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Transposition of mini-Mu containing only one of the ends of bacteriophage Mu

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Transposition of mini-Mu containing only one of the ends of bacteriophage Mu was studied. Transposition was observed when plasmids containing this mini-Mu were used as the donor in a mating-out assay with the F factor POX38, which lacks all known transposable elements, as the target. Analysis of the plasmids isolated from the recipient strain showed that in most cases the mini-Mu containing plasmid and POX38 were fused and that a part of the plasmid had been duplicated, indicating the formation of co-integrates. To obtain the DNA sequences of the junctions between donor and recipient DNA, an F factor was constructed that made it possible to analyse these junctions directly. The results showed that several sequences can be used as an alternative end in transposition and that these alternative ends show homology with the consensus for a strong A binding site. Moreover, the first base pair at the junction was always a (T/A) base pair. This base pair is situated at exactly the same position with respect to the sequence which has homology with the consensus for a strong A binding site as in the ends of Mu.

Key words: DNA protein interactions/DNA recombination/transposition/evolution

Introduction

A transposable element is in general characterized as a DNA segment whose ends are more or less perfect inverted repeats varying in size between 16 and 40 bp.

In bacteriophage Mu the organization of the ends is more complex (Figure 1). Like in other transposable elements, inverted sequences play an essential role in the transposition of bacteriophage Mu, but they are not located exactly at the ends (Groenen *et al.*, 1985; Groenen and van de Putte, 1986). These sequences are specific binding sites for the Mu transposase, the A protein (Craigie *et al.*, 1984).

The complex organization of A binding sites in the ends of Mu is possibly related to the property of Mu to transpose at the very high level of 100 times during one lytic cycle (~45 min). Moreover, the differences in the organization of the left and right end (Figure 1) might be related to Mu being a phage, ensuring that different processes, such as replication (Wijffelman and van de Putte, 1977; Goosen, 1978), transcription (Bade, 1972; Wijffelman *et al.*, 1974) and packaging (Bukhari and Taylor, 1975) can proceed at appropriate times with the same polarity. Efficient transposition of bacteriophage Mu is known to require both ends of the phage genome in their proper relative orientation on a single DNA molecule (Schumm and Howe, 1981; Mizuuchi, 1983). (Special constructions however can be made *in vitro* where this is no longer the case. See Craigie and Mizuuchi, 1986.)

Using a sensitive transposition assay we found that mini-Mu containing only one end of Mu can also transpose, though at strongly reduced frequencies. 'One-ended' transposition has been described for Tn*3* (Arthur *et al.*, 1984) and related transposons such as Tn*1721* (Mötsch and Schmitt, 1984) and Tn*21* (Avila *et al.*, 1984). These results suggested an asymmetrical replicative transposition reaction (Mötsch *et al.*, 1985). We show that during transposition of one-ended mini-Mu particular sequences on the plasmid can function as an alternative second end, supporting a symmetrical transposition model such as that proposed by Shapiro (1979). In this model both ends of the transposon first have to be brought together with the target site, and the subsequent strand transfer reactions have to occur at both ends in a concerted fashion.

Results and discussion

Transposition of mini-Mu that contain only one end of Mu was studied using the F factor POX38 as the target for transposition. Because POX38 lacks all known transposable elements, the background level of transfer of the plasmids containing the mini-Mu is much lower than when an F′ *pro*$^+$ *lac*$^+$ episome is used as the target, as was done in previous experiments (Groenen *et al.*,

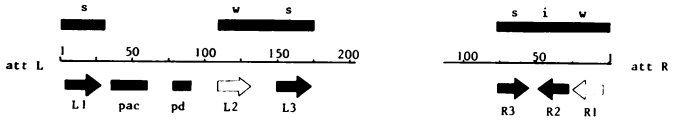

Fig. 1. A representation of the left (*att*L) and right (*att*R) end of bacteriophage Mu. The scale is in base pairs. The filled boxes at the top indicate the regions that are protected from nuclease digestion by the Mu A protein (Craigie *et al.*, 1984). S, strong binding: I, intermediate binding: W, weak binding of the A protein to that region. All these binding sites share a common sequence that is homologous to the consensus AARYRCGAAAR (indicated with an arrow). In the sequences L2, L3, R2 and R3 this consensus can be extended to YGtTTCAYtNNAARYRCGAAAR (indicated with a filled arrow). The arrows denote the orientation of these sequences. The position of the 12-bp palindrome (pd) between nucleotides 80 and 91 of the left end and the position of the site for packaging of the Mu DNA (pac) between nucleotides 35 and 58 of the left end (Groenen and van de Putte, 1985) are shown.

Table I. Transposition frequency of mini-Mu

Plasmid	Mu genes present	Ends of Mu present	Transposition frequency (%)
pGP618	*nerAB*	LR	5
pGP640	*nerAB*	L	4×10^{-3}
pGP618 Δ12	*nerAB*	R	4×10^{-3}
pGP641	*nerAB*	–	3×10^{-5}
pGP644	–	–	3×10^{-5}
pGP630 Δ66	*nerAB*	L + 27 bp of R	2×10^{-2}

The transposition frequency was measured using the mating out assay described in Materials and methods with the F factor pGP655 as the target. The transposition frequency is expressed as the percentage of the exconjugants (Str^R Tc^R) that are also CAM^R.

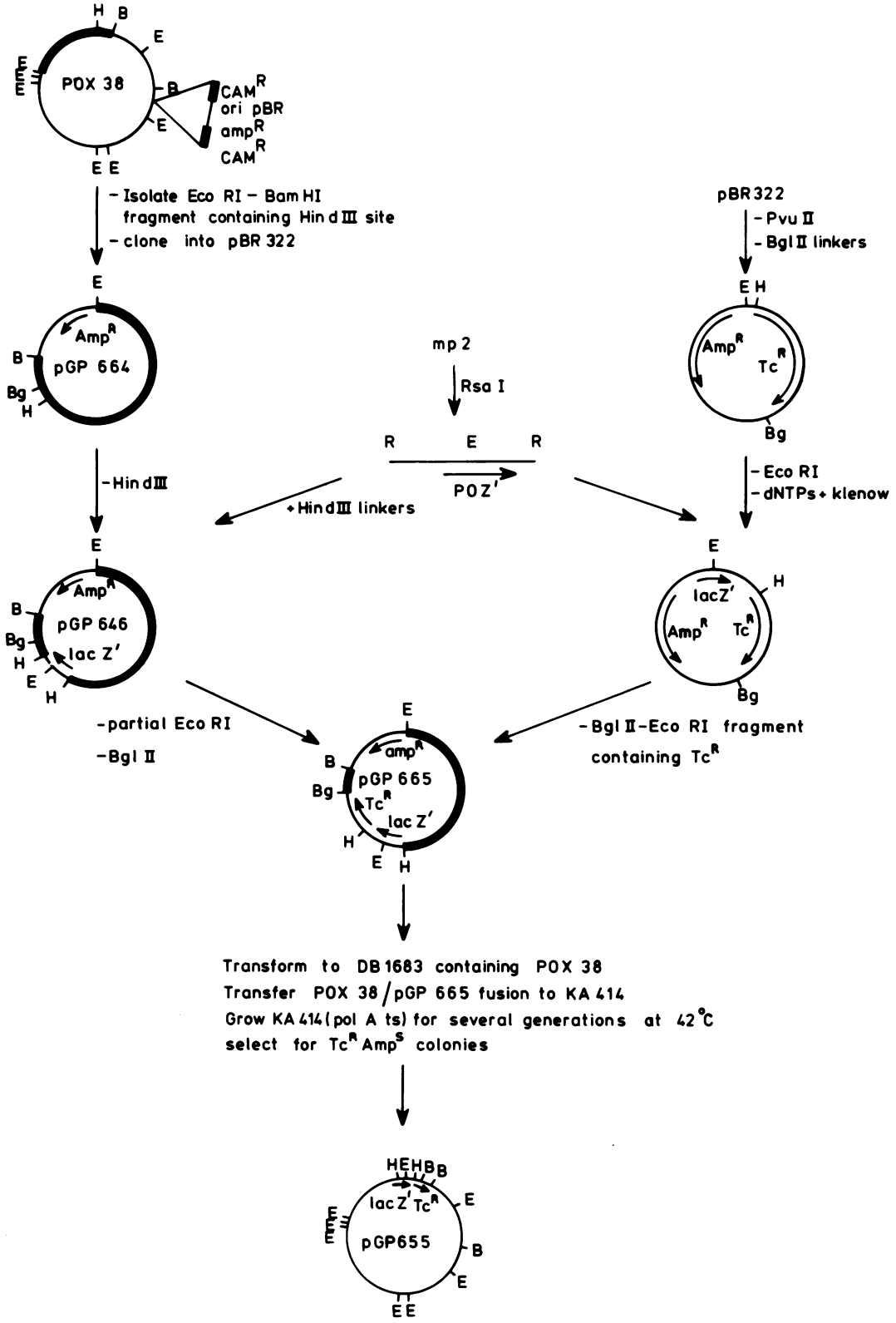

Fig. 2. Construction of pGP655. Plasmids are not drawn to scale. The F factor pGP655 was constructed by cloning the gene conferring Tc^R and the gene coding for the N-terminal part of the LacZ protein into the *Hin*dIII site of POX38. The *Eco*RI–*Bam*HI fragment of POX38 containing the *Hin*dIII site was isolated from a co-integrate between POX38 and pGP618 because this co-integrate has a much higher copy-number due to the presence of the origin of replication of pBR322. This fragment is indicated with a thick line. pGP655 was generated from pGP665 and POX38 by a double recombination event. Selection for this event was as indicated in the figure. The structure of pGP655 was verified by restriction enzyme analysis.

1985; Groenen and van de Putte, 1986). When pGP640 (Figure 4) or pGP618 Δ12 (Groenen *et al.*, 1985) which only contain either the left or right end of Mu respectively, were used in the transposition assay with POX38 as the target, it was found that induction of the *A* and *B* genes of Mu leads to an increased transfer of these plasmids. This was not observed when the ends

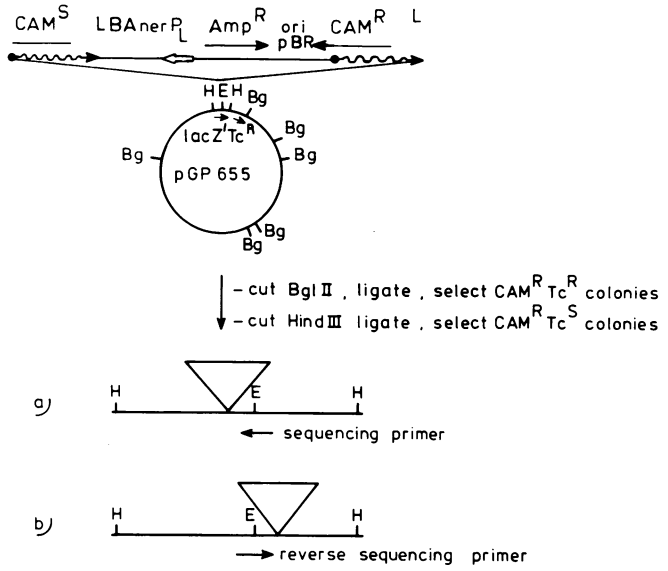

Fig. 3. Schematic representation of the isolation and sequencing of pGP655/pGP640 fusions. Shown is a co-integrate between pGP655 and pGP640 where a sequence in the middle of the CAM^R gene (●) is used as the alternative right end of the mini-Mu. Because a co-integrate is formed, one copy of the CAM^R gene is still intact, leading to a CAM^R phenotype. Simple insertions will be CAM^S and are not found as the selection is for CAM^R exconjugants. DNA of the fusions between pGP655 and pGP640 were isolated using the alkaline lysis method (Birnboim and Doly, 1979). The *Hin*dIII fragment of these fusions, containing the insertion into the *lacZ* gene were isolated as shown. Depending on the site of insertion into the *lacZ* gene the double-stranded DNA was sequenced using the m13 sequencing or reverse sequencing primers as described in Materials and methods. (E = *Eco*RI, H = *Hin*dIII, Bg = *Bgl*II.)

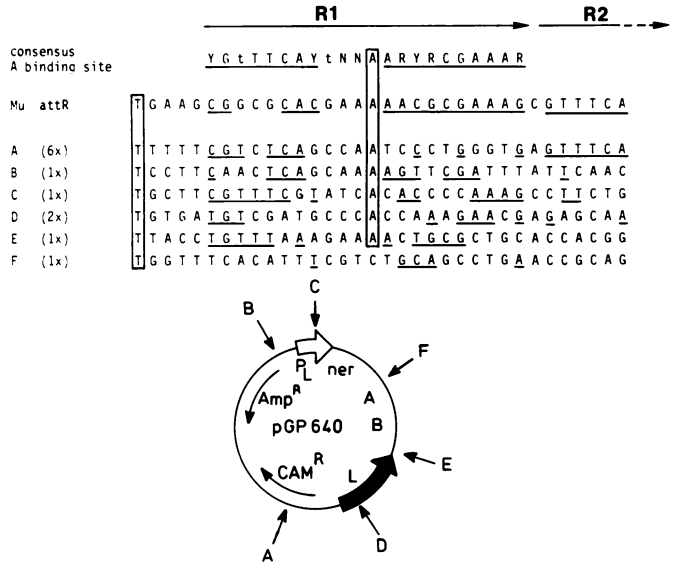

Fig. 4. DNA sequences used as an alternative end in one-ended transposition. The DNA sequence of the right 33 bp of Mu and the consensus for a strong A binding site are also shown. The T at position 1 and the A at position 17 are boxed. Further DNA homology with the conserved base pairs in the strong A binding sites are underlined. The position of the sequences in pGP640 are also shown.

of Mu were absent. Thus the increased transfer of the plasmids is dependent on the expression of *A* and *B* and on the presence of at least one of the ends of Mu. The frequencies of transposition of these one-ended mini-Mu were determined using the F factor pGP655 and are shown in Table I. Analysis of the plasmids from the recipient strain indicated that in most cases a fusion had been formed between the mini-Mu containing plasmid and the F factor and that a part of the plasmid had been duplicated, indicating the formation of co-integrates (data not shown). Thus the predominant end-products, as is found with mini-Mu containing both ends (Groenen *et al.*, 1985) are co-integrates. These fusions could have been formed by either one of the following mechanisms. (i) An asymmetrical replicative process could have taken place as proposed for one-ended transposition of Tn*21* and Tn*1721* (Mötsch *et al.*, 1985). According to this model, initiation of replication starts at the end of the transposon and the replication fork subsequently travels round the donor plasmid, passes through the original site of initiation and then terminates close to that site. (ii) Sequences on the plasmid homologous to either one of the ends of the transposon could have functioned as an alternative end. To examine which mechanism is involved in 'one-ended' transposition of the mini-Mu, an F factor was constructed that contains the gene conferring Tc^R and the N-terminal part of the *lacZ* gene (pGP655; Figure 2). Using this F factor as the target for transposition and pGP640, which contains the Mu left end (Figure 4), as the donor plasmid, in ~0.1–0.01% of the Tc^R CAM^R exconjugants the insertion had occurred into the *lacZ* gene. These Lac^- exconjugants were used for further analysis of the junctions of the insertions. The *Hin*dIII fragment of these F factors, which contains the mini-Mu insertion in the *lacZ* gene, was isolated and recloned in two steps as shown in Figure 3. Depending on the position of the insertion in the *lacZ* gene, either the sequencing primer or the reverse sequencing primer was used for sequencing. Using this system only one of the junctions of the fusion can be sequenced.

It was found that all insertions had occurred between position −30 and +60 with respect to the beginning of the *lacZ* gene. This suggests that some site preference exists for the integration of the mini-Mu, or alternatively that integration downstream of position 60 in the *lacZ* gene does not lead to a Lac^- phenotype. Because Mu integration can occur in either orientation we expected that in approximately half of the cases the sequence of the junction would be the normal left end of Mu while in the other half it would be an alternative sequence. In 14 out of the 26 independent insertions sequenced the first base pair of the left end of Mu was fused to *lacZ* sequences. This indicates that one of the junctions of the fusions always occurs precisely at the left end of the mini-Mu. The terminal sequences of the remaining 12 insertions map at six different positions on plasmid pGP640. These sequences and their position on plasmid pGP640 are shown in Figure 4. Five of these sequences show some homology (35–40%) with the right 27 bp of Mu, which contains the weak A binding site R1 (Figure 1). However, the homology between these sequences and the consensus sequence for a strong A binding site is considerably higher (42–73%). This suggests that if only one A binding site is present instead of three, as is the case in the right end of Mu, a strong binding site is preferred. Interestingly, at the left end, A binding site L1, which is not flanked by other A binding sites, is also a strong one.

The sequence that is found in the middle of the CAM^R gene (sequence A, Figure 4) was used in six of the 12 insertions. The homology between this sequence and the right end of Mu includes in addition half (GTTTCA) of binding site R2. We previously showed (Groenen *et al.*, 1985) that the presence of R2 is important for transposition of a mini-Mu. The transposition frequency of a deletion derivative that still contained the sequence GTTTCA of R2 was 20-fold higher than a deletion derivative which lacked it. Therefore the reason that sequence

A (Figure 4) is used most frequently as an alternative end is probably related to the presence of its homology with both R1 and R2. Although none of the conserved base pairs in the A binding site is present in all the sequences shown in Figure 4, the A at position 17 is present in five of these six sequences, indicating the importance of this A at this position in the A binding sites. Moreover, the only sequence where this A is not present (sequence F, Figure 4) has no apparent homology with the consensus for an A binding site.

The results show that these fusions most likely are generated by a mechanism where sequences on the plasmid function as an alternative second end. The only exception seems to be sequence F (Figure 4) which has no apparent homology with the consensus for the A binding sites. However, because the Mu A protein has general DNA-binding properties non-specific binding of A to DNA may occasionally lead to a transposition event.

The extreme ends of bacteriophage Mu form the 2-bp terminal inverted repeat 5′ ($^{TG}_{AC}$) 3′. It was shown that mutations of the first base pair ($^{T}_{A}$) severely reduced transposition (Burlingame *et al.*, 1986) while a transposition of the ($^{G}_{C}$) to an ($^{A}_{T}$) base pair, had hardly any effect on transposition (Groenen and van de Putte, 1986). This is in good agreement with the present results since all the sequences shown in Figure 4 have a T at position 1, whereas any nucleotide seems to be allowed at position 2. Mizuuchi (1984) showed that during the strand transfer reaction at the initiation of transposition, a single-stranded cut is made adjacent to the first base pair of the end of Mu and that subsequently this 3′ end of Mu is attached to the 5′ protruding end of the target DNA. Our results indicate that this reaction can only take place if the first base pair is ($^{T}_{A}$).

The results presented above are also interesting in view of possible models for the evolutionary origin of transposable elements. Our results support an evolutionary model like the one proposed by Sasakawa *et al.* (1983). According to this model, a simple transposable element may have evolved from a gene encoding a DNA binding protein with topoisomerase activity. Initially one (or even both) of the ends of the transposon may not have been precisely defined and sites with affinity for the protein may have been used as an end. Due to selective pressure, certain sites eventually may have evolved to the 'real' ends of the transposon. The complex organization of binding sites in the ends of Mu may have been generated by duplication of transposase binding sites and/or transposition next to other potential binding sites. Such events would only have been advantageous if there was selective pressure for a high level of transposition. This seems to be the case with bacteriophage Mu which for its replication is dependent on transposition which therefore has to be very efficient.

Materials and methods

Recombinant DNA techniques and DNA sequencing

Restriction endonuclease, T4 DNA ligase and Klenow fragment of DNA polymerase I were purchased from Boehringer Mannheim, Bethesda Research Laboratories and Promega Biotec. *Hin*dIII linkers d(CAAGCTTG) were from Amersham and *Bgl*II linkers d(CAGATCTG) were a gift from J.Maat of Unilever Research Laboratories in Vlaardingen. Incubation conditions were as recommended by the suppliers. Dideoxy-sequencing reactions (Sanger *et al.*, 1977) using CsCl purified dsDNA as template were done as follows. Plasmid DNA was linearized with *Hin*dIII in 10 μl (6 mM Tris−HCl pH = 7.4, 6 mM $MgCl_2$, 6 mM DTT, 50 mM NaCl). After addition of sequence primer, the DNA was denatured for 4 min at 100°C and immediately cooled on ice. Sequencing reactions were performed as described by Sanger *et al.* (1977).

Bacterial strains and plasmids

The bacterial strains used were KMBL1164 [Δ (*lac-pro*)XIII, *thi*209, *sup*E; our laboratory), PP135 [KMBL1164 (λ)], ED8817 (*met*B, *lac*Z, Δm15, *sup*F, *sup*E, *hsd* δ^-; Prof. W.J.Brammar), PP1884 [ED8817 (λ)], PP1885 [a streptomycin resistant (Str^R) derivative of PP1884], DB1683 (Δ*trp*E5, contains POX38; Dr D.E.Berg) and KA414 (*pol*A12, *thy*204, *rha*104, *lac*212, Str^R, su^+; strain MM383 of Dr M.Monk). Plasmid pC1857 (Remaut *et al.*, 1981) contains the temperature-sensitive repressor CI857 of bacteriophage λ. POX38 (Guyer *et al.*, 1979) is a 55-kb F factor lacking all known transposable elements. pGP618, pGP618 Δ12 and pGP630 Δ66 have been described previously (Groenen *et al.*, 1985). pGP640 was constructed by deleting the *Bam*HI−*Hin*dIII fragment of pGP630 (Groenen *et al.*, 1985) containing the right 216 bp of Mu. pGP644 was constructed by cloning the *Pst*I fragment of Tn*9* containing the CAM^R gene into the *Pst*I site of the Amp^R gene in pPLc2833. pGP641 is a derivative of pGP644 containing the *ner*, *A* and *B* genes of Mu under control of the P_L promoter of bacteriophage λ. The other plasmids used are described in Figure 2. Plasmids and F factors were isolated using the alkaline/SDS method (Birnboim and Doly, 1979).

Assay of transposition by conjugation

Transposition of mini-Mu sequences to an F factor was monitored by a conjugation assay as described (Groenen *et al.*, 1985). The F factors used are POX38 and pGP655. The latter is a derivative of POX38 containing the gene conferring tetracyclin resistance (Tc^R) (Figure 2). Using pGP655, the percentage of the exconjugants (Str^R, Tc^R) that is chloramphenicol resistant (CAM^R) is a measure for the transposition frequency of the mini-Mu. The values given for the transposition frequency are means of the values of 3−5 independent experiments.

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