Insertions of resistance genes into Tn21-like transposons

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The evolution of Tn21 and related multiresistance transposons of natural and artificial origin is described. Variations resulting from insertions and deletions in these transposons indicate the presence of specific recombination sites. There is evidence that these 'hot spots' are flanking several resistance genes. We examined these structures on the basis of the aadA gene, mediating resistance to spectinomycin and streptomycin. Comparison of the DNA sequences of Tn21, Tn7 and pSa indicate the wide spread of these recombination sites.

Introduction

Evolution as reflected in phenotypic variation depends on the aberration of genetic material. Mechanisms of homologous and illegitimate recombination are involved in these variations (Holliday, 1964; Kleckner, 1981).

On the basis of Tn21 and related transposons we developed a scheme describing the possible evolution and the relationship of several transposons, including in-vivo and in-vitro recombinants. Tn21 is known to harbour genes encoding resistance towards mercuric chloride (mer), sulphonamides (sul), spectinomycin/streptomycin (aadA) and the transposition functions transposase (mpA) and resolvase (mpR) (De la Cruz & Grinsted, 1982). One source for Tn21 is plasmid R538-1, synonymous with R100.1.

In some Tn21-related transposons such as Tn2424 and Tn2426 we found several additional genes next to the aadA gene, a 3′(9)-O-nucleotidyltransferase. It is predicted that this gene would be flanked by recombinational 'hot spots' (Schmidt, 1984). These recombination sites seem to be one mechanism for the evolution of multiresistance transposons (Wiedemann et al., 1985). Recently we described sequence variations in a genetic area between the mer and the sul genes, based on the existence of IS161 (Meyer et al., 1985). As the aadA gene is known to be widespread, we compared several Tn21-related transposons with Tn7 and the plasmid pSa, containing the resistance gene.

Methods

The plasmids and bacterial strains used are listed in Table I. Methods, including electron microscopy, DNA ligation, cloning experiments and transformation procedures, have been described before (Meyer, Nies & Wiedemann, 1983).

Resistances towards different drugs are abbreviated as follows: HgR (mercuric chloride) 500 mg/l, SuR (sulphonamides) 500 mg/l, SmR (streptomycin) 500 mg/l, SpecR 85

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Table I. Bacterial strains, plasmids and transposons

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant characteristics*</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>E. coli W3110</td>
<td>Nx^r lac</td>
<td>Grinsted et al. (1972)</td>
</tr>
<tr>
<td>E. coli JC2926</td>
<td>rpsL recA thi arg his lac</td>
<td>Bachmann (1983)</td>
</tr>
<tr>
<td>E. coli SK1592</td>
<td>gal thi ton sbc815 hsr rec</td>
<td>Kushner (1978)</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1767</td>
<td>Ap^R Su^R Sm^R Spec^R Cm^R As^R Te^R Hg^R Coll tra</td>
<td>Richmond &amp; Wiedemann (1974)</td>
</tr>
<tr>
<td>NR79</td>
<td>Hg^R Su^R Spec^R Ami^R Cm^R Te^R tra</td>
<td>Beneviste &amp; Davies (1971)</td>
</tr>
<tr>
<td>pBP84</td>
<td>Ami^R Sm^R Spec^R Gm^R</td>
<td>This paper</td>
</tr>
</tbody>
</table>

| Transposons   |                           |                                 |
| pBR322::Tn241 | Ap^R Te^8 Hg^R Su^R Sm^R/Spec^R | Kratz et al. (1983)             |
| pBR322::Tn242 | Ap^R Te^8 Hg^R Su^R Sm^R/Spec^R | Kratz et al. (1983)             |
| pBR322::Tn243 | Ap^R Te^8 Su^R Sm^R/Spec^R    | Kratz et al. (1983)             |
| pUB307::Tn244 | Ap^R Hg^R Su^R Sm^R/Spec^R Cm^R Ami^R | Meyer et al. (1983) |
| pUB307::Tn246 | Hg^R Su^R Cm^R Ami^R Gm^R Sm^R/Spec^R | This paper                     |

* See Methods for abbreviations.

(spectinomycin) 50 mg/l, Cm^R (chloramphenicol) 30 mg/l, Te^R (tetracycline) 20 mg/l, Ap^R (ampicillin) 50 mg/l, Ami^R (amikacin) 8 mg/l and Gm^R (gentamicin) 10 mg/l.

The designation of genotypes follows the nomenclature of Bachmann (1983).

Results

Insertions in Tn21-related transposons

The conjugative R-factor system R1767 (Richmond & Wiedemann, 1974) is known to be intrinsically unstable, even in recA strains (Wiedemann, 1981). This observation resulted in the discovery of the TN21-related transposon Tn2411. A recombination derivative of Tn2411 is Tn2410, where the aadA gene is substituted by an OXA gene (Nies et al., 1985) (Figure 1).

Related variations have also been observed in the study of Tn2424 (Hg^R Su^R Cm^R Ami^R Sm^R; Meyer et al., 1983), a transposon from plasmid NR79, carrying an insertion of a Cm and Ami resistance encoding region in front of the aadA gene. Another related, but artificial, transposon with phenotypic aberrations is Tn2426. Via recombination of Tn2424 with pBP201 (Schmidt & Klopfer-Kaul, 1984), the ANT-2' gene, coding for a gentamicin-modifying enzyme in pBP201, has been inserted behind the aadA gene, resulting in Tn2426 (Hg^R Su^R Cm^R Ami^R Sm^R Gm^R). R46, a plasmid including a Tn21-related region, has been another source of frequent recombinations (Figure 2). In Tn21 and Tn2427 no similar variations could be detected.

Deletion analysis of pBP84

To examine these recombinational events more precisely a PstI BamHI-fragment of Tn2426 has been cloned in pBR322 resulting in the plasmid pBP84 (Ami^R Sm^R Gm^R). Spontaneous deletions in this plasmid resulted in several products, named pBP84 1–10,
Insertions in Tn21-related transposons

Figure 1. Schematic representation of the homology between Tn21 and the related transposons received from R1767.

including deletions of single genes as well as those of adjacent genes (Figure 3). In Escherichia coli W3110 (rec\(^+\)) the deletion occurs with frequencies of \(10^{-2}\), in E. coli JC2926 (recA) these frequencies decreased to \(10^{-3}\) (Table II).

In electron microscopy of heteroduplex structures the terminals of deletion could always be located at the ends of the \(aadA\) gene, about 6±0.12 kb respectively 5.1±0.08 kb away from the right IR of Tn2426. These are the same loci acquired for insertions. Tn7 also shows a similar recombination activity (Barth, Grinter & Bradley, 1978).

Structural analysis of recombination sites

To predict the structure of these recombinational 'hot spots' we compared \(aadA\) genes of different origin. It could be shown that the genes taken from structures without

Figure 2. Schematic structures of several Tn21-related transposons, and the recombination of Tn2424 with pBP201 resulting in Tn2426 and its derivatives.
increased deletionary frequencies were lacking a functional promoter of their own. This had been proved by inhibiting the coupled transcription in Tn21 with the \textit{sul} gene. Cloning a fragment including a stop codon into the \textit{sul} gene made these clones become sensitive to spectinomycin.

Electron microscopy of hybridized promoter-deficient and promoter-including genes showed no aberrations, so that the recombination sites are probably smaller than 100 bp (resolving power of electron microscopy). Restriction analysis predicts a length of about 50 bp.

With the experiment shown in Figure 4 we have been able to demonstrate that the deleted fragments can build separate plasmids, as predicted for direct repeats (Albertini \textit{et al.}, 1982). Reinserting the deleted fragment, the precise insertion in front of the \textit{aadA} gene suggests that the deleted structures carry one copy of the recombination sequence. The frequencies of reinsertions were about $10^{-7}$ to $10^{-8}$ in

\begin{table}[h]
\centering
\caption{Deletion frequencies}
\label{table:deletion_frequencies}
\begin{tabular}{lll}
\hline
 & \textit{E. coli} W3110 (\textit{rec}+) & \textit{E. coli} JC2926 (\textit{recA}) \\
\hline
\texttt{pBP84} & $4 \times 10^{-2}$ & $4 \times 10^{-3}$ \\
\texttt{Tn2426} & $5 \times 10^{-3}$ & $6 \times 10^{-3}$ \\
\texttt{R46} & $2 \times 10^{-2}$ & $2 \times 10^{-3}$ \\
\texttt{Tn21} & 0 & 0 \\
\texttt{Tn2427} & 0 & 0 \\
\hline
\end{tabular}
\end{table}
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Figure 4. Representation of the experiment to isolate and reactivate the deleted aacA gene for reinsertion.

E. coli W3110 (rec+); in E. coli JC2926 (recA) the deletion frequency was outside the detection limits.

Recently some aadA genes have been sequenced and the flanks of these genes partly include potential sequences similar to the predicted 'hot spots'. The sequences of Tn21 (Hollingshead & Vapnek, 1985), Tn7 (Fling, Kopf & Richards, 1985) and the plasmid pSa (Tait et al., 1985) have been published (Figure 5). While Tn7 includes proximal and distal repeats, Tn21 and pSa lack the proximal repeat. The proximal repeat of Tn7 has a length of 54 bp and includes a functional promoter region (Rosenberg & Court, 1979). The homologies of the distal repeats exceeded 74%; Tn21 and Tn7 differed only in the insertion of one base.

Discussion

Published data suggest that several mechanisms are involved in the evolution of multiresistance transposons. In addition to the recombination via IS-elements (Meyer et al., 1985) we described recombinational 'hot spots' as one possibility for evolution of resistance plasmids. The predicted recombination loci seem to flank the genes as direct repeats of 54 bp length, including a functional promoter in the proximal repeat.

The results imply that two different mechanisms could be involved for insertion and deletion. While deletions seem to be a recA independent mechanism, insertion seems to afford recA functions. The deletions could be explained with a slipped mismatch; a possible negation of the repeat flanked region by DNA-polymerase during replication (Albertini et al., 1982). This explanation is supported by the fact that the direct repeats include inverted repeats as a secondary structure (Fling et al., 1985), which are predicted to be necessary for exact excision of procaryotic transposable elements (Albertini et al., 1982). A possible mechanism for the evolution of Tn21-related transposons is shown in Figure 6.
Figure 5. Nucleotide sequences of several aadA gene flanking regions. I shows the promoter region in the proximal repeat of Tn7. II compares the proximal and distal repeats in Tn7, showing a homology of about 90.8%. III demonstrates the homology of the distal flanks of Tn7 (upper line), Tn21 (middle line), and pSa (lower line).

The high ratio of insertions and deletions as well as the wide spread of these recombination sites suggest that this mechanism could be a basic step in evolution of multiresistance, mediated by recombination of resistance genes in a block. This is supported by the fact that these sites have been found in different structures, such as Tn21, Tn7 and pSa. Figure 7 shows the possible evolutionary relationships of several multiresistance transposons involving these sites. The possible existence of Tn21X is supported by the fact that Tn21 includes a few base pairs of the predicted promoter structure (proximal repeat) in front of the aadA gene. An insertion, deletion or substitution could have destroyed this sequence, resulting in a promoter-deficient aadA gene.

Figure 6. Model for the evolution of Tn21-related transposons. Deletion of a resistance gene including one recombination sequence (black box) leads to a distinguished structure able to insert beside an aadA gene, including intact direct repeats.
It may be possible to find related sequences flanking other genes in other transposons and plasmids also responsible for evolution. To determine whether those structures belong to a group of degenerated transposons as implied by Heffron (1983), will require further experiments.

Acknowledgements

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References


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