Transposon targeting determined by resolvase
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Abstract

The Mu-related transposon Tn5090, also called Tn402, was observed to be highly selective for targets clustered in or close to recombination sites of serine-type recombinases in plasmids R388 and RP1. Transposition to the par area of RP1 responded strongly to a deletion in the gene of resolvase ParA. A search in sequence databanks revealed further insertions of Tn5090/Tn402 close to different genes of resolvases. These results imply that the target selection of Tn5090 depends on a property that is shared among several serine recombinases. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Transposon; Target selection; Resolvase; Site-specific recombinase; Phage Mu; Integron carrier

1. Introduction

Transposons are discrete nucleic acid sequences carrying instructions for relocating in the DNA. The transfer of transposons is unrestricted by sequence homology and the target site selectivity varies widely among different transposons [1]. In this work we describe a new transposon targeting mechanism which is highly dependent on site-specific recombinases of the serine (resolvase/invertase) class [2].

The ends of transposons form inverted repeats where a specialised protein, transposase, binds and executes coordinated cleavage and joining reactions in the transfer process. A few transposons known as Mu-like (e.g. Tn5090/Tn402 [3,4], Tn7 [5], Tn552 [6] and Tn5053 [7]) carry extended patterns of transposase binding motifs on either end and require at least one protein in addition to the transposase for efficient transposition. All Mu-like elements encode a nucleotide binding protein (TniB in Tn5090/Tn402 [4]) whereas a cryptic protein (ORF6 in [4], TniQ in [7]) is exclusive for Tn5090/Tn402 (Fig. 1) and the related Tn5053.

The transposons within the Mu family show wide variation with respect to their target selectivity. Tn7 is highly preferent for transposition to a unique site in the chromosomes of several bacterial species [1,8] whereas transposon Tn552, by contrast, inserts close to randomly [9]. Mu integrates with an even distribution in the Escherichia coli genome but careful study has disclosed patterns of region and nucleotide preference [10].

Transposons Tn502, Tn5053 and Tn5090/Tn402 have been reported to integrate in par of RP1 [7,11,12]. This target selectivity of Tn5090/Tn402 was further analysed in this work with the goal of disclosing a mediator protein. The border sequences of more than 30 independent insertions in IncP plasmid RP1 and several in plasmid R388 of IncW were determined.

2. Materials and methods

2.1. E. coli strains and plasmids

The strains JSR58b harbouring a lambda::Tn402 prophage [3] and HB101 (F- hsdS20 rK mK recA13 ara-14 proA2 lacY1 galK2 rpsL20 (Smr) xyl-5 mtl-1 supE44 lambda−) carrying pLKO650 were used as transpositional donors. For transposon isolations by the mating-out method, C600 (F' lacY1 leuB6 supE44 thi-1 rpsL20 (Smr) rpsL20 (Tet) thi-1 lonA21 1′ (Nam)') was used as recipient. Transposition to non-conjugative plasmids was studied using JSR58b as donor. Transposition was assayed after retransformation of plasmid to DH5α (supE44 ∆lacU169 (φ80lacZ' M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA) and DH5α (supE44 ∆lacU169 (φ80lacZ' M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA) and DH5α (supE44 ∆lacU169 (φ80lacZ' M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA). The following antibiotics were used at 50 µg ml⁻¹: ampicillin (Ap), chloramphenicol (Cm), kanamycin (Km) and trimethoprim (Tp), while tet-

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racycline (Tc) and nalidixic acid (Nal) were used at 25 µg ml⁻¹. Sulfathiazole (Su) was used at 0.5 mM. Selection with Su or Tp was made using Iso Sentitest medium (Oxoid). Plasmids used in this study are listed in Table 1. The S11C mutation in ParA cloned to yield pLKO821 was introduced using the method described by Vandeyar et al. [13]. The mutagenic primer 5'-CTACCTGCGCGCCAGAGACGAGACGAGACGAG-3' was used.

2.2. Transposition assays

Transposition to the conjugative plasmids RPIΔA1 or R388Δ1 was examined in exconjugants from matings between a strain harbouring Tn5090/Tn402 and the recipient strain C600 (Nal'). The donor strains harboured the transposon on the chromosome (JSR58b) or on a plasmid (pLKO650). The constructs were restreaked twice and incubated for a total of 3 days at 30°C before mating. The mixed mating cultures were applied to filter membranes on the surface of non-selective plates. After conjugation for 3 h at 30°C the cells were plated with selection. When RPIΔ1 was used as recipient the mixed growth was plated on Km/Nal and Km/Nal/Tp media. Matings of R388Δ1 were plated on Su/Nal and Su/Nal/Tp media. The transposition frequencies were calculated as a ratio of the number of colonies on the plates containing Km/Nal and Km/Nal/Tp, respectively. The transposition frequency was calculated as a ratio of the number of colonies on Ap/Tp plates to the total number of transfectants (Ap plates).

Non-conjugative plasmids carrying various resolution systems were passaged through JSR58b. The transposition constructs were restreaked twice as described above. The target plasmid pool was isolated by using QIAprep Spin Miniprep (QIAgene) and retransformed to DH5α by electroporation at 200 Ω, 25 µF and 2.5 kV in a 0.2-cm cuvette, using a Bio-Rad Gene Pulser II. Selection for plasmids harbouring or lacking Tn5090 was achieved by plating the cells on media containing Ap/Tp and Ap, respectively. The transposition frequency was calculated as the ratio of the number of colonies on Ap/Tp plates to the total number of transformants (Ap plates).

3. Results and discussion

Tn5090/Tn402 insertions in RPIΔA1, from 27 independent experiments, revealed eight unique target sites in two clusters within 144 bp of the par region. One cluster of three sites (cluster 1) coincided with subsite II of the resolvase-binding site, res [14]. The second cluster (cluster 2) of five sites was located in parC, a gene in the same operon as the resolvase gene, parA [15] (Fig. 2A). Most insertions of cluster 2 were within 5 bp with an outside 24 bp upstream of the others. The insertions in cluster 1 were oriented with the i-end of Tn5090/Tn402 toward parA where-as all insertions in cluster 2 appeared with the t-end pointing toward parA (Fig. 2A).

Sequence analysis of 11 experimentally independent insertions of Tn5090/Tn402 in R388Δ1 (Table 1) revealed 10 identical insertions and a single insertion a short distance (19 bp) away (Fig. 2B). The lower transposition frequency to R388Δ1 compared to RPIΔ1 could be a partial immunity effect due to the presence of a resident transposon end in the former plasmid (Fig. 2B). All Tn5090/Tn402 insertions in R388Δ1 were unidirected. The two IncW plasmids R388Δ1 and pSa are supposed to be identical apart from different integrons. A sequence of pSa retrieved from nucleotide sequence databases (accession number U30471) revealed the presence of a likely resolution system close to the targeted area (Fig. 1). The primary target site in R388/pSa was localised 51 bp upstream of the resolvase gene, orf2, and not far from a probable res site (Fig. 2B).
Sequence comparisons among target sites observed in experiments or retrieved from databases (see below) did not disclose a consistent nucleotide consensus for insertion sites. However, one of the sites in RP1 contained the 5-bp target duplication of ATTTA, which was identical to the duplication of the most abundantly used site in R388Δ1 (Fig. 2).

Thus Tn5090/Tn402 transposition was highly biased for insertion at the binding sites of serine recombinases in RP1 and R388. To test if the RP1 resolvase was used in target determination, a new recipient plasmid (pLKO821) harbouring the parCBA operon and the res site of RP1 was constructed. A parA-truncated derivative (pLKO822) was constructed in parallel. The plasmid with an intact resolvase gene, pLKO821, was an efficient target (transposition at 10^{-4}). The transposition frequency was 1000-fold lower when using the parA-deficient plasmid pLKO822 as target (Table 1). This decrease indicates that targeting depends on the presence of resolvase with an intact DNA-binding domain.

The invariant serine around position 10 in the serine family of site-specific recombinases is documented to be essential in DNA strand cleavage and joining [2]. To investigate whether recombinase activity is required for target recognition of Tn5090/Tn402 the serine in position 11 of parA was substituted by a cysteine (pLKO821-S11C). The transposition frequency using this mutant as a target was similar to that observed with the wild-type plasmid pLKO821 (Table 1). This rules out that progressing site-specific recombination is required to generate targeting determinants.

Not all tested resolution systems documented a targeting function. In our assay neither TnIC (pLKO655, see Table 1; Kamali-Moghaddam et al., unpublished) [4,7] nor the closely related TnpR of Tn163 (pAUM2) [16] gave detectable transposition. Also TnpR of Tn5 [17] and the Gin/gix inversion system of Mu [18] (pGP273 containing gix and Gin expressed from pLKO824) were targeting-negative.

A database search recorded all sequenced insertions of Tn5090/Tn402 that are close to resolvase genes (Fig. 1). Some of these belong to plasmid segregational mechanisms (e.g. RP1 [14]) whereas others belong to class 2 transposons including TnpR of Tn21 [19]. The postulated Tn21 ancestor, Tn2163 [20], and another similar progenitor element [21] are supposed to have attracted independent insertions of Tn5090/Tn402 into equivalent internal sites close to res to form Tn21 and Tn1403, respectively.

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The par locus of RP1 [15] is missing in the related IncPβ plasmid R751 [22] that is the documented source of Tn5090/Tn402 [3,4]. It is possible that Tn5090/Tn402 in R751 originate from two original copies of the transposon in par. These could have recombined with one another to create a deletion of par. Such an event is indicated also by the absence of the usual 5-bp target duplication at the ends of the resident Tn5090/Tn402 in R751 [4]. Several RPl1::Tn5090/Tn402 isolated in this work were sequenced on both ends which confirmed that the target duplication comprises 5 bp (Fig. 2).

The resolvase-determined targeting mechanism pre-
Table 1

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Relevant characteristics</th>
<th>Transposition frequency</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC18/19</td>
<td>ApR, ColEl ori</td>
<td>&lt; 10⁻⁸</td>
<td></td>
</tr>
<tr>
<td>pSU18/19</td>
<td>CmR, p15A ori</td>
<td>&lt; 10⁻⁸</td>
<td></td>
</tr>
<tr>
<td>RPIΔ1</td>
<td>ApR, KmR, Te3ΔTnI</td>
<td>1 x 10⁻⁴</td>
<td>Two contiguous BsrEI fragments of tnpRA in TnI of RP1 [25] were deleted</td>
</tr>
<tr>
<td>R388Δ1</td>
<td>SuR, TpR, res-orf2 of R388</td>
<td>5.3 x 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>pLKO8022⁺</td>
<td>ApR, res-parCB/Δ(parA)</td>
<td>5 x 10⁻⁸</td>
<td>HindIII-SphI fragment in pLKO821, encoding 43 amino acids in the C-terminal of ParA, deleted</td>
</tr>
<tr>
<td>pLKO650B⁺</td>
<td>CmR, TpR, Tn5090/Tn402</td>
<td>&lt; 10⁻⁸</td>
<td>8.5-kb PstI–PstI fragment of R751 comprising the entire Tn5090/Tn402 inserted in pSU19</td>
</tr>
<tr>
<td>pLKO823⁺</td>
<td>ApR, res-tnpR of Tn3</td>
<td>&lt; 10⁻⁸</td>
<td>845-bp PCR fragment of Tn3 (R141d19) in HindIII–XbaI of pUC19</td>
</tr>
<tr>
<td>pAUM2</td>
<td>TeR, res-tnpR of Tn163</td>
<td>&lt; 10⁻⁸</td>
<td>[16]</td>
</tr>
<tr>
<td>pGP273</td>
<td>TeR, gix of Mu</td>
<td>&lt; 10⁻⁸</td>
<td>[18]</td>
</tr>
<tr>
<td>pLKO824⁺</td>
<td>CmR, gin of Mu</td>
<td>&lt; 10⁻⁸</td>
<td>XbaI–BamHI fragment of pGP1188 [18] in pSU19</td>
</tr>
</tbody>
</table>

*Fragments containing the respective recombinase gene and res transcribed from the lac promoter in the vector plasmid.

*Effect of Gin in trans assayed.

sent in this work was recently published by others when this article was still in manuscript [23]. There are, however, interesting distinguishing results in these two reports. The report by Minakhina et al. [23] describes responses to deletions in the subsite I of res in Tn1721, where DNA strand breakage and exchange occurs. This could indicate that resolvases must be bound to res to efficiently promote targeting. Only sporadic and very infrequent transposition at the usual target sites (not shown) was observed in the presence of resolvase mutation that eliminates DNA binding (Table 1). The efficient transposition to par in RP1 in the presence of the S11C mutation in pLKO821 (Table 1) indicates that targeting is independent of the catalytic capacity of the resolvase. Despite a high similarity between Tn5090/Tn402 and Tn5053 [7] the former element was found in this work to be inserted in both orientations in two clusters in par (Figs. 1 and 2A). Minakhina et al. [23] describe unidirected insertion of Tn5053 in cluster 1 in the presence of parAB and parDE. Both orientations have been found in the absence of parAB or parDE [23]. Tn5090/Tn402 transposition disclosed an additional cluster of sites in RP1 (cluster 2) in parC and just outside res. Insertions in clusters 1 and 2 occurred at a similar frequency (Fig. 2A) but in opposite orientation.

Because targeting is independent of the catalytic activity of the resolvases (see above) and requires a complete res (or at least a res containing subsite I) [23] it is possible that the ‘correct’ nucleoprotein assemblies formed by resolvases [2] define the targets. Although recognition could depend on direct contacts between resolvase and transposase, other mechanisms are also possible. The proteins TnIB and TnI Q [4] should be given particular attention in the target recognition process.

Tn5090/Tn402 and Tn7 mediate high selectivity for target sites and thus connect their mobile gene cassettes to programmed horizontal transfer [11]. Otherwise, the bio-

 logical purpose of transposon targeting in the vicinity of resolution systems is not obvious. One explanation could be an addiction mechanism for maintaining Tn5090/Tn402 on plasmids. In several of the instances reported here, transposition has occurred directly into the res sites. The transposon’s ends could rescue the function of res as was described in R46 [24] or the resolution system, TniC, of Tn5090/Tn402 could substitute for loss of the external resolution system. One point with clustering transposons close to res is that it potentially facilitates the acquisition of resolution systems to transposons. The high degree of sequence similarity between the resolution systems in Tn163 and Tn5090/Tn402, contrasting with the Tn3-like organisation of the former and Mu-like organisation of the latter, illustrates that resolution systems are reshuffled amongst different transposons.

Acknowledgements

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