KpnI family members were immobilized on nitrocellulose filters and hybridized with 32P-labelled RNA synthesized in vitro in the presence or absence of α-aminitin. A human Alu sequence cloned in the BamHI site of pBR322 (Blur 8) and a human β-actin cDNA sequence served as controls. Figure 3A shows that in the absence of α-aminitin, transcripts of the Alu sequences (lane g), the β-actin gene sequences (lane h) and all but one (that represented by plasmid pBK(1.820), lane d) of the KpnI families (lanes a–f) were synthesized.

Figure 3B shows that 0.6 µg ml⁻¹ α-aminitin inhibited transcription of all but one of the KpnI sequences, and of the β-actin gene. Alu sequences and sequences homologous to pBK(1.951), however, continued to be transcribed, although at a reduced rate. Plasmid pBK(1.951) actually contains an Alu-like sequence, so it was not possible to determine which of the RNA polymerases was responsible for the transcription of the KpnI sequences in this plasmid. 200 µg ml⁻¹ of α-aminitin inhibited transcription of all the KpnI sequences and the Alu sequences. Thus, unlike the Alu family sequences, which are transcribed extensively by RNA polymerase III16, the KpnI family sequences are transcribed by RNA polymerase II. These results suggest that either polymerase II promoters exist in KpnI DNAs or that their transcription occurs by extension of RNA polymerase II transcripts initiating upstream from the sequences. In the latter case, KpnI repeats must be close to these polymerase II transcription units since nascent chains extend only about 500 bases in the in vitro system17.

That the polymerase transcribes the KpnI sequences asymmetrically was indicated by the following experiment. The separated, complementary strands of the insert in pBK(1.211) and in pBK(1.554) were hybridized to HeLa nuclear RNA labelled in vitro with 32P-orthophosphate. As shown in Fig. 4, the labelled RNA hybridized predominantly with the slow strands of both inserts. Experiments with RNA labelled with 32P-UTP in the in vitro system also indicated asymmetric transcription of both strands. In this case, RNA sequences homologous to the slow strands of KpnI 1.2 and 1.5 kb DNAs were 2.5–3.5 times more abundant than transcripts homologous to the corresponding fast strands.

We thank Drs L. Kedes and P. Ponte for the use of the human cytoskeletal β-actin cDNA clone, and Ms R. Alterman and Dr A. Skoulitch for their help. This work was supported by NIH grant CA16790 (NCI) and Core support for the Cancer Research Center, NIH NCI-P30-CA1330. B.S.-Z. is supported by NIH Research Training in Medical Genetics, no. ST3GM07001, and P.J.Z. is supported by National Research Award ST32 CA09060-08.

Received 16 January; accepted 18 May 1983.

transposition. This 16–18-bp sequence contains the 8–9-bp small inverted repeat present at each end of IS50 plus a 9-bp sequence which is homologous to an interrelated sequence present in four copies in the chromosomal origin of replication in a variety of Gram-negative bacteria. This sequence organization suggests that the ends of Tn5 may function to provide a recognition site for the Tn5 transposase adjacent to a sequence recognized by the host replication system.

Tn5 is an example of a composite prokaryotic transposable element. It contains two physically similar but genetically different 1,534-bp insertion sequences (IS50R and IS50L) in inverted orientation flanking a 2.7-kilobase (kb) segment of unique DNA which contains a gene encoding an aminoglycoside antibiotic modifying function. A derivative of Tn5 was constructed containing the outer 186 bp of the element in inverted orientation flanking a 2-kb segment of DNA encoding tetracycline resistance. While this structure is unable to transpose in the absence of a functional copy of IS50R, transposition of Tn5-320 from a ColE1 plasmid to λ was detected when a wild-type copy of Tn5 was present on an F plasmid. This complementation is inefficient, as Tn5-320 only transposed at 1–3% of the frequency of the wild-type element. Table 1 shows that complementation remains inefficient when the donor Tn5 is present on a multi-copy plasmid. However, when the wild-type donor is located on the same replicon within 420 bp of Tn5-320, both elements transpose at approximately equal frequencies. This result indicates that Tn5-320 contains all the DNA sequence information required for efficient translocation when the transposase is synthesized in close proximity. The inability of the transposase to act efficiently in trans has been demonstrated in several studies with Tn5 (refs 2–4) and other Tn5-like transposable elements (for example, Tn10, Tn903, IS196). These results indicate that IS50 is required for efficient transposition.

pRZ341 was constructed so that a unique BamHI restriction site was located at nucleotide 53 from the left end of the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Complementation of Tn5-320</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>Tn5 elements present</td>
</tr>
<tr>
<td>A</td>
<td>CoIE1::Tn5-320</td>
</tr>
<tr>
<td></td>
<td>&lt;6.6x10&lt;sup&gt;-9&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>RSFI010::Tn5-wt</td>
</tr>
<tr>
<td>C</td>
<td>CoIE1::Tn5-320</td>
</tr>
<tr>
<td></td>
<td>Tn5-wt</td>
</tr>
</tbody>
</table>

Cultures of RZ502A bba (E. coli W3110 lac recA456 srl str acrB57 h515 h519 nim 5am 7r) containing the various plasmids were heat induced and the resulting lysates used to lysogenize Hf1–1 as described previously. The transposition frequency measures the kanamycin (Kan<sup>-</sup>) of Tn5-wild-type, wt) or tetracycline (Tet<sup>-</sup>) resistant transductants per plaque-forming unit of the phage. Expt B, no IS50R functions are supplied, except B, IS50R functions are supplied on the multi-copy compatible plasmid RSFI100; expt C, wild-type Tn5 was transposed onto CoIE1::Tn5-320 and is located — 420 bp from Tn5-320 with IS50R oriented adjacent to Tn5-320. In expt C, 3% of the Kan<sup>-</sup> transductants were also Tet<sup>-</sup> and 11% of the Tet<sup>-</sup> transductants were also Kan<sup>-</sup> due to transposition of Tn5-320::Tn5-wild-type. The doubly resistant transductants were not included in the values shown. Tn5-320 was constructed as elsewhere described. Wild-type Tn5 was transposed onto RSFI101 by Jerry Yin.

Fig. 1 Homology at the ends of Tn5 with a repeated sequence in the chromosomal origin of replication (oriC). The Tn5 sequence from nucleotides 8–16 is given with the four copies (R1–R4) of the interrelated sequence in the oriC region of six different Gram-negative bacteria. A large letter indicates that the nucleotide is totally conserved; a small letter indicates it is the predominant nucleotide. C<sup>+</sup> indicates the location of a single base pair change in the E. coli oriC which inactivates its function.

The oriC-like sequence found at the outer ends of Tn5 may serve to direct host replication functions to the ends of the element during transposition. The requirement for the sequence at the end distal to the site of transposase synthesis suggests that transposition of Tn5 may involve replication.
Table 2  Transposition assays of Tn5 derivatives containing various amounts of the left inverted repeat

<table>
<thead>
<tr>
<th>Plasmid present</th>
<th>Structure of Tn5</th>
<th>Left end sequence present</th>
<th>Transposition frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRZ190</td>
<td></td>
<td>1–1,534</td>
<td>$9.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>pRZ191</td>
<td></td>
<td>1–118</td>
<td>$1.1 \times 10^{-5}$</td>
</tr>
<tr>
<td>pRZ341</td>
<td></td>
<td>1–56</td>
<td>$1.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>pRZ346</td>
<td></td>
<td>1–56</td>
<td>$1.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>pRZ340</td>
<td></td>
<td>0</td>
<td>$&lt;5.0 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

Transposition assays of Tn5 derivatives containing various amounts of the left inverted repeat. Transposition assays were performed in RZ102a bbn (RZ102 = E. coli MO recA56 str) as described in Table 1 legend. pRZ190 is ColE1-Tn5 containing a 2.7-kb BglII restriction fragment which carries the tetracycline resistance determinant inserted into the BamHI site in Tn5 (S. J. Rothstein and W. R., unpublished). pRZ191 has the DNA from the SalI site in pRZ190 to the HaeIII site in the left inverted repeat deleted. pRZ341 was constructed by ligating a 120-bp EcoRI-Sau3A restriction fragment, which contains the outer 56 bp of IS50L plus ColE1 sequences, from pRZ102 to pBR322 between the EcoRI site and the BamHI site, regenerating the BamHI restriction site. An EcoRI-SalI fragment from this plasmid was then substituted for the left inverted repeat of pRZ190. pRZ346 was made in an analogous manner starting with a Tn5 insertion into pBR322 with IS50R located in the tetracycline resistance region about 45 bp from the EcoRI site. Therefore, the left end of Tn5-346 is actually derived from IS50R and is adjacent to pBR322 sequences. pRZ340 contains non-Tn5 DNA at its left end and was constructed by ligating an EcoRI–SalI fragment from pRZ4006 (a pBR322 derived plasmid containing the lacPO between the EcoRI and BamHI sites) into pRZ190.

Table 3  Transposition assays of Bal31 generated deletions into the left inverted repeat of Tn5

<table>
<thead>
<tr>
<th>Tn5 mutant</th>
<th>Transposition frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn5-341</td>
<td>$1.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>Tn5-341:25</td>
<td>$1.3 \times 10^{-5}$</td>
</tr>
<tr>
<td>Tn5-341:21</td>
<td>$1.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>Tn5-341:18</td>
<td>$2.8 \times 10^{-6}$</td>
</tr>
<tr>
<td>Tn5-341:15</td>
<td>$&lt;1.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>Tn5-341:13</td>
<td>$&lt;1.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>Tn5-341:7</td>
<td>$&lt;1.0 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

Transposition assays on Bal31-generated deletions into the left inverted repeat of Tn5. The nucleotide sequence of the outer 30 bp of Tn5 is given along with the location of the BamHI containing oligonucleotide (in italics) present in the deletion mutants. A dot indicates the wild-type nucleotide is present. The arrow depicts those bases which are repeated in inverted orientation at the inner end of IS50 and the underlined sequence contains the nucleotides which are homologous to a conserved sequence found in four copies in the oriC region (see Fig. 1). The transposition assays were performed in RZ201a bbn as described in the Table 1 legend. The deletion mutants were constructed as follows. pRZ341 (Table 2) was linearized at its BamHI restriction site and subjected to Bal31 exonuclease (New England Biolabs) digestion at 1 U ml$^{-1}$ in a 100-μl reaction volume containing 0.2 M NaCl, 12 mM CaCl₂, 12 mM MgCl₂, 1 mM EDTA, 20 mM Tris–HCl, pH 7.9, and 2.4 pmol of DNA terminus. The reaction was terminated after 30–60 s at 30°C by the addition of diethyl pyrocarbonate and EDTA to 0.05% and 30 mM, respectively. After ethanol precipitation, the 0.2 pmol of termini were ligated overnight at 15°C in a 30–50 μl reaction volume with 24 pmol of BamHI containing oligonucleotides (New England Biolabs) which were phosphorylated using polynucleotide kinase and ATP. The reaction mixture was then heated to 65°C for 10 min to inactivate any remaining ligase and digested with 30 U of BamHI for 4 h. The reaction was again heated to 65°C for 10 min and the plasmid DNA containing BamHI sticky ends was circularized in the presence of T4 DNA ligase (gift of R. Simon) at a plasmid terminus concentration of approximately 1 nM and transformed into RZ211 (Δ(lac-pro) recA6 srl 157). The exact end points of the deletions were determined using the DNA sequencing methods of Maxam and Gilbert.

proceeding from both ends in a symmetrical manner. This type of mechanism may differ from the mechanism of transposition of other elements which is believed to involve polarized replication beginning at the end proximal to the site of transposase synthesis (for example, Tn9 (ref. 16)). Perhaps this difference is reflected in the very low frequency of co-integration events promoted by Tn5 in the absence of homologous recombination, in contrast to many other transposons (including Tn9). We have searched the outer 100 bp of several other transposable elements, including Tn10, IS1/Tn9, Tn3 and bacteriophage Mu, and have found no significant homology to the oriC repeated sequence.

We thank L. Munson for initially pointing out the homology to oriC and J. Yin and S. Stibitz for valuable discussion. R.C.J. was supported in part by an NIH training grant (GM07215). Additional support was from grants from the NSF (PCM791086) and the NIH (GM19670) to W.S.R.

Received 20 April; accepted 17 May 1983.