A New Generalizable Test for Detection of Mutations Affecting Tn*10* Transposition

Olivier Huisman[1] and Nancy Kleckner

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT

We describe here a new rapid screen that allows easy detection of transposon or host mutations that affect Tn*10* transposition in *Escherichia coli*. This test involves a new Tn*10* derivative called the "mini-*lacZ-kanR* fusion hopper" or mini-Tn*10*-LK for short. This element does not direct expression of β-galactosidase when present at its original starting location on a suitably engineered plasmid or phage genome because it lacks appropriate transcription and translation start signals. However, transposition of this element into the chromosome of *E. coli lacZ*$^-$ bacteria leads to productive fusions in which the *lacZ* gene within the transposon is expressed from external chromosomal signals. Such fusions are readily detectable on MacConkey lactose indicator plates as red (Lac$^+$) papillae inside of white (LacZ$^-$) colonies. The length of time required to see red papillae appearing in a white colony sensitively and accurately reflects the transposition frequency of the mini-transposon within the colonies. Differences in times for color formation are sensitive enough that 10-fold differences in transposition frequency can readily be detected. This papillation assay can be used to identify mutant clones in which the frequency of Tn*10* transposition is either increased or decreased. We have successfully used the assay to identify mutations in the terminal sequences of Tn*10*; mutations in the Tn*10* transposase gene or the bacterial host can be isolated just as easily. This screen should be readily adaptable to transposable elements other than Tn*10*.

MOBILE elements are involved in many types of DNA rearrangements in bacteria. Because of their importance, they have been extensively studied (for reviews, see KLECKNER (1981) and GRINDLEY and REED (1985)]. In *Escherichia coli*, several transposons and insertion sequences have been completely sequenced, and molecular and genetic analysis has disclosed the existence and the role of element-encoded transposase genes and of essential sequences at the ends of each element. Biochemical approaches, although only at their beginning, have begun to reveal host factor requirements (D. MORISATO and N. KLECKNER, personal communication) and other aspects of the transposition reaction.

Despite the importance of the transposition process, very few direct searches for mutations affecting transposition have yet been conducted, although this approach has turned out to be very successful when it has been used. Recently, for example, mutant isolation has led to the discovery of a role for DNA adenine methylation in regulation of Tn*10* and IS*10* transposition (ROBERTS *et al.* 1985). Further isolation of host mutations should allow identification of host factors that participate directly in transposition, and mutations affecting the transposase or the essential terminal sequences should help to improve our understanding of the functions of these two types of determinants and of their interaction. Despite the obvious advantages of such a genetic approach, it has not yet been widely used, probably in large part because it has been difficult to identify the desired mutants. We present here a new strategy that allows efficient and easy detection of all types of mutations affecting the activity of transposon Tn*10* in *E. coli* and the successful application of this strategy for isolation of mutations in the Tn*10* terminal sequences. Our approach could, with appropriate engineering of mini-transposon derivatives, be extended to analysis of other transposable elements. The new mini-Tn*10* transposon we present here as part of this strategy can also be used as a new useful transposon tool for genetic analysis *in vivo* (HUISMAN *et al.* 1987).

MATERIALS AND METHODS

Bacterial strains: NK7800 is a *recA56* transductant of NK7254 (*trp31, his1, argG6, rpsL104, tonA2, lacZdel-r1, supE44, xy17, mt12, metB1, tsx, leu6*). The F'*lacI*^{q}*L8pro* episome (FOSTER *et al.* 1981) has been introduced in this strain by standard conjugation assay (MILLER 1972). The strain NK6641 which is used as recipient in the mating out assay is *lac-proXIII, recA56, malR, strA* (FOSTER *et al.* 1981).

Plasmids: pNK1206 is pNK81 (FOSTER *et al.* 1981) digested with *Bcl*I and religated in the presence of the *Bam*HI-*lacZ* fragment of pMC1871 (CASADABAN *et al.* 1983) and the *Bam*HI-*kanR* fragment of pNK862 (WAY *et al.* 1984). The *Bcl*I digest of pNK81 deleted the inside part of Tn*10*

[1] Present address: Departement de Biotechnologie, Institut Pasteur, 29 rue du Docteur Roux, 75015 Paris, France.

leaving only the 70 outermost basepairs (bp) at the ends of the element at the two extremities of the backbone. The *Bam*HI digest of pMC1871 generated a truncated *lacZ* fragment starting at bp 22 of the *lacZ* coding sequence; in pNK1206, the amino-terminal end of *lacZ* is adjacent to the terminal sequences at the IS*10*-Right end of Tn*10*, and the *kanR* segment is located between the distal end of *lacZ* and the terminal sequences at the IS*10*-Left end of Tn*10*. pNK1207 has been made by substitution of the *Bgl*II-*Pst*I segment of pNK1206, which lies upstream of the IS*10*-Right end of Tn*10*, by a *Bam*HI-*Pst*I piece of pRS391 (SIMONS, HOUMAN and KLECKNER 1987), containing repeated copies of the *rrnB* transcriptional terminator sequence. In pNK1207, the terminator sequences prevent nonspecific transcription of the *lacZ* gene. pNK1214 was used for isolation of the mutations in the terminal sequences of Tn*10*. It derives from pNK1207 by several steps. First the *Bam*HI site at the *lacZ/kanR* junction has been destroyed by filling in with the Klenow fragment of DNA polymerase. The left outside end of Tn*10*, adjacent to the *kanR* determinant, has then been replaced by the right outside end of Tn*10* extended to the *Acc*I site [for restriction map of Tn*10*, see WAY *et al.* (1984)]. Finally, a *Sal*I linker has been inserted in the *Nru*I site of this new end. pNK1215 is a *Bgl*II-*Bcl*I deletion of the Tn*10* outside end adjacent to the *kanR* segment in pNK1214.

Bacteriophages: λNK1039 is a derivative of λRP167 (MAURER, MEYER and PTASHNE 1980), containing the *Eco*RI *his* fragment of pNK75 (FOSTER *et al.* 1981). λNK1205 has been obtained by homologous recombination between the homologous histidine regions contained in pNK1207 and λNK1039. The phage recombinant has been selected as prophage able to render lysogenic bacteria resistant to kanamycin and contains the mini-Tn insert within this segment.

Media, enzymes and chemicals: Biological media were prepared as described by MILLER (1972). When used, supplements were added at the following concentrations: amino acids, 100 μg/ml; ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; streptomycin, 200 μg/ml; tetracycline, 10 μg/ml; isopropyl-1-thio-β-D-galactopyranoside (IPTG), 1 mM. Bacteriological supplies were purchased from Difco. The MacConkey lactose medium was made fresh each time and prepared according to the instructions. Enzymes for *in vitro* DNA work were purchased from New England Biolabs.

Procedure for the "papillation test": Competent bacteria were transformed by the required plasmids according to MANIATIS, FRITSCH and SAMBROOK (1982). They were then diluted 20-fold in LB medium and incubated at 37° for 90 min with good aeration. Appropriate dilutions were spread on freshly poured MacConkey lactose plates and incubated at 37° for the appropriate time. Since the incubation was sometimes very long, the plates were protected against light and evaporation.

Mutagenesis procedure: The *Bcl*I *Sal*I fragment from the pNK1214 that contains the outermost 70 bp of Tn*10* has been mutagenized following the procedure described by MEYERS and MANIATIS (1985). The mutagenized fragment preparation was then subcloned back into plasmid pNK1214. After overnight ligation at 16°, the ligation mixture was transformed directly into NK7800/F*lacI*^{q}*L8pro*; after 30 min expression in LB medium, transformants were selected on MacConkey lactose plates containing ampicillin. Further details will be published elsewhere (O. HUISMAN, P. ERRADA and N. KLECKNER, unpublished results).

Conjugational "mating out" assay for transposition: For each strain to be tested, three single colonies were chosen, individually inoculated into LB broth containing IPTG and grown to about 1 × 10^8 bacterial/ml with good aeration. One milliliter of each such culture was mixed with 2.5 ml of a culture of the recipient strain grown to a concentration of 4 × 10^8 cells/ml in LB broth. After 60 min of incubation with good aeration, the mating mixture was vortexed vigorously, placed on ice, and aliquots were plated. The total frequency of exconjugants was determined by direct selection of Pro$^+$Sm^R clones emerging from the mating. The frequency of transposition exconjugants was determined by selection of Pro$^+$Km^{R}Sm^R clones. Transposition frequencies in such experiments are expressed as the ratio of Pro$^+$Km^{R}Sm^R to Pro$^+$Sm^R exconjugants.

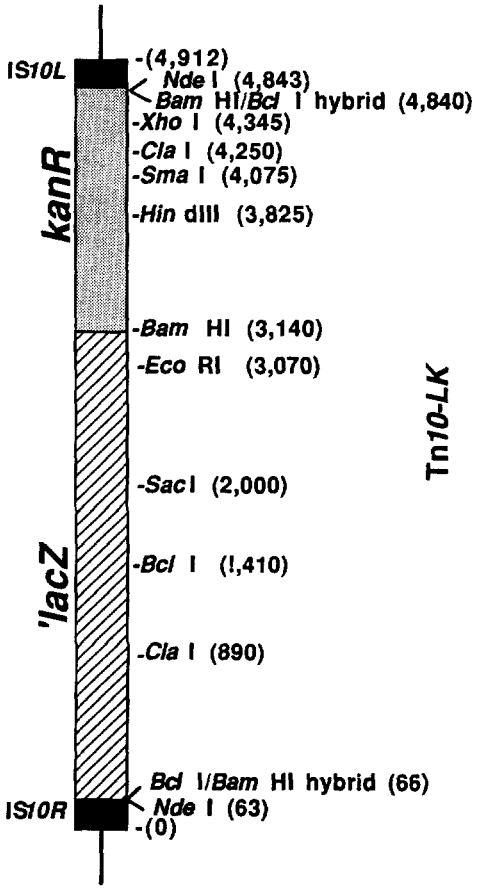

FIGURE 1.—Structure of the mini-*lacZ-kanR* transposon (Tn*10*-LK). Numbers represent the distance in nucleotides of each restriction enzyme cut site from the left-most end of the element. *LacZ* transcription is from left to right. The sequence of the Tn*10* across the junction to the truncated *lacZ* gene, beginning with bpl of the IS10 sequence, is CTG ATG AAT CCC CTA ATG ATT TTG GTA AAA ATC ATT AAG TTA AGG TGG ATA CAC ATC TTG TCA TAT *GAT* CCC GTC, etc. *GAT* is the first codon of the *lacZ* coding sequence.

RESULTS AND DISCUSSION

Basis of the screening assay: To facilitate the isolation of mutants affected for transposition, we wished to develop an assay by which differences in transposition frequency among different bacterial clones could be recognized by simple visual inspection of isolated single colonies.

For this purpose, we constructed *in vitro* the 4.7-kb artificial transposon shown in Figure 1. This mini-

lacZ-kanR element, Tn*10*-LK, has the appropriate terminal sequences of Tn*10* flanking the *E. coli lacZ* gene and the *kanR* determinant of Tn*903*. The *lacZ* gene was truncated so that it lacks both transcriptional and translational initiation signals, and a series of transcription terminators is present upstream of the *lacZ* gene, but outside of the transposon, to eliminate fortuitous readthrough transcription. As a result of these features, the mini-*lacZ-kanR* element produces only a very low level of β-galactosidase. This level is insufficient to render a LacZ$^-$ bacterial strain LacZ$^+$, even when the transposon construct is present on a multicopy plasmid. However, the *lacZ* coding sequence has been connected to one of the terminal sequences of Tn*10* in such a way that a correct open reading frame extends from the very end of the element, across the terminal sequences, and into the *lacZ* gene. Thus, insertion of this mini-transposon in the correct orientation and reading frame into an efficiently expressed *E. coli* gene can lead to high level expression of β-galactosidase and a LacZ$^+$ phenotype. The exact sequence across the Tn*10* terminus and the start of the *lacZ* gene are given in the legend to Figure 1.

We have used the Tn*10* mini-*lacZ-kanR* transposon to detect transposition mutants by taking advantage of the fact that Lac$^+$ and Lac$^-$ bacteria can easily be discriminated on MacConkey lactose indicator plates, where they form red and white colonies respectively. A LacZ$^-$ strain containing a non-transposing mini-*lacZ-kanR* element forms white colonies on such plates. In contrast, a LacZ$^-$ strain carrying a transposition-proficient mini-element forms colonies that are initially white but eventually develop red papillae or, at higher transposition rates, turn completely red due to the occurrence of multiple Lac$^+$ insertion events within the colony. In fact, the rate at which the colonies develop red papillae is directly and sensitively related to the frequency of transposition within the colony. (Note: for simplicity, we will describe all colony morphologies with the terms "papillae" or "papillation" even though the colonies that turn completely red do not actually exhibit discrete fast-growing outgrowths.)

Fidelity and sensitivity of the assay: This method was first tested using a *lacZ*$^-$ *recA*$^-$ strain, NK7800, carrying two compatible plasmids: pNK1207 that contains the mini-*lacZ-kanR* transposon, and pNK629 that provides high levels of the essential Tn*10* transposase function that is lacking from the mini-element construct. Transformants containing both plasmids were plated on MacConkey lactose plates supplemented with appropriate selective drugs and incubated at 37°. All of the resulting colonies were initially white but had turned red by 48 hr of incubation. Occurrence of red colonies exhibits the same requirements as Tn*10* transposition: presence of transposase and integrity of the transposon termini. Transformants of the *lacZ*$^-$ *recA*$^-$ strain carrying only the mini-transposon plasmid pNK1207, or transformants carrying the transposase plasmid plus pNK1215, a mini-transposon plasmid lacking the terminal Tn*10* sequences adjacent to the *kanR* gene, both give rise exclusively to uniform white colonies after 48 hr of incubation at 37°.

The Lac$^+$ character of the red papillae is not the result of internal rearrangements within the mini transposon contained in the plasmid molecule. Indeed, we analyzed ten independent Lac$^+$ clones isolated from ten different colonies exhibiting red papillae. Each of the ten were shown by physical characterization and genetic analysis to contain the two original wild type plasmids (data not shown). We thus conclude that the formation of red colonies primarily reflects transposition of the mini-*lacZ-kanR* element from the plasmid into appropriate locations in the bacterial chromosome.

The fidelity and sensitivity of the papillation assay were further assessed by genetically varying the transposition frequency of the mini-*lacZ-kanR* element and comparing in a single set of strains the rate of red papillae-containing colony formation on MacConkey lactose plates and the quantitative transposition frequency as measured in a standard "mating-out" assay (see MATERIALS AND METHODS for further description). This analysis, described in Table 1 and below, shows that the rate at which colonies turn red decreases from 48 to 96 hr as the relative frequency of transposition drops 1000-fold. Most important, it reveals that transposition frequencies differing by as little as a factor of 10 should be distinguishable by this test. Finally, these reconstruction experiments provide background information that allows one to select exactly the appropriate conditions needed to identify mutants having the desired increase or decrease in transposition.

A 1000-fold range in transposition frequencies was achieved by combining different levels of transposase with high or low transposon copy numbers. The frequency of Tn*10* transposition is known to be roughly proportional to the level of transposase present in the cell (MORISATO *et al.* 1983). In our first experiments, described above, the transposase was provided from a multicopy plasmid and its synthesis was under control of a very strong heterologous p*tac* promoter. Lower transposase levels were achieved by using a single copy prophage vector and/or by using normal Tn*10* transposase promoter pIN which is much weaker than p*tac* (SIMONS *et al.* 1983); transposon copy numbers were reduced from the original multicopy level by placing the mini-*lac-kan* element in the cell on a single copy λ prophage.

The results presented in Table 1 show that the

TABLE 1

Length of time for papillation as a function of tranposition frequency

Mini-*lacZ-kanR* vector	Source of transposase	Frequency of KanR transposition into *FlacI^{q}L8pro*	Average time to get red papillae in >99.9% of colonies (hr)
Multicopy plasmid (pNK1207)	Multicopy plasmid p*tac*::Tase (pNK629)	1.5×10^{-2}	48
Single copy (λNK1205)	Multicopy plasmid p*tac*::Tase (pNK629)	1.3×10^{-3}	60
Multicopy plasmid (pNK1207)	Single copy p*tac*::Tase (λNK1046)	1.7×10^{-4}	72
Single copy (λNK1205)	Single copy p*tac*::Tase (λNK1046)	2.4×10^{-4}	72
Multicopy plasmid (pNK1207)	Single copy pIN::Tase (λNK1194)	2.3×10^{-5}	96
Single copy (λNK1205)	Single copy pIN:Tase (λNK1194)	1.3×10^{-5}	96

"p*tac*::Tase" means that the synthesis of the transposase is controlled by the heterologous p*tac* promoter (AMANN, BROSIUS and PTASHNE 1983; ROBERTS, KACICH and PTASHNE 1979). The pIN::Tase expression means that on those vectors the synthesis of the transposase is controlled by the wild type Tn*10* transposase promoter (SIMONS *et al.* 1983). The "frequency of KanR transposition" has been measured by the standard mating out transposition assay (see MATERIALS AND METHODS). The "average time to get red papillae" represents the time necessary for all of the 2000 colonies examined to exhibit red papillae. Additional controls have shown that, after prolonged time of incubation, colonies containing the Lac-Kan transposon but no transposase will eventually turn red (data not shown). However, this happens only after the incubation times of around 96 hr in the case where the element is carried on a multicopy plasmid and around 120 hr in the case where the element is present in a single copy. In the case of the multicopy plasmid, the appearance of Lac$^+$ bacteria was due to transposase-independent rearrangements on the plasmid rather than to any real transposition event (data not shown).

frequency of transposition is correlated with the length of incubation required for every colony to exhibit red papillae. For example, if the frequency of transposition is 1.5×10^{-2}, all colonies exhibit red papillae after 48 hr of incubation at 37°; when the transposition frequency drops to 1.3×10^{-3}, the colonies remain white after 48 hr, but all have red papillae after 60 hr; even at a transposition frequency of 1.3×10^{-5}, all colonies have red papillae by 96 hr, while colonies of transposition-negative control strains are still all uniformly white. In some cases, the transposition frequency remains constant despite a variation in transposon copy number (compare lines 3 and 4, and lines 5 and 6). One possible explanation is that the level of transposase in these cases is so low as to be the limiting factor for transposition. The important point for the analysis here is that even in these unusual situations, the time necessary to observe the formation of red papillae or colonies is still correlated with transposition frequency.

Use of assay to isolate transposition mutants: We have successfully used the mini-*lacZ-kanR* red papillae screen to isolate mutations in the terminal sequence of Tn*10* that decrease the level of transposition. After an efficient mutagenesis targeted to one terminal sequence of a plasmid-born mini-transposon (see MATERIALS AND METHODS), plasmid molecules were introduced into an appropriate *lacZ*$^-$ *recA*$^-$ strain containing the compatible transposase overproducer plasmid pNK629. The resulting transformants were plated on MacConkey lactose plates and incubated at 37°. After 48 hr, 13 of the 5200 colonies observed remained white, while all others presented red papillae. Further analysis of the mutant candidates by mating-out transposition assays revealed that 8 of the 13 did in fact exhibit reduced transposition frequencies. DNA sequence analysis of these mutants confirms that 5 of them had suffered a single base change within the Tn*10* terminal sequence (the 3 others were deletions at the end of the transposon). The changes identified in this experiment were: a G to T transversion at bp 6, a T to C transition at bp 9, and a C to T transition at bp 11. One of the point mutations identified at bp 9 was in fact identical to a mutation previously identified by more laborious methods (WAY and KLECKNER 1984). Further characterization of those single mutations and their effects on Tn*10* transposition will be described elsewhere. The five white colonies exhibiting normal transposition phenotype are presumed to have suffered alterations in the *lacZ* determinant that did not affect transposition. Three other independent mutageneses of the mini-*lacZ-kanR* element followed by mating-out assays on mutant candidates have confirmed the conclusions from the first analysis: this screen allows the easy detection of transposons harboring mutations in the terminal transposon sequences.

The papillation screen is very versatile. It has thus far been used only to isolate mutations in the mini-transposon itself, but it should in the same way reveal mutations in the transposase gene or in any host gene encoding an important transposition factor. It is

straightforward to search specifically for mutations in only one of these determinants, because the terminal sequences, the transposase gene and the host genome can each be mutagenized and manipulated separately. Also, the papillation assay allows detection of mutations that either increase or decrease the efficiency of transposition.

We expect that this method will greatly facilitate the genetic dissection of Tn*10* transposition. This approach should be also generally adaptable to genetic analysis of elements other than Tn*10*. Other transposons that can promote formation of *lacZ* fusions have already been described in some cases (CASADABAN and CHOU 1984; KROOS and KAISER 1984) and can be readily engineered in most others.

Application to genetic analysis in vivo: The mini *lacZ-kanR* element transposon described here also presents several advantages for transposon analysis. Like other mini-Tn*10* elements described previously (WAY *et al.* 1984), it can transpose at a high frequency, but the resulting insertions are stable because the transposase gene is not present within the element itself. Most important, the presence of the truncated *lacZ* gene in an open reading frame through the end of the Tn*10* terminus allows one to obtain protein fusions if insertion occurs in the correct orientation and open reading frame. Isolation and analysis of insertions and protein fusions are described in HUISMAN *et al.* (1987).

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