Functional and physiological characterization of the Tn21 cassette for resistance genes in Tn2426

MICHAEL T. ZÜHLSDORF and BERND WIEDEMANN

Institut für Medizinische Mikrobiologie und Immunologie, University of Bonn, Germany

(Received 11 June 1992; revised 5 November 1992; accepted 18 December 1992)

The Tn21 subgroup of class II transposons plays an important role in the dissemination of resistance genes and especially in the epidemic spread of multi-resistance. This ability reflects the variety of resistance genes that associate with the streptomycin/spectinomycin-resistance gene (aadA) of Tn21. Deletion experiments with Tn2426, a typical member of the Tn21 subgroup, and sequencing of the region that accommodates additional resistance genes revealed significant structural characteristics. Each resistance gene was flanked by short, directly repeated recombinationally active sequences with unexpected variability in their sequence and length. The consensus for a recombinationally active sequence appeared to be 13 bp in length (TAAAACAANGNNA), compared to previous estimates of 54 bp. This sequence, in combination with the product of the integrase gene, is responsible for the genetic variability of members of the Tn21 family of transposable elements and the dissemination of multi-resistance.

Introduction

Bacterial resistance is one major reason for the failure of antimicrobial chemotherapy. High-level antibiotic resistance can be attributed mainly to specific resistance genes originating from cellular precursor genes via mutation or by the acquisition of additional genes from other bacteria. R-plasmids are the major vector for these acquired genes and many of these plasmids carry transposons which harbour the resistance genes and play an important role in their dissemination. Beside genes encoding antibiotic resistance or other additional functions, all transposons share the property of encoding specific enzymes that promote and/or regulate their own transposition.

According to Kleckner (1981), transposons can be divided into two classes: class I transposons or composite transposons are flanked by two copies of an IS element whereas the more homologous class II transposons are flanked by short inverted repeats. The class II transposons divide into two subgroups, the Tn3- and the Tn21-like families. The elements in both subgroups encode two transposition proteins: the transposase, TnpA, which interacts with the highly specific, repeated outer extremities of the transposon, and the resolvase, TnpR, which interacts with the internal resolution site (IRS or res) to mediate the resolution of the cointegrate intermediate (Grinsted et al., 1990). The families differ mainly in the orientation of the transposition genes and their regulation.

Whether or not the regulator of resolvase, TnpM (Hyde & Tu, 1988), found exclusively in some members of the Tn21 family (Tn21 and Tn501) is involved in the regulation of the transposition is still in doubt (Grinsted et al., 1990).

Another basic difference between the Tn3- and Tn21-like transposons is the diversity of their resistance genes and the stability of the basic backbone. Tn3-like elements encode mainly TEM-β-lactamase enzymes whereas the Tn21-like transposons encode a great variety of resistance genes (Wiedemann et al., 1986). This variability is primarily due to the presence of a site-specific integration system (Grinsted et al., 1990), consisting of an integrase (Mercier et al., 1990) and short, recombination sequences (RSs), that act as targets for the integrase (Wiedemann et al., 1986). Mapping studies on several Tn21-like transposons (Fig. 1) have identified two specific recombination sites in Tn21 flanking the aadA
gene. These loci are involved in all observed recombinational events within the region, including integration, substitution and deletion events.

In this work, we have investigated this recombinationally active region of the Tn21-like transposon Tn2426 and compared it to analogous sequences from other Tn21-like transposons. The sequence data might be expected to reveal information about the RSs and the insertion or junction points of the additional genes in the so-called 'gene cassette' (Sundström et al., 1991) or 'integron' (Hall et al., 1991).

### Methods

**Bacterial strains and plasmids.** All bacterial strains and plasmids used are listed in Table 1.

**Preparation of DNA and plasmid techniques.** Plasmid DNA was prepared by the cleared lysate method of Kupersztoch-Portnoy et al. (1974) and purified by caesium chloride/ethidium bromide dye-bouyant density-gradient centrifugation (Radloff et al., 1967).

Restriction endonucleases and T4 DNA ligase were used according to the conditions recommended by the manufacturers. DNA fragments were separated by electrophoresis in agarose gels with concentrations of 1–1.5% (Meyers et al., 1976).

**Subcloning and DNA sequencing.** Nested deletions were obtained by the ExoIII site-directed digestion method (Henikoff, 1984) using the 'nested deletion kit' from Pharmacia-LKB with deletion rates of 200 bp min⁻¹.

DNA sequencing was carried out with the T7 sequencing kit and [α⁻²⁵P]dATP (Pharmacia-LKB) according to the protocol recommended by the manufacturer. Sequence gels were exposed to Fuji RX film, without intensifying screens, at room temperature for 12–30 h. Detailed analysis of DNA sequence data was performed with the DNASTAR software package (Pharmacia-LKB). Homology scans were done with HUSAR in the EMBO sequence database at the EMBL in Freiburg, Germany.

**Predicting proteins.** DNASTAR calculates the significance of proteins from ORFs by the Test Code, a correlation coefficient. The algorithm calculates the base/position preferences compared to preferences of known proteins. Good Test Codes exceed 0.9.

**Other methods.** These have been described previously (Meyer et al., 1983; Wiedemann et al., 1986).

### Results

**Deletion analysis**

As described above, sequence-specific recombination is one of the most important features of Tn21-like transposons for generating variability among the

### Table 1. Bacterial strains and plasmids

Resistance towards different drugs are abbreviated as follows: HgR (mercuric chloride), 20 mg l⁻¹; SuR (sulphonamides), 250 mg l⁻¹; SmR (streptomycin), 100 mg l⁻¹; SpR (spectinomycin), 50 mg l⁻¹; CmR (chloramphenicol), 30 mg l⁻¹; TcR (tetracycline), 20 mg l⁻¹; ApR (ampicillin), 50 mg l⁻¹; AmR (aminoglycoside resistance genes); mer, mercury-resistance gene; sulI, sulphonamides resistance gene; tnpI, integrase gene; tnpA, transposase gene; tnpM, modulator of transposase gene; tnpR, resolvase gene.

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli W3110</td>
<td>NxR lac⁺</td>
<td>Grinsted et al. (1972)</td>
</tr>
<tr>
<td>E. coli IC2926</td>
<td>rpsL311 reca13 lacZ1 thi-1 thr-1 leuB6 his-4 argE3</td>
<td>Bachmann et al. (1972)</td>
</tr>
<tr>
<td>E. coli C6065</td>
<td>rpsL reca lacZ1 thi-1 thr-1 leuB6 tonA21 supE44</td>
<td>Cohen et al. (1972)</td>
</tr>
<tr>
<td>E. coli JM83</td>
<td>ara Δ(lac-proAB) rpsL Φ(80lacZ M15)</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>pBR322::Tn21</td>
<td>ApR HgR SmR/SpR SuR TcR</td>
<td>Kratz et al. (1983)</td>
</tr>
<tr>
<td>pUB307::Tn2426</td>
<td>AmR CmR GmR HgR SmR/SpR SuR</td>
<td>Wiedemann et al. (1986)</td>
</tr>
<tr>
<td>pBP90</td>
<td>AmR ApR GmR SmR/SpR</td>
<td>This paper</td>
</tr>
<tr>
<td>pBP91</td>
<td>ApR</td>
<td>This paper</td>
</tr>
<tr>
<td>pUC18/19</td>
<td>ApR</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>pGEM-7Zf(+)</td>
<td>ApR</td>
<td>Promega</td>
</tr>
</tbody>
</table>
members of the Tn21- family. To determine the presence and the activity of these genes, we subcloned a 4.6 kb BamHI/PstI fragment of Tn2426 (Fig. 2) containing the resistance determinants Am\(^R\), Sm/Sp\(^R\), and Gm\(^R\) in pUC19. The resulting clone was designated pBP90. Since pBP91 encodes resistance to ampicillin, amikacin, streptomycin/spectinomycin and gentamicin, deletion of the 1.1 kb PstI/HindIII fragment left the aac\(^A\) gene intact. For the generation of orientation-directed subclones, two restriction endonuclease pairs were used: Apal/BamHI for deletions starting at the tnpl gene and HindIII/NsiI for deletions starting downstream of the aac\(^A\) gene. Deletions starting at the HindIII site of the aac\(^A\) gene resulted in a general loss of expression of all aminoglycoside resistance genes when the deletions exceeded 1 kb. This latter finding indicates a common promoter for the resistance genes aad\(^B\), aad\(^A\), and aac\(^A\), located approximately 1 kb downstream of the BamHI restriction site, and supports the finding of Cameron et al. (1986).

DNA sequencing

Plasmid pGEM-7Zf(+) has the 3'-overhanging restriction sites on the outer extremities of the polylinker and the 5'-overhanging restriction sites in the middle of the polylinker, was used as the vector for generation of deletion mutants. A 3.5 kb EcoRI/HindIII fragment from pBP90 was cloned into this vector and the resulting clone was named pBP91 (Fig. 2). Since pBP91 encodes resistance to ampicillin, amikacin, streptomycin/spectinomycin and gentamicin, deletion of the 1.1 kb PstI/HindIII fragment left the aac\(^A\) gene intact. For the generation of orientation-directed subclones, two restriction endonuclease pairs were used: Apal/BamHI for deletions starting at the tnpl gene and HindIII/NsiI for deletions starting downstream of the aac\(^A\) gene. Deletions starting at the HindIII site of the aac\(^A\) gene resulted in a successive loss of expression of the resistance genes, whereas deletions starting at the BamHI site resulted in a general loss of expression of all aminoglycoside resistance genes when the deletions exceeded 1 kb. This latter finding indicates a common promoter for the resistance genes aad\(^B\), aad\(^A\), and aac\(^A\), located approximately 1 kb downstream of the BamHI restriction site, and supports the finding of Cameron et al. (1986).

Sequence of Tn2426

The sequence of the 3.5 kb fragment is shown in Fig. 3. The putative promoters identified using DNASIS were found at bp 1203–1208 (−35 region) and bp 1180–1185 (−10 region) in 3'−5' orientation and bp 1035–1040 (−35 region) and bp 1058–1063 (−10 region) in 5'−3' orientation. The second promoter is located in the region identified by the deletion analysis described above. This promoter appears to be responsible for the transcription of all resistance genes, as was also supposed by Hollingshead & Vapnek (1985) and Cameron et al. (1986). ORFs exceeding 50 amino acid residues and a Test Code of greater than 0-99 are shown in Table 3.

Four ORFs (ORF 2, 5, 6, and 8) could be assigned to previously described genes. ORF 2 (bp 1888–2676) codes for the aminoglycoside-modifying enzyme 3'−(9)-O-adenyltransferase, AadA, conferring resistance to streptomycin and spectinomycin. The predicted Mr of 29330 agrees well with the experimentally found value of 30000 (Dempsey & McIntire, 1979; Fling & Elwell, 1980; Tait et al., 1985). This gene is flanked by two directly repeated putative RSs, of length 50 and 53 bp, that belong to a family of RSs, as predicted by Wiedemann et al. (1986). Searches for homologous genes in the EMBO database revealed five homologous structures: 99-7% homology with R538–1 (Hollingshead & Vapnek, 1985) and Tn21

![Fig. 2. Maps of Tn2426 and the subclones described. The underlined region in Tn2426 represents the insert of pBP90. In the restriction map of pBP91, solid arrows show the localization and orientation of identified genes; the open arrows represent the two promoter structures P\(_{\text{int}}\) and P\(_{\text{ext}}\). Abbreviations of restriction sites: A, Apal; B, BamHI; C, ClaI; E, EcoRI; H, HindIII; I, NsiI; N, NruI; P, PstI; S, SalI; and V, PvuII. Other abbreviations, see Fig. 1 legend.](image-url)
Fig. 3. Sequence of the 3.5 kb BamHI-HindIII fragment of pBP91. The coding region of each gene is boxed-in, the promoter regions are over- and underlined, and the RSs are marked by solid (H; 54 bp structures) or dotted (O; 14 bp structures) bars. The ORF from bp 140-1150 codes for the integrase gene in 3'-5' orientation. The ORFs from bp 1299-1829 bp, 1888-2676 bp and 2775-3329 bp represent the aadB, aadA and aacA genes.
The Tn21 gene cassette in Tn2426

1646 1655 1664 1673 1682 1691 1700 1709 1718
Ile Ala Gly Arg Pro Val Arg Cys Ala Ser Thr Gly Ala Ala Asp Glu Val Pro Val
ATC GCC GGG CCA GTC CTT TAT ACC TCG TGG GAG GCC ATC TCC CAT CAT CAT CAT GAC TGG CTA CCA CCA GCT GCT GCT GCT GCT

1727 1736 1745 1754 1763 1772 1781 1790 1799 1808
Asp Thr Pro Thr Lys His Ile Glu Ser Tyr Arg Leu Ala Cys Thr Ser Leu Gly Ala Lys Val Glu Val Leu Arg Ala
GAC TGG CTT ACA AGC CAA ATA CAA GAG CTC ACC TGC TAC GAG GCA CTC CCA CCA CCA CCA CCA CCA GCT

1817 1826 1835 1843 1853 1863 1873 1883 1892 1901
Phe Arg Ser Arg Tyr Ala Ala

***

Fig. 3. (continued)

(Sundström et al., 1988); 99-4% homology with the aadA genes in pCN1 of Shigella flexneri (Chinault et al., 1986) and in Tn7 (Fling & Richards, 1985); and 83% homology detected with the sequence of the aadA gene of pSA of Agrobacterium tumefaciens (Tait et al., 1985). The sequences of the corresponding enzymes encoded by pBP91, Tn21 and R538-1 are identical, since all of the changes are in degenerate codons. pCN1 and Tn7 have a

Downloaded from www.microbiologyresearch.org by IP: 54.70.40.11 On: Sun, 04 Aug 2019 08:17:25
Table 3. ORFs detected in the insert of pBP91 and properties of their possible gene products

<table>
<thead>
<tr>
<th>ORF</th>
<th>Position (bp)</th>
<th>Length (bp)</th>
<th>Orientation</th>
<th>Peptide</th>
<th>Length (amino acids)</th>
<th>M&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1543-1743</td>
<td>201</td>
<td>5'-3'</td>
<td></td>
<td>67</td>
<td>7540</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>1888-2676</td>
<td>789</td>
<td>5'-3'</td>
<td></td>
<td>263</td>
<td>29330</td>
<td>AadA</td>
</tr>
<tr>
<td>3</td>
<td>209-610</td>
<td>402</td>
<td>5'-3'</td>
<td></td>
<td>134</td>
<td>14439</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>752-1057</td>
<td>306</td>
<td>5'-3'</td>
<td></td>
<td>102</td>
<td>11444</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>1299-1829</td>
<td>531</td>
<td>5'-3'</td>
<td></td>
<td>177</td>
<td>19872</td>
<td>AadB</td>
</tr>
<tr>
<td>6</td>
<td>2775-3329</td>
<td>555</td>
<td>5'-3'</td>
<td></td>
<td>185</td>
<td>21263</td>
<td>AacA</td>
</tr>
<tr>
<td>7</td>
<td>1504-1217</td>
<td>288</td>
<td>3'-5'</td>
<td></td>
<td>96</td>
<td>10320</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>140-1150</td>
<td>1011</td>
<td>3'-5'</td>
<td></td>
<td>337</td>
<td>38413</td>
<td>TnpI</td>
</tr>
<tr>
<td>9</td>
<td>1926-2309</td>
<td>385</td>
<td>3'-5'</td>
<td></td>
<td>128</td>
<td>14608</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>797-1040</td>
<td>243</td>
<td>3'-5'</td>
<td></td>
<td>81</td>
<td>9105</td>
<td>Unknown</td>
</tr>
<tr>
<td>11</td>
<td>1919-2177</td>
<td>258</td>
<td>3'-5'</td>
<td></td>
<td>86</td>
<td>9093</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Table 4. Structural and functional properties of sequences sharing more than 90% homology to the corresponding region of tnpI in pBP91

<table>
<thead>
<tr>
<th>Localization of tnpI</th>
<th>Resistance genes</th>
<th>Homology to tnpI of Tn4246 (%)</th>
<th>Recombination sequences*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn21</td>
<td>aadA</td>
<td>99.6</td>
<td>RS1, RS2</td>
<td>Sundström et al. (1988)</td>
</tr>
<tr>
<td>R46</td>
<td>oxa2</td>
<td>99.5</td>
<td>ΔRS2</td>
<td>Hull &amp; Vockler (1987)</td>
</tr>
<tr>
<td>Tn2603</td>
<td>oxa1</td>
<td>99.5</td>
<td>ΔRS1, ΔRS2</td>
<td>Ouellette et al. (1987)</td>
</tr>
<tr>
<td>pLMO20</td>
<td>dhfrV</td>
<td>99.4</td>
<td>ΔRS1, RS2</td>
<td>Sundström et al. (1988)</td>
</tr>
<tr>
<td>Tn21</td>
<td>aadA</td>
<td>99.4</td>
<td>RS2</td>
<td>Mercier et al. (1990)</td>
</tr>
<tr>
<td>pDGO100</td>
<td>aadB</td>
<td>99.4</td>
<td>RS1, RS2</td>
<td>Cameron et al. (1986)</td>
</tr>
<tr>
<td>Tn1696</td>
<td>aacC</td>
<td>99.2</td>
<td>RS2</td>
<td>Wohleben et al. (1989)</td>
</tr>
<tr>
<td>pBWH100</td>
<td>aacA</td>
<td>99.3</td>
<td>ΔRS1, RS2</td>
<td>Tenover et al. (1988)</td>
</tr>
<tr>
<td>R46</td>
<td>oxa2</td>
<td>99.1</td>
<td>ΔRS2</td>
<td>Mossakowska et al. (1989)</td>
</tr>
<tr>
<td>E. coli</td>
<td>dhfr</td>
<td>98.7</td>
<td>ΔRS1, RS2</td>
<td>Zolg &amp; Hägg (1981)</td>
</tr>
<tr>
<td>pLMO229</td>
<td>dhfrI</td>
<td>99.3</td>
<td>RS2</td>
<td>Sundström &amp; Sköld (1990)</td>
</tr>
<tr>
<td>pLMO150</td>
<td>dhfrI</td>
<td>99.1</td>
<td>ΔRS1, RS2</td>
<td>Sundström &amp; Sköld (1990)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>AAC-6'-II</td>
<td>98.9</td>
<td>RS1, RS2</td>
<td>Shaw et al. (1989)</td>
</tr>
<tr>
<td>R538-1</td>
<td>aadA</td>
<td>96</td>
<td>RS1, RS2</td>
<td>Hollingshead &amp; Vapnek (1985)</td>
</tr>
<tr>
<td>Tn4000</td>
<td>aadB</td>
<td>97.6</td>
<td>RS1, RS2</td>
<td>Schmidt et al. (1988)</td>
</tr>
<tr>
<td>R751</td>
<td>dhfr</td>
<td>93.1</td>
<td>RS2</td>
<td>Flensburg &amp; Steen (1986)</td>
</tr>
</tbody>
</table>

*Δ indicates a truncated RS.

deletion of three bases (positions 2593–2595 in pBP91), resulting in the loss of a glutamine residue at position 235 (numbering from AadA of pBP91) in the enzymes encoded. All of the genes have similar flanking regions, implying a Tn21-like structure, and a recombinational sequence at the 3’-end. In addition, Tn7 and pCN1 each have a second recombination sequence at their 5’-ends, as found in pBP91. The genes located in Tn21 and R538-1 possess a 14 bp sequence, also found at the 5’-end of the aadB gene (ORF 5, see below).

ORF 5 (bp 1299–1829) codes for the aminoglycoside-modifying enzyme 2”-O-adenyltransferase, AadB, conferring resistance to amikacin, kanamycin and tobramycin. Sequences 100% homologous to ORF 5 were found in pDGO100 (Cameron et al., 1986) and Tn4000 (Schmidt et al., 1989). ORF 5 is flanked by RSs: a 53 bp sequence at the 3’-end and a 14 bp sequence at the 5’-end. The same structures are found in pDGO100 and Tn4000.

ORF 6 (bp 2775–3329) codes for the aminoglycoside-modifying enzyme 6'-N-acetyltransferase, AacA, conferring resistance to amikacin, kanamycin and tobramycin. The predicted protein has an M<sub>f</sub> of 21263, which corresponds well with that (M<sub>f</sub> 20500) reported by Tenover et al. (1988). The sequence reported by Tenover et al. (1988) is 99.6% homologous to the corresponding region of pBP91 and exceeds the coding region, as it reaches the terminal HindIII site of the cloned insert. The aacA gene of pBP91 is flanked by a 50 bp recombination sequence at the 5’-end and a truncated 10 bp element, homologous to the central region of the 14 bp sequence at the 3’-end.

ORF 8 (bp 140–1150) corresponds to the integrase gene (int) as identified by Mercier et al. (1990). The
coding region of this gene is transcribed divergently with respect to the identified resistance genes in the transposon. Its putative promoter, $P_{\text{int}}$, is located at bp 1179–1184 (−10 region) and 1203–1208 (−35 region). The Int protein shows homology with a group of sequence-specific recombinases responsible for the integration of several phages (Mercier et al., 1990). Searches in the EMBO database revealed several homologous structures: the sequences sharing >90% homology are listed in Table 4.

None of the remaining ORFs could be identified as encoding known proteins, and their involvement in encoding or regulating the expression of the resistance genes or of transposon functions is therefore unknown.

**Discussion**

In this work, we investigated a so-called ‘resistance gene cassette’ of a member of the Tn21-like group of transposons. This region is interesting because a variety of resistance genes are inserted at the flanks of the $aadA$ gene. Short RSs (Wiedemann et al., 1986) and a site-specific integrase (Martinez & de la Cruz, 1988; Mercier et al., 1990) are responsible for recombination events involved.

Tn2426 (Wiedemann et al., 1986) is a typical member of the Tn21 family of class II transposons. Beside the resistance genes typical for Tn21, e.g. mer, sulI, and $aadA$, Tn2426 harbours additional resistance genes. $aadB$, conferring resistance to gentamicin, is located at the 5'-end of $aadA$, and $aacA$, conferring resistance to amikacin, is located at the 3'-end of $aadA$. $aacA$ is accompanied by a $cat$ gene, conferring resistance to chloramphenicol.

The region of Tn2426 harbouring the aminoglycoside-resistance genes was subcloned to permit the demonstration via deletion experiments of the presence and different activities of the RSs. The deletion frequencies in $rec^+$ ($6 \times 10^{-3}$) and $recA$ ($2 \times 10^{-3}$) backgrounds showed the independence of the recombinational activities from host functions. Comparable experiments carried out by Albertini et al. (1982) with synthetic directly repeated sequences of variable length revealed, in contrast, a dependence of the deletion frequencies on the recombinational proficiency of the host. In these experiments, the deletion frequencies varied by a factor of about 25 between $rec^+$ and $recA$ strains, implying involvement of the RecA enzyme. In addition, the absolute frequencies of deletions generated by the test system varied enormously: we found frequencies in the order of $10^{-3}$ while Albertini et al. (1982) found frequencies of the order of $10^{-9}$. These differences imply the involvement of different mechanisms in the two systems. Albertini et al. (1982) discuss the possibility of a ‘slipped mismatch’, which involves a loop structure with the stem built by the repeats during replication and a ‘read through’ of the polymerase which results in a deletion of the loop in the daughter-DNA strand. In this case, the homology and the length of the duplications are important for the formation and stability of the loop. Albertini et al. (1982) were able to describe the dependence of deletion events on the length and degree of homology of the direct-repeated sequences used in their experiments; the highest frequencies were found with the longest repeated sequences. In contrast, in our system deletion events are not limited to sequences flanked by highly homologous, repeated sequences. The highest frequencies were found for the $aacA$ gene, which is flanked by a 50 bp RS at the 5'-end and a 10 bp RS at the 3'-end. This implies a $recA$-independent mechanism which requires the RSs at the ends of the processed region.

To investigate further the structures involved in these rearrangements, the sequence of the 3.5 kb BamHI/HindIII fragment was determined. As expected, all resistance genes are flanked by RSs showing homology with the predicted RS consensus sequence (Wiedemann et al., 1986). While the RSs at the flanks of the $aadA$ gene show a high degree of homology to the predicted RS, the RS at the outer ends of the gene cassette showed a much lower degree of homology to the consensus sequence: only the initial 14 bp (5'-end of the cassette) or 10 bp (3'-end) showed homology to the 54 bp consensus sequence. The length of the RS seems to have no influence on its activity as the shortest sequence at the 3'-end of the $aacA$ gene (10 bp) is the one with the highest deletion activities. This RS is actually the shortest functional RS currently known. Shorter RSs, such as AAGTT or GTTA, as described by Ouellette et al. (1987), Nobuta et al. (1988) and Sundström et al. (1988) seem not to represent the active site, as they are not embedded in the minimal consensus sequence deduced from our experiments. This minimal consensus sequence is as follows: TAAAACAANGNNA (where N represents any of the four nucleotides). Whether this sequence is the shortest active sequence will require further experiments.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to B. Wiedemann, University of Bonn, Germany (grant number Wi 361/131).

**References**


Cameron, F. H., Obbink, D. J. G., Ackerman, V. P. & Hall, R. M.
(1986). Nucleotide sequence of the AAD(2') aminoglycoside adenyltransferase determinant aadB. Evolutionary relationship of this region with those surrounding aadA in R538-1 and dftrII in R388. *Nucleic Acids Research* 14, 8625-8635.


Sundström, L. & Sköld, O. (1990). The dftrI trimethoprim resistance gene of Tn7 can be found at specific sites in other genetic surroundings. *Antimicrobial Agents and Chemotherapy* 34, 642-650.


