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Specificity of Mini-Mu Bacteriophage Insertions in a Small Plasmid

BEATRIZ A. CASTILHO†* AND MALCOLM J. CASADABAN

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois 60637

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Target site selection for bacteriophage Mu transposition was studied in pools of over 10^7 independent mini-Mu insertions in pUC9, selected by transduction of the plasmid. Insertions in both orientations were clustered in three regions and, within these, at preferred sites.

Bacteriophage Mu insertions within defined genetic intervals have indicated that insertions occur preferentially at certain locations (3, 4, 6, 12, 13). Insertional hot spots have been found for most transposable elements, with consensus sequences often present at the location of the insertions (2, 8, 10, 15, 17, 19).

The insertion specificity of Mu at the nucleotide level was investigated with pools of insertions of the mini-Mu Mu dII4041 (4) in pUC9 (16, 18), selected as described in Fig. 1. This procedure yields approximately 5×10^4 independent transductants per ml of lysate, 95% of which contain pUC9 plasmids with mini-Mu insertions. These should represent, on a random basis, the saturation of pUC9 with insertions at all possible positions, in both orientations. In order to reduce the statistical fluctuations in the insertion distribution, the experimental procedures were scaled up to obtain pools of over 10^7 transductants.

*Bam*HI digestion of plasmid DNA extracted from pools of the Km^r transductants should result in two sets of fragments: one, ranging from 7.5 to 10.2 kb, containing the left end of Mu dII4041 plus a variable length of plasmid sequences; and another set, from 117 bp to 2.8 kb, containing the right terminus of Mu dII4041 and the remaining plasmid sequences. If insertions were random, these would each form a smear on a gel within their size ranges, except for interruptions from the lack of insertions in the essential replication region of the plasmid. Figure 2 shows the pattern of the small *Bam*HI fragments on an acrylamide gel, implying that insertions are clustered in discrete regions. This pattern is reproducible in different lysates and with different lysogens, except for one set of fragments (C) present only in pools 4 and 5. Pooled insertion plasmid DNA cut with *Eco*RI, which has only one recognition site in the pUC9 plasmid and none in the mini-Mu, generated only one fragment of 10.2 kb, indicating that the majority of the plasmids in the pool had only one insertion of Mu dII4041 without detectable rearrangements (data not shown).

The orientation of the insertions originating this pattern of fragments was determined (Fig. 3). Plasmid pool 1 DNA was digested with *Bam*HI (lane B) or with *Eco*RI (lane E-B), which has a unique restriction site in the insertion plasmids, 10 bp from the *Bam*HI site in pUC9. The 5′ ends were labeled with γ-^{32}P. The *Eco*RI-digested DNA was then digested with *Bam*HI. Both samples were electrophoresed on a 4% acrylamide gel. The size standard was pBR322 plasmid DNA digested with *Hin*fI and end labeled with γ-^{32}P. A similar experiment was performed to have the insertions in the (+) orientation only labeled, digesting the pool with *Sal*I, instead of *Eco*RI, which confirmed these results (data not shown). By labeling the plasmid pools at the *Bam*HI ends, fragments F and G, corresponding to insertions adjacent to the plasmid *Bam*HI site, could also be visualized. Three preferred regions for Mu insertions in pUC9 were detected: one at each side of the β-lactamase gene and the third in the *lac* promoter-operator and initial *lacZ* sequences.

In order to aid in the nucleotide position analysis of the insertional hot spots, 47 independent individual insertions were isolated and their *Bam*HI digestion patterns were compared to that of an insertion plasmid pool. Forty-one of these insertions were in the hot spot regions, corresponding to the major bands observed in the pools: 13 in the Lac region (6 in A or A′ and 7 in F or G), 21 in the region upstream (13 in B and 8 in E), and 7 in the region downstream (2 in C and 5 in D) of the β-lactamase gene. The precise locations of 15 of the insertions occurring in the hot spots were determined by sequencing across the Mu right end, from the *Bam*HI site (Fig. 5). These data indicate a preference for G/C in the center of the 5-bp repeats originated during transposition, as observed for other Mu insertions (9, 12).

Mu dII4041 insertions at the Lac region and at the β-lactamase upstream region were analyzed at the nucleotide level (Fig. 4). Insertion pool DNA was digested with *Bam*HI and end-labeled with γ-^{32}P. For insertions in the Lac region, the A and A′ fragments were then digested with either *Hin*fI or *Rsa*I. The A fragments in the *Hin*fI digestion were reduced to sizes ranging from approximately 11 to 330 bp, with the *Hin*fI site located at position 622, while the A′ fragments were larger than 1 kb, with a *Hin*fI site at position 1676. In the *Rsa*I digestion, the A′ fragments were reduced to between approximately 117 and 354 bp, with the *Rsa*I site at position 2159. B fragments, labeled at the *Bam*HI ends, were digested with *Xmn*I (*Xmn*I site located at position 2278 in pUC9), yielding fragments from 300 to 550 bp. The isolated fragments were run on 6% denaturing gels. The bands observed in the pools were matched to a nucleotide position in the purine sequencing ladders (11) prepared from single insertion plasmids that had the Mu dII4041 at or near the region being analyzed. This would indicate how much

* Corresponding author.
† Present address: Disciplina de Microbiologia, Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Rua Botucatú, 862 CEP 04023, São Paulo, SP, Brazil.

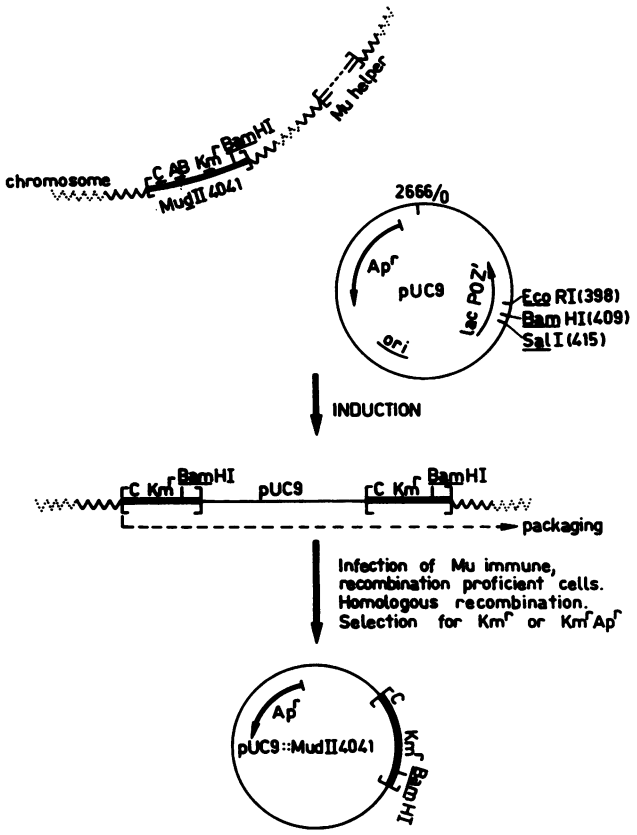

FIG. 1. Selection of Mu dII4041 insertions in pUC9. *E. coli* BAC101 [F⁻ *araD139* Δ(*ara leu*)$_{7697}$ Δ(*proAB argF lacIPOZYA*)$_{XIII}$ *strA recA56 srl*::Tn*10* Mu *cts62* Mu dII4041] (4) containing the plasmid pUC9 in monomeric form is induced at 42°C for transposition of the Mu elements. Transposition of Mu dII4041 to pUC9 can result in a cointegrate structure, where a copy of the plasmid, flanked by two copies of the Mu dII4041 element, in direct orientation, is inserted in the chromosome. The packaging mechanism of Mu allows the encapsidation of up to 38 kb of DNA, starting specifically at a Mu left terminus. Thus, the entire cointegrate structure can be encapsidated. Upon infection of a Mu lysogenic cell (M8820Mu c^+) (4), homologous recombination between the two copies of the Mu dII4041 results in a plasmid molecule containing Mu dII4041 inserted at the site of the original transposition in pUC9.

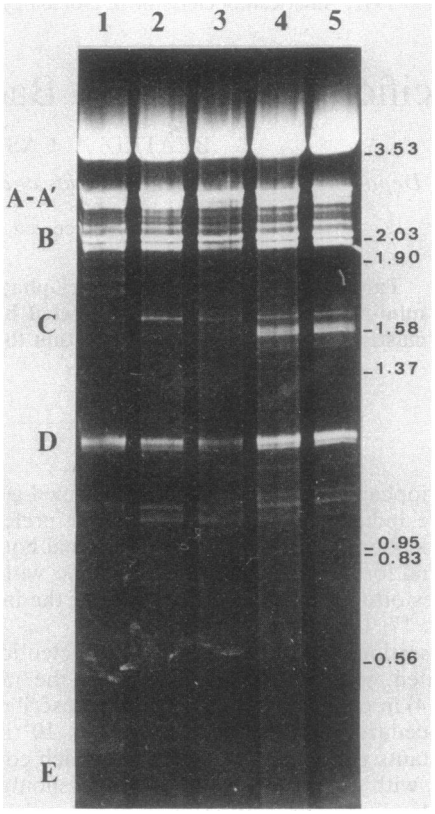

FIG. 2. Hot spots for Mu dII4041 integration; 4% acrylamide gel electrophoresis of insertion plasmid pools digested with *Bam*HI. Insertion plasmid pools were obtained by infecting M8820Mu c^+ cells with a 200-ml lysate prepared from BAC101, at a multiplicity of infection of 1. Transductants were obtained by two rounds of selection in liquid cultures. Each lane contains 5 μg of DNA. The groups of fragments were labeled alphabetically, according to their sizes. Pool 1 was obtained from transductants selected only for the mini-Mu element (kanamycin, 20 μg/ml). The other pools were obtained with additional selection for the plasmid (carbenicillin, 100 μg/ml). Pools 2 and 3 were obtained from the same lysate as pool 1 but from independent transduction experiments. Pools 4 and 5 were obtained from another lysate and independent transductions. The size standard was lambda DNA digested with *Eco*RI and *Hin*dIII (only fragments in the relevant size range are indicated).

smaller the fragments in the pools were in relation to the same fragment on the individual insertions used in the ladders. The numbers obtained were converted to the approximate location of the insertions on the pUC9 nucleotide sequence (Fig. 5). Insertions occurred at a few defined positions in the preferred regions. The insertions located away from the most frequently used positions in the A region appear to be distributed more uniformly, with an average spacing of 3.6 bp. Some of the most used positions correspond to sites of individual insertions. Figure 5 indicates the approximate positions for the insertions in the A, A', and B regions.

Upon a close examination of the most frequently used sites in each region, sequence similarities were found which partially matched sequences present in the termini of Mu where the Mu A protein binds: 5'-PuPyPuCGAAAPu-3' (5). In the hot spot at position 293, the sequence 5'-GC GAAAG-3' can be found 19 bp from the left end of Mu, as part of a large palindromic region centered in nucleotides 308 to 309. In the hot spot at position 2569, the sequence 5'-CGAAAA-3' is present 13 bp from the Mu left end, as part of a partial palindrome. In the hot spot at position 527, no homology as extensive could be found.

This analysis defines a high specificity for insertion site selection at regional and nucleotide levels. The regional specificity for Mu insertions does not seem to be correlated with the AT content of these regions; the *lacPOZ'* segment is not exceptionally AT rich, as are the β-lactamase upstream and downstream regions. Transcription of the target sequence does not seem to affect its usage as the target for Mu transposition, as it inserts very often at the *lacZ* gene segment and its promoter and operator sequences.

Two rules for Mu insertions may exist: one requiring a G/C nucleotide at the center of the 5-bp repeats (9, 12, and this work) and another, represented by the most used sites, requiring partial homology of the target with the protein A

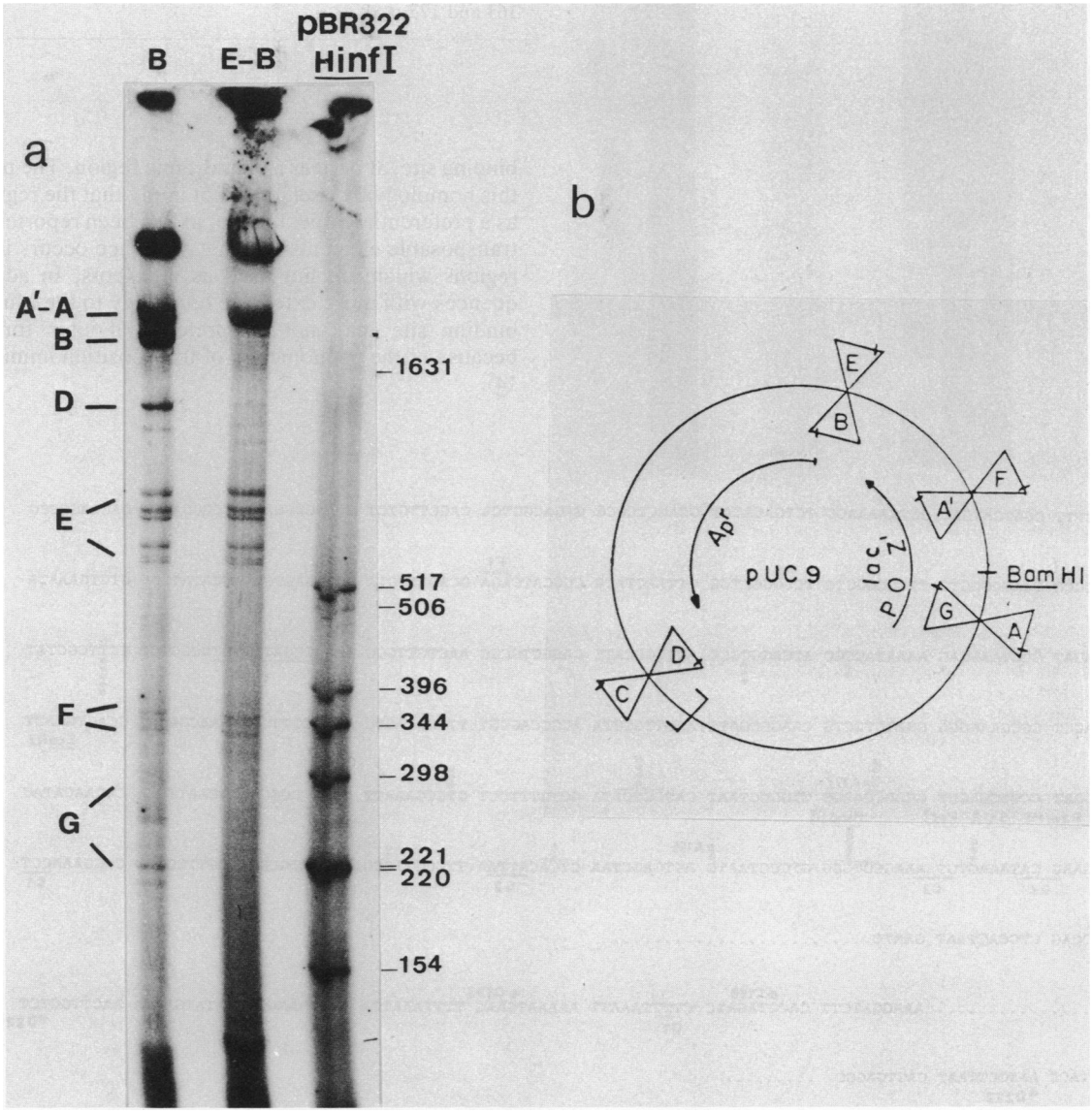

FIG. 3. Localization of insertions in the plasmid pools. (a) Determination of orientation of insertions. Plasmid pool 1 DNA was digested with *Bam*HI (lane B) or with *Eco*RI (lane E-B), labeled, and electrophoresed as described in the text. Insertions in both orientations are visible in lane B; insertions in the (−) orientation are visualized in lane E-B. Insertions in the (+) orientation lost a 10-bp *Eco*RI-*Bam*HI fragment from the plasmid sequences, which had the labeled terminus. (b) Map of insertions in pUC9. Insertional hot spots of Mu dII4041 in pUC9, as determined in previous sections, are represented as triangles around the plasmid molecule. The *Bam*HI site at the right terminus of Mu is indicated as a bar on the triangles, thus indicating the orientation of the insertions. Inside the circle, the insertions are in the (+) orientation (Mu repressor *c* gene transcribed in a clockwise direction); outside the circle, the insertions are in the (−) orientation. A bracket in the plasmid molecule represents the Tn*3* element terminus.

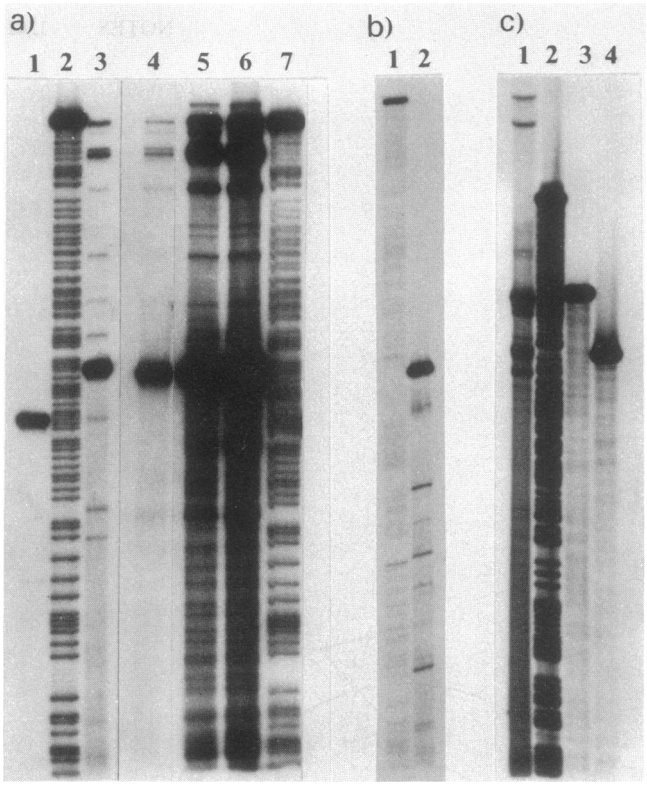

FIG. 4. Nucleotide level mapping of the host spots. (a) Autoradiogram of two denaturing gels resolving the A fragments from two independent plasmid pools (lanes 3 and 4 to 6). Lanes 4, 5, and 6 contain dilutions of the same DNA. Lane 1 has the 199-bp *Bam*HI-*Hin*fI fragment from insertion plasmid 155; lanes 2 and 7 have a purine ladder from the corresponding fragment from insertion plasmid 177. (b) Autoradiogram of a denaturing gel with the A′ fragments (lane 2); lane 1 represents the purine ladder from the *Rsa*I-*Bam*HI fragment from insertion plasmid 164, labeled at the *Bam*HI site. (c) Autoradiogram of denaturing gel with the B fragments (lane 1); lane 2 contains a purine ladder of the *Rsa*I-*Bam*HI fragment of insertion 164. Lanes 3 and 4 contain plasmid insertions 163 and 173.

binding site, at or near a palindromic region. The presence of this homology by itself does not imply that the region is used as a preferential target for Mu, as has been reported for other transposable elements (8); that sequence occurs in pUC9 in regions which are not used as hot spots. In addition, sequences with more extensive homology to the Mu A protein binding site may not be preferential sites for insertion because of the phenomenon of transposition immunity (1, 7, 14).

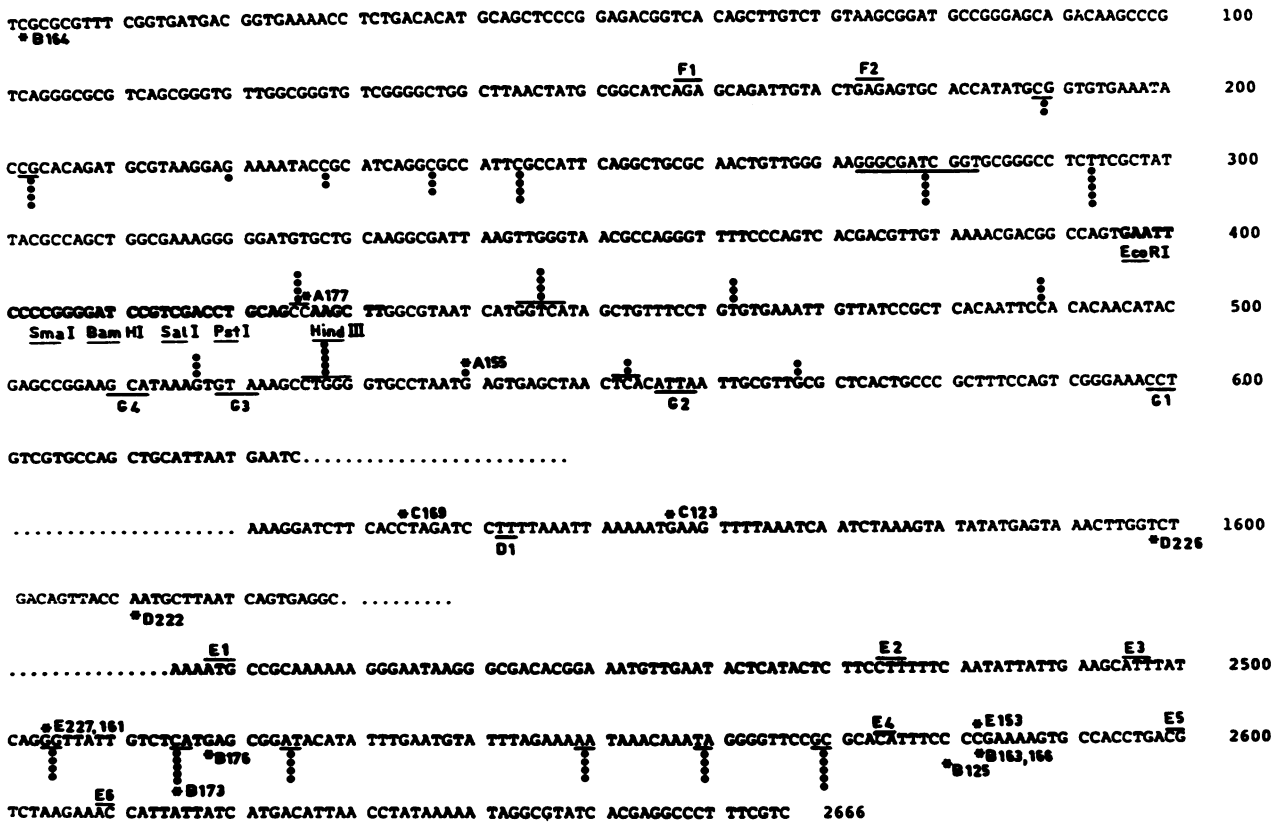

FIG. 5. Insertion hot spots. Partial nucleotide sequence of the pUC9 plasmid showing the locations of the preferred target sites for Mu insertions. The nucleotide assignments correspond to the middle base of the 5-bp repeats originated in the transposition event. Hot spot regions, derived from the gels shown in Fig. 4, are indicated by filled circles, the number of which represents the preferential usage of that insertion site as deduced from the intensity of the bands. Where the positions could not be accurately mapped, the hot spot is depicted by a bar above or below the approximate location. From data acquired from Fig. 3, insertions corresponding to fragments E, F, and G were also approximately mapped and indicated in this figure. Symbols placed above the nucleotide sequence correspond to insertions in the (−) orientation; those below the sequence correspond to insertions in the (+) orientation. Individually mapped insertions are indicated by asterisks, followed by the insertion plasmid numbers.

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