to another (for a review see KLECKNER 1981). In the case of simple IS (Insertion Sequence) elements, no obvious advantageous phenotypic trait is conferred to the cell, and almost the entire coding capacity is often taken up by functions that promote and in some cases modulate their transposition. When IS sequences flank a segment of DNA encoding a function, such as an antibiotic resistance gene, the entire region can transpose in a coordinate manner. Such units have been termed composite transposons. It is understandable how IS sequences survived once they became responsible for promoting the translocation of a selectable gene that can increase the fitness or survival of a cell. It is perhaps less understandable how these entities evolved prior to the introduction of a selectable marker. Recent studies comparing the growth in chemostat cultures of strains containing Tn*5* or Tn*10* with their nontransposon counterparts have suggested that transposable elements may provide some beneficial function or generate beneficial mutations which provide a selective advantage to their host (BIEL and HARTL 1983; CHAO *et al.* 1983). An additional possibility was suggested by DOOLITTLE and SAPIENZA (1980) and ORGEL and CRICK (1980) who have postulated that transposable elements may have evolved as DNA parasites on the cell. In the extreme interpretation of this model, a transposable element

[1] Present address: Biology Division, California Institute of Technology, Pasadena, California 91125.
[2] To whom reprint requests should be sent.

may want to transpose at a high rate which will allow it to proliferate at its maximum potential. On the other hand, each transposition event runs a rather high risk of introducing a detrimental mutation on the cell which would consequently be deleterious to the survival of the transposon. Therefore, one might predict that transposable elements would have evolved some sort of balance where the transposition rate could be maintained at a level to ensure its survival and dispersal but not lead to a high risk of introducing multiple mutations or become a load on the replication machinery of its host.

Tn*5* is an example of a compound transposable element which contains an antibiotic resistance determinant to neomycin and kanamycin flanked by inverted copies of IS*50* (BERG *et al.* 1975; JORGENSON, ROTHSTEIN and REZNIKOFF 1979). Tn*5* has previously been shown to encode an inhibitor of its own transposition as measured by the inhibition of transposition of an infecting Tn*5* by cells containing resident copies of the element (BIEK and ROTH 1980; ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982). This inhibitory function was found to be mediated by a protein product from one of the IS*50* elements, IS*50R*. Further analysis revealed that the smaller but more abundant protein synthesized from IS*50R*, protein 2, was sufficient to mediate the inhibition (ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982). Protein 1, which contains an additional 40 amino acids at its *N*-terminus as compared with protein 2, was found to be absolutely required for the promotion of transposition (ROTHSTEIN *et al.* 1980a; ISBERG, LAZAAR and SYVANEN 1982; R. C. JOHNSON and W. S. REZNIKOFF, unpublished results). The comparable proteins from IS*50L*, proteins 3 and 4, do not contain either the transposase or the inhibitory activities due to a DNA sequence difference between the IS*50* elements which results in premature termination of the IS*50L* proteins with respect to the IS*50R* proteins (ROTHSTEIN *et al.* 1980b).

In this present study, we have investigated the regulation of Tn*5* under steady state conditions by measuring the combined and individual transposition rates when the number of copies of the element in a cell is varied. The results indicate that there is a fine control over the transposition of Tn*5* with regard to copy number which is, at least in part, mediated by the concentration of protein 2 in the cell.

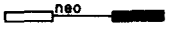
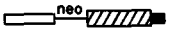
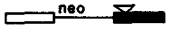
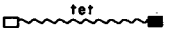
MATERIALS AND METHODS

Media: Bacteria were standardly grown in LB (DAVIS, BOTSTEIN and ROTH 1980) which was supplemented with 10 mM $MgSO_4$ and 5 mM $CaCl_2$ when the cultures were to be used for phage titering or transductions. For transductions, well-saturated LB cultures were centrifuged and resuspended in λ dilution buffer (DAVIS, BOTSTEIN and ROTH 1980) plus 5 mM $CaCl_2$ and shaken at 37° for 30 min prior to phage infections. Cells were grown in superbroth (DAVIS, BOTSTEIN and ROTH 1980) for induction of λ lysogens. Kanamycin was used at 25 μg/ml (corrected for potency), tetracycline at 15 μg/ml, gentamycin at 2.5 μg/ml and naladixic acid at 30 μg/ml (all purchased from Sigma).

Bacterial strains, bacteriophage and plasmids: Table 1 lists the bacterial strains, phage and plasmids used in this study as well as the genotypes and the relevant structures of the Tn*5*-containing plasmids. These plasmids are all derivatives of ColE1 or pBR322. The F-derived pOX38-Gen plasmid was constructed by ligating a *Hin*dIII restriction fragment from pSK2 (S. KAGAN and J. DAVIES, unpublished results) into the unique *Hin*dIII site of pOX38 (GUYER *et al.* 1980). This

TABLE 1

Strains of bacteria phage and plasmids used in this study

Strains	Relevant markers/structure[a]	Source/derivation
Bacteria		
RZ102	F⁻ *recA56 rpsL*	JOHNSON, YIN and REZNIKOFF (1982)
RZ103	pOX38::Tn*5-102*/RZ102	Tn*5-102* transposed onto pOX38; transferred to RZ102
RZ104	pOX38::Tn*5-191*/RZ102	pOX38::Tn*5-191* (JOHNSON, YIN and REZNIKOFF 1982) transferred to RZ102
RZ211	Δ(*lac-pro*) *recA56 ara rpsL srl*	JOHNSON, YIN and REZNIKOFF (1982)
RZ212	pOX38-Gen/RZ211	This paper
RZ222	*polA* Δ(*lac-pro*) *ara nal rpsL*	Spontaneous λ^r (Mal⁻) in RZ221 (JOHNSON, YIN and REZNIKOFF 1982)
RZ432	*supB recA56* Δ (*lac-pro*) *metB argE araD rpoB nalA srl*	JOHNSON, YIN and REZNIKOFF (1982)
RZ433	pOX38::Tn*5-191*/RZ432	pOX38::Tn*5-191* transferred to RZ432
Hfl-1	*hfl-1 ser trp leu ilv lys rpsL*	BELFORT and WULFF (1971)
LE392	*supE supF*	L. ENQUIST
Phages		
λ*bbn*	λ*cI857 b515 b519 nin5 Sam7*	R. YOUNG
λ*bbn*::Tn*5-102*	λ*bbn*::Tn*5-102* (Kan^r)	Tn*5-102* transposed onto λ*bbn*
λ*bbn*::Tn*5-191*	λ*bbn*::Tn*5-191* (Tet^r)	Tn*5-191* transposed onto λ*bbn*
λi^{21}::Tn*5-341*	λi^{21} *nin5*::Tn*5-341* (Tet^r)	Tn*5-341* transposed onto λi^{21} (N. KLECKNER)
Plasmid		
pRZ102		JORGENSEN, ROTHSTEIN and REZNIKOFF (1979)
pRZ191		ROTHSTEIN *et al.* (1980a)
pRZ112		JORGENSEN, ROTHSTEIN and REZNIKOFF (1979)
pRZ174		ROTHSTEIN *et al.* (1980a)
pRZ149		ROTHSTEIN *et al.* (1980a)
pRZ204		ROTHSTEIN and REZNIKOFF (1981)
pRZ201		ROTHSTEIN and REZNIKOFF (1981)
pRZ236		ROTHSTEIN and REZNIKOFF (1981)
pRZ401		JOHNSON and REZNIKOFF (1981)
pRZ352		JOHNSON, YIN and REZNIKOFF (1982)
pRZ320		JOHNSON, YIN and REZNIKOFF (1982)

[a] The structure of Tn*5* is diagrammed where appropriate. Solid bars: DNA from IS*50R*. Open bars: DNA from IS*50L*. Hatched bars: deleted DNA. Wavy line: non-Tn*5* DNA. Δ indicates a short deletion.

plasmid was then transferred by conjugation into RZ211 to give RZ212. Tn*5-341* is similar to Tn*5-191* except that it contains only the outer 56 base pairs (bp) of IS*50L* as opposed to Tn*5-191* which contains the outer 118 bp of IS*50L* (JOHNSON and REZNIKOFF 1983).

λ *induction assay for transposition*: The λ induction assay was similar to that described by ROTHSTEIN *et al.* (1980a). Tn*5*-containing cells which were lysogenic for λ*bbn* were thermoinduced at midexponential growth phase. Approximately 5×10^7 phage from the lysates were mixed with 5×10^8 Hfl-1 cells and incubated at 30° for 60 min. The number of resulting antibiotic-resistant transductants upon plating was compared to the number of plaque-forming units of the phage as assayed on LE392. To correct for variation in the transduction frequency using different antibiotic resistances, parallel reconstruction assays were performed. About 5×10^2 λ*bbn*::Tn*5-102* (Kan^r) or λ*bbn*::Tn*5-191* (Tet^r) phage were mixed with 5×10^7 λ*bbn* phage and lysogenized. The lysogeny frequency varied between 10 and 30%, and the appropriate value was used to standardize the transposition assays to 100% lysogenization.

Mating-out assay for transposition: One-tenth milliliter of fresh overnight cultures of RZ212 λi^{21}::Tn*5-341*, which contained pOX38-Gen plus one or more Tn*5*s, and RZ221 (*polA*, *nalA*) was mixed with 1 ml of LB and gently shaken for 6 hr. The number of Tn*5*-containing exconjugants (Kan^r or Tet^r, Gen^r, Nal^r), in which the Tn*5* had transposed from the λ or the ColE1 onto the F-derived plasmid pOX38-Gen, was compared to the total number of exconjugants (Gen^r, Nal^r) to give the transposition frequency.

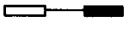
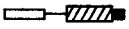
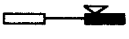
RESULTS

*Transposition remains constant independent of the number of copies of Tn*5: We first observed that the transposition frequency of Tn*5* was similar in cells containing either one or multiple copies of the element. This result is illustrated in Tables 2, 3 and 4, where the transposition frequency in cells containing a single copy of Tn*5* located on an F plasmid (Table 2) or on a λ prophage (Table 3) is compared to cells containing additional copies of Tn*5* on a multicopy plasmid. Little difference is observed in the overall transposition frequency in any of these experiments indicating that a constant rate of transposition is occurring in *E. coli* cells regardless of the number of copies of the element present.

To follow the transposition frequency of an individual element within a population of elements, we introduced multiple copies of Tn*5* which conferred a different antibiotic resistance than the resident element. In the experiment in Table 2, transposition of the single-copy Tn*5-191* (Tet^r) was compared with the transposition rate of multiple copies of Tn*5-102* (Kan^r). Although the sum of the transposition events remained equivalent to a cell containing only one copy of Tn*5-191*, Tn*5-191* transpositions made up only about 6% of the total transpositions in the presence of multiple copies of Tn*5-102*. This result is consistent with the relative proportion of Tn*5-191* in the cells containing 20–25 additional copies (the copy number of ColE1; CLEWELL and HELINSKI 1972) of Tn*5-102*; Tn*5-191* makes up 5–10% of the total number of Tn*5* elements under these conditions. (The copy number of Tn*5-102* is assumed to be approximately the same as ColE1 as determined by CLEWELL and HELSINKI 1972). It does not appear to be a reflection of the experimental conditions (*e.g.*, the sequences surrounding the transposon) since the same result occurs when the antibiotic resistance markers are reversed on the Tn*5*s residing on the F and colicin plasmids as in Table 3 or when a mating-out assay is performed with the single-copy Tn*5* located on the chromosome as a λ prophage (Table 4).

TABLE 2

λ induction assays in RZ104 (pOX38::Tn5-191)

Plasmid	Structure[b]	Transposition frequency[a] Tn5 element	Transposition/ pfu[c]	% Transposition[d]	Total[e]
ColE1		Tn*5-191*	2.5×10^{-5}	100	
pRZ191		Tn*5-191*	2.6×10^{-5}	104.4	
pRZ102		Tn*5-191*	1.6×10^{-6}	6	102
		Tn*5-102*	2.4×10^{-5}	96	
pRZ149		Tn*5-191*	1.1×10^{-6}	4.4	
pRZ112		Tn*5-191*	2.2×10^{-5}	88	
pRZ174		Tn*5-191*	2.3×10^{-5}	92	
pRZ201		Tn*5-191*	2.4×10^{-5}	96	
pRZ236		Tn*5-191*	7.7×10^{-7}	3.1	
pRZ401		Tn*5-191*	1.6×10^{-6}	6.4	
pRZ352		Tn*5-191*	6.5×10^{-8}	0.3	

[a] The assay measures the transposition frequency of the single-copy Tn*5* element Tn*5-191* or Tn*5-102* located on the F-derived plasmid pOX38 in the presence of Tn*5* mutants on multicopy plasmids. The transposition frequency of the transposon in multiple copies is also given where appropriate.

[b] Structure of the Tn*5* contained on the multicopy plasmid. Solid bars: DNA from IS*50R*. Open bars: DNA from IS*50L*. Hatched bars: deleted DNA. Wavy line: non-Tn*5* DNA.

[c] Transpositions per plaque-forming unit (pfu) measured in RZ104 λ*bbn* (pOX38::Tn*5-191*).

[d] Compares the transposition frequency relative to cells containing ColE1.

[e] Sum of the percent transpositions of both transposons.

*Multiple copies of IS*50R *provide the inhibition*: It was found that a functional IS*50R* was sufficient to mediate the inhibition, whereas IS*50L* had little effect. This is most directly illustrated by comparing the effects on transposition of the single-copy element by multiple copies of IS*50R* which is contained on pRZ149 or pRZ204 *vs.* multiple copies of IS*50L* which is contained on pRZ201 in Tables 3 and 4. pRZ149 or pRZ204 are equally as effective as pRZ102 (ColE1::Tn*5*-wild type) in inhibiting the transposition of a single-copy element, whereas pRZ201 has no significant effect. Derivatives of Tn*5* which leave both of the outer ends (target sites for the transposase) but carry deletions removing the coding sequence for the IS*50R* proteins such as Tn*5-112* or Tn*5-320* are similarly inactive. In addition, an insertion into the *Hin*dIII site of IS*50R* (Tn*5-174*, Table 2), which has previously been shown to inactivate both IS*50R* proteins (ROTHSTEIN *et al.* 1980a), results in the inability of the element to provide inhibition.

pRZ236 maps the difference between IS*50R* and IS*50L* with respect to copy number control to be located between the *Pvu*II and the *Bgl*II restriction sites of IS*50* (located at nucleotides 1424 and 1516, respectively, from the outer

TABLE 3

λ induction assays in RZ103 (pOX38::Tn5-102)[a]

Plasmid	Structure[b]	Transposition frequency			
		Tn*5* element	Transposition/ pfu[c]	% Transposition[d]	Total[e]
ColE1		Tn*5-102*	2.0×10^{-5}	100	
pRZ102		Tn*5-102*	2.1×10^{-5}	105	
pRZ191	tet	Tn*5-102*	1.9×10^{-6}	9.5	90
		Tn*5-191*	1.6×10^{-5}	80	
pRZ149		Tn*5-102*	1.2×10^{-6}	6.0	
pRZ201		Tn*5-102*	1.5×10^{-5}	75	
pRZ320		Tn*5-102*	1.9×10^{-5}	95	96
		Tn*5-320*	2.3×10^{-7}[f]	1.2	

[a] The assay measures the transposition frequency of the single-copy Tn*5* element Tn*5-191* or Tn*5-102* located on the F-derived plasmid pOX38 in the presence of Tn*5* mutants on multicopy plasmids. The transposition frequency of the transposon in multiple copies is also given where appropriate.

[b] Structure of the Tn*5* contained on the multicopy plasmid. Solid bars: DNA from IS*50R*. Open bars: DNA from IS*50L*. Hatched bars: deleted DNA. Wavy line: non-Tn*5* DNA.

[c] Transpositions per plaque-forming unit (pfu) measured in RZ103 λ*bbn* (pOX38::Tn*5-102*).

[d] Compares the transposition frequency relative to cells containing ColE1.

[e] Sum of the percent transpositions of both transposons.

[f] Transposition of Tn*5-320* is a result of complementation by Tn*5-102*.

end of IS*50*). pRZ236, which efficiently inhibits transposition of the single-copy Tn*5-191* (Table 2), is identical with pRZ201 (containing IS*50L*) except that the DNA between these two restriction sites was replaced with the analogous sequences from IS*50R*. This 92-bp region contains the sequence difference between the IS*50*s which results in an ochre codon in IS*50L* terminating these proteins 26 amino acids before the IS*50R* proteins (ROTHSTEIN *et al.* 1980b).

The concentration of protein 2 is responsible for controlling transposition in steady state conditions: These results imply that a product from IS*50R* is responsible for mediating the inhibition. A very likely candidate for this function is the previously identified IS*50R*-encoded inhibitor, protein 2, and the experiments that will be described support this conclusion. That a protein product is responsible is demonstrated by suppressing the ochre allele of IS*50L*. pRZ112 and pRZ201 are inactive in inhibiting transposition of a single-copy element in a *sup*^{0} background (Table 2) but are significantly active in a strain containing the *supB* ochre suppressor (Table 5). A mutant of IS*50R* demonstrates that protein 2 is sufficient for mediating copy number control. pRZ401 contains a frameshift mutation located within the unique 40 amino acids of protein 1 prior to the translation initiation region for protein 2. This mutation results in the synthesis of only protein 2, and, therefore, the mutant IS*50R* is unable

TABLE 4

Mating-out assay for copy number control[a]

Plasmid	Structure[b]	Transposition frequency			
		Tn*5* element	Transposition/ exconjugants[c]	% Transposition[d]	Total[e]
None		Tn*5-341*	2.1×10^{-5}	100	
pRZ102		Tn*5-341*	8.6×10^{-7}	4.1	142
		Tn*5-102*	2.9×10^{-5}	138	
pRZ149		Tn*5-341*	9.0×10^{-7}	4.3	
pRZ201		Tn*5-341*	2.2×10^{-5}	105	
pRZ352		Tn*5-341*	1.1×10^{-7}	0.5	

[a] The assay measures the transposition frequency of the single-copy element Tn*5-341*, which resides on a λ prophage, onto the conjugatable plasmid pOX38-Gen in the presence of Tn*5* mutants located on multicopy plasmids. The transposition frequency of the transposon in multiple copies is also given where appropriate.

[b] Structure of the Tn*5* contained on the multicopy plasmid. Solid bars: DNA from IS*50R*. Open bars: DNA from IS*50L*.

[c] Transpositions per exconjugant measured in RZ212 $\lambda i^{21} nin5$::Tn*5-341* containing the various plasmids as described in MATERIALS AND METHODS.

[d] Compares the transposition frequency relative to cells containing no additional Tn*5* sequences.

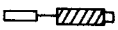
[e] Sum of the percent transpositions of both transposons.

to transpose (R. C. JOHNSON and W. S. REZNIKOFF, unpublished results). However, pRZ401 is equally as efficient in providing inhibitory function as pRZ149, which is the identical plasmid without the frameshift mutation.

Several lines of evidence indicate that the decrease in transposition is dependent upon the concentration of protein 2 present in the cell. pRZ352 contains the *lacPUV5* promotor driving the expression of protein 2. Under inducing conditions, it synthesizes eight to ten times more protein 2 than wild-type Tn*5* as measured by β-galactosidase activity when protein 2 is fused to *lacZ* (R. JOHNSON, unpublished results). As shown in Tables 2 and 4, transposition of a single-copy element in the presence of pRZ352 is less than 0.5% of its uninhibited transposition frequency, an effect significantly more severe than multiple copies of wild-type Tn*5* confer. The reduced inhibitory effect seen by suppressed IS*50L* as compared with IS*50R* is also consistent with the amount of protein 2 determining the amount of inhibition. *SupB*-suppressed IS*50L* proteins are synthesized at about 20% the rate of IS*50R* proteins (R. C. JOHNSON and W. S. REZNIKOFF, unpublished results). This level of synthesis correlates well with the data in Table 5 in which the transposition of the single-copy element was inhibited by suppression of IS*50L* to about 30% of its control value, as compared to less than 5% with multiple copies of IS*50R*. Interestingly, the sum of the transposition frequencies of the suppressed multiple copies of Tn*5-112* plus the single-copy Tn*5-191* in Table 5 is approximately equivalent

TABLE 5

λ induction assays in RZ433 (pOX38::Tn5-191 supB)

Plasmid	Structure	Transposition frequency			
		Tn*5* element	Transposition/ pfu	% Transposition	Total
ColE1		Tn*5-191*	1.3×10^{-5}	100	
pRZ102		Tn*5-191*	4.8×10^{-7}	3.7	81
		Tn*5-102*	1.0×10^{-5}	77	
pRZ112		Tn*5-191*	3.5×10^{-6}	27	90
		Tn*5-112*	8.2×10^{-6}	63	
pRZ204		Tn*5-191*	1.3×10^{-7}	1.0	
pRZ201		Tn*5-191*	3.8×10^{-6}	29	

The conditions were the same as described in Table 2 except that the transpositions were performed in the ochre-suppressing strain RZ433 (pOX38::Tn*5-191 supB*) λ*bbn*.

to the transposition of Tn*5-191* in the absence of additional copies of Tn*5* or Tn*5-191* plus multiple copies of wild-type Tn*5*. This result indicates that, although the overall transposition rate in a cell remains constant, the rates of individual elements within a population can be varied by changing the relative amounts of IS*50R* proteins that they synthesize.

DISCUSSION

Previous studies on the regulation of Tn*5* transposition have measured the inhibitory effect that resident Tn*5*s confer on the transposition of an infecting Tn*5* that is introduced into the cell on a phage DNA vector (BIEK and ROTH 1980; ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982). In experiments with *E. coli*, multiple copies of Tn*5* (IS*50R*) reduced the transposition of the infecting Tn*5* about 100-fold as compared with transposition in a cell containing no Tn*5* sequences (ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982). The experiments described in this paper examine the control of Tn*5* transposition in a steady state situation. Under these conditions, we have shown that the transposition of a single resident Tn*5* is reduced about 20-fold in the presence of multiple copies of additional Tn*5* elements. Analysis of mutants of Tn*5* demonstrated, like the previous studies, that the inhibition was mediated by the level of protein 2 present in the cell. The consequence is a rather exquisite control on the transposition of a population of Tn*5* elements in a cell. Although the overall transposition frequency remains constant regardless of the number of copies of Tn*5* present in the cell, the transposition frequency of individual Tn*5* elements is inversely related to the total number of copies in the cell. Thus, one copy of Tn*5* in a population of approximately 20 additional copies transposes at about 5% its rate in the absence of additional Tn*5*s.

The number of copies of Tn*5* appears to be mechanistically sensed by the number of protein 2-producing equivalents in the cell. Elements that are deficient in protein 1 expression, and are, therefore, unable to transpose themselves, are equally active in reducing the transposition of a single-copy element provided they synthesize normal amounts of protein 2. Elements that are manipulated to synthesize more or less than the normal amount of protein 2 inhibit transposition of a single-copy element to a corresponding extent.

Several observations lead us to question whether protein 2 is the only factor responsible for limiting transposition of Tn*5*. Derivatives of Tn*5* that alter the expression of protein 2 relative to protein 1 do not appreciably effect the transposition frequency of the element. No increase in the transposition frequency is found with a mutant that synthesizes less protein 2 than the wild type, and only a 50% decrease in the transposition frequency of an element occurs when protein 2 expression is increased to about seven-fold over the wild-type level (J. YIN and W. S. REZNIKOFF, unpublished results; R. C. JOHNSON and W. S. REZNIKOFF, unpublished results). These results suggest that in addition to protein 2 another factor (or factors) is playing a role in limiting transposition of Tn*5*. Such a factor could be a limiting host function that is required for Tn*5* transposition. This suggestion is supported by the finding of a sequence at the ends of Tn*5* which is homologous to a repeated sequence located within the chromosomal origin of replication (JOHNSON and REZNIKOFF 1983). This sequence may be a recognition site for host replication factors. Host replication factors, which function to initiate chromosomal replication, may be present in relatively low quantities and, thus, may serve to limit the transposition of Tn*5*.

In summary, relatively high concentrations of protein 2 inhibit the transposition of a resident Tn*5*. A limiting host function(s) may also be responsible for controlling the maximum rate of Tn*5* transposition. Both the Tn*5*-encoded inhibitor and the host may be acting in concert to control the transposition of Tn*5* in the steady state. Transposition is controlled in a manner that ensures continuing transposition to allow dispersal of the transposon, but it prevents high rates of transposition with increasing copies of the element which reduces the risk of deleterious mutations and the accumulation of excess DNA.

This work was supported by grants from the National Science Foundation (PCM 8215021) and the National Institutes of Health (GM 19670).

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Corresponding editor: B. GLICKMAN