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Definition of three resolvase binding sites at the *res* loci of Tn*21* and Tn*1721*

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The dual functions of resolvase, site-specific recombination and the regulation of its own expression from *tnpR*, both require the interaction of this protein with the DNA sequence at *res*, but the specificity of this interaction differs between groups of Tn*3*-like elements. In this study, DNA fragments that contained *res* from Tn*21* or Tn*1721* were subjected to either cleavage by DNase I or methylation by dimethyl sulphate in the presence of the purified resolvase from Tn*21* or Tn*1721*. These experiments showed that each resolvase bound to the same three sites (I, II and III) within *res* from Tn*1721* and to an equivalent series of three sites on Tn*21*: the differences in the amino acid sequences of the two proteins did not affect their interaction with either DNA. The DNA sequences at each site had some similarities and, in conjunction with data from the related transposon Tn*501*, a consensus was established. However, the three sites are functionally distinct: site I (*tnpR*-distal) spans the recombination cross-over point and sites II and III (*tnpR*-proximal) overlap the promoter of *tnpR*. The binding sites on these transposons were compared with those in the γδ/Tn*3* system: the similarities between the two groups of transposons revealed some general features of resolvase-DNA interactions while the differences in fine structure elucidated the specificity of each resolvase.

Key words: consensus sequence/DNA protection experiments/ resolvase binding/resolution site

Introduction

The transposition of Tn*3*-like elements involves two sequential steps, the formation of a co-integrate followed by its resolution: the latter step, a reciprocal recombination between specific sites (*res*) that are present in direct repeat on the co-integrate, is catalysed by resolvase (reviewed by Heffron, 1983). The purified resolvases encoded by the transposable elements γδ and Tn*3* can carry out *in vitro* site-specific recombination between cognate *res* sequences: these two proteins are functionally interchangeable (Reed, 1981; Kitts *et al.*, 1983; Krasnow and Cozzarelli, 1983). Resolvase has unique properties: recombination occurs only if two *res* sites are present on the same supercoiled replicon, it exhibits a strong preference for directly oriented *res* sites and it leads to catenanes interlinked only once, implying that strand exchange occurs with a unique geometry (Krasnow and Cozzarelli, 1983). The structure of the *res* site is therefore of special interest. *In vitro* studies with purified resolvase from γδ served to identify the cross-over point and three sites (I, II and III) within the *res* sequence to which the resolvase bound (Reed and Grindley, 1981; Grindley *et al.*, 1982). The cross-over occurs at site I but the other sites are also required for efficient resolution of co-integrates (Kitts *et al.*, 1983; Wells and Grindley, 1984). On γδ and Tn*3*, the binding of resolvase to *res* also represses the expression of the divergently transcribed genes, *tnpA* (transposase) and *tnpR* (resolvase): *res* is located between these genes and site I overlaps the *tnpA* Pribnow box while site II overlaps the entire *tnpR* promoter (Wishart *et al.*, 1983).

The transposons Tn*21*, Tn*501* and Tn*1721* are more closely related to each other than to γδ and Tn*3* (Schmitt *et al.*, 1984). Unlike γδ and Tn*3* the *tnpR-tnpA* genes of Tn*21* and its relatives have the same transcription polarity, *res* is located upstream of both genes but close to the 5′ end of *tnpR*, and only the expression of *tnpR* is regulated by resolvase (Altenbuchner and Schmitt, 1983). This group of elements has diverged sufficiently from the γδ/Tn*3* group so that the transposition functions are not exchangeable between groups, whereas this is possible within each group (Grinsted *et al.*, 1982; Diver *et al.*, 1983). Therefore, an analysis of the site-specific recombination systems in Tn*21*-related elements should reveal new insights into these processes: similarities with the γδ/Tn*3* system might identify the general features that are required for all resolvase-DNA interactions, while differences between the Tn*21* group and γδ/Tn*3* might account for the specificity of these proteins for a particular DNA sequence. The DNA sequences of *res* and *tnpR* in Tn*21*, Tn*501* and Tn*1721* have been determined (Diver *et al.*, 1983) and the

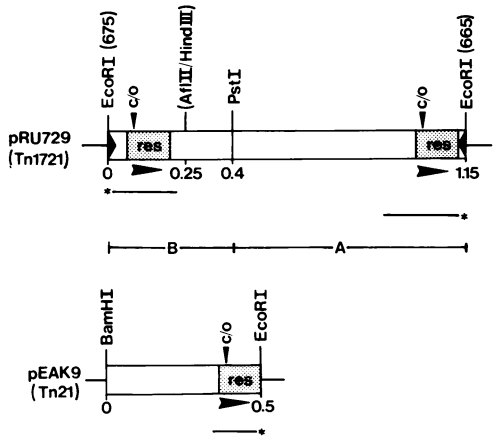

Fig. 1. DNA fragments used for footprinting and G protection experiments. Fragments of the Tn*1721* and Tn*21*-derived recombinant plasmid pRU729 (Rogowsky, 1985) and pEAK9 (Halford *et al.*, 1985) are shown as open boxes. Resolution sites (*res*) are shaded, their relative orientation indicated by arrows pointing to the *tnpR* gene (deleted in the recombinants); 'c/o' marks the cross-over point (Rogowsky and Schmitt, 1984); A and B denote the 0.75-kb and 0.4-kb fragments of pRU729 generated by an *Eco*RI/*Pst*I double digestion. Asterisks indicate the ^{32}P-labeled 5′ ends of DNA fragments. Relevant restriction sites and their coordinates (in kb) are shown. The *Afl*III/*Hind*III junction on pRU729 was constructed by blunt-end ligation of end-repaired DNA. The *Eco*RI sites in pRU729 were generated by insertions 665 and 675 of transposon Tn*1725* containing an *Eco*RI site in each terminal repeat (Altenbuchner *et al.*, 1983); cleavage with *Eco*RI leaves the terminal 15 bp of the transposon (arrowheads within the boxes).

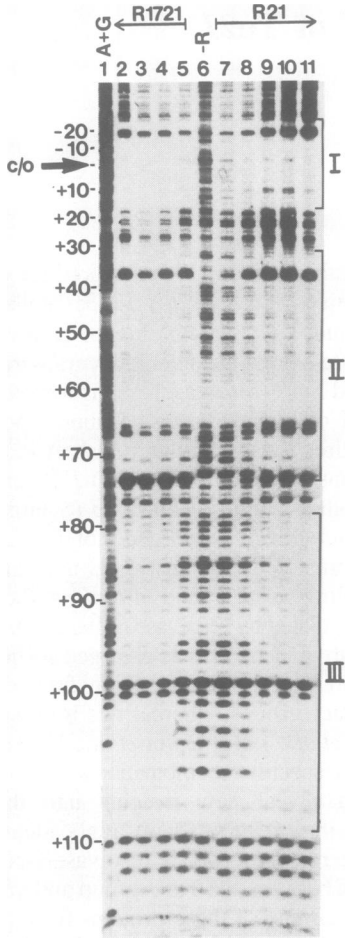

Fig. 2. Protection of Tn*1721 res* DNA from DNase by Tn*21* and Tn*1721* resolvases (footprinting). All lanes contain the 0.75-kb *Eco*RI-*Pst*I fragment from Tn*1721* 5′-labelled at the *Eco*RI site (A in Figure 1). **Lane 1**: sequence marker generated by the A+G Maxam and Gilbert (1980) reaction. **Lane 6**: cleavage by DNase I in the absence of resolvase (−R). Reactions in **lanes 2−5** contained Tn*1721* resolvase (R1721), the amounts increasing from right to left (arrow): **lane 5**, 25 ng; **lane 4**, 125 ng; **lane 3**, 250 ng; **lane 2**, 500 ng. Reactions in **lanes 7−11** contained increasing amounts of Tn*21* resolvase (R21): **lane 7**, 5 ng; **lane 8**, 13 ng; **lane 9**, 25 ng; **lane 10**, 250 ng; **lane 11**, 500 ng. Numbers indicate base pairs in the sequence ladder relative to the cross-over point (c/o; Rogowsky and Schmitt, 1984). The protected sites I, II and III are indicated.

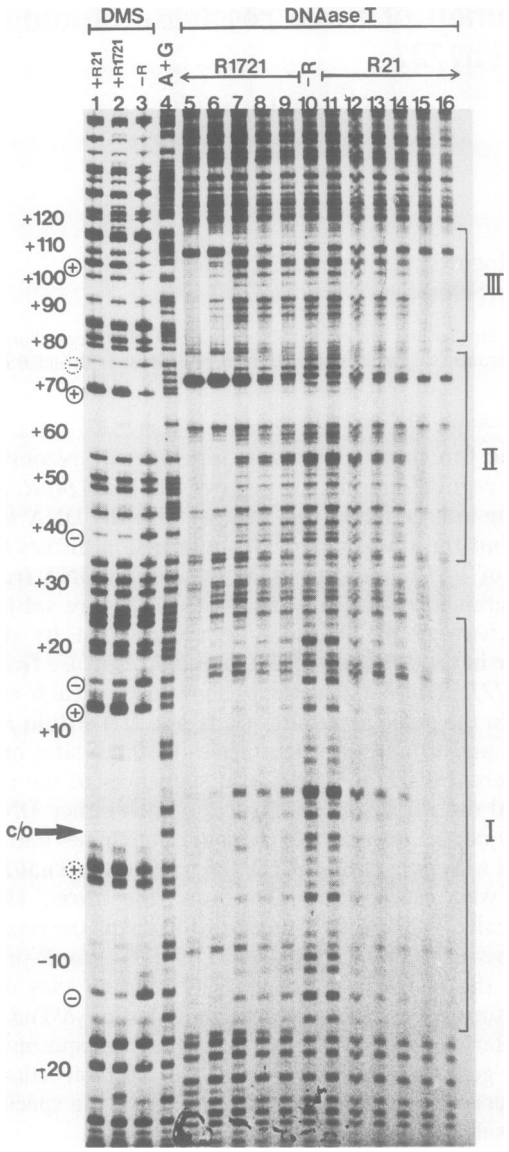

Fig. 3. Protection of Tn*1721 res* DNA from guanine methylation (G protection) and from DNase I (footprinting) by R21 or R1721. All lanes contain the 0.45-kb *Eco*RI-*Pst*I fragment from Tn*1721* (5′-labelled at the *Eco*RI site as in Figure 1). The A+G sequence marker, numbering of the sequence ladder and the protected sites are as in Figure 2. The methylation of guanine by dimethyl sulphate (DMS) was tested without resolvase (−R) and in the presence of 500 ng R21 or R1721 (**lanes 1−3**). As indicated at the margin, methylation of guanine was slightly (⊖) or strongly (⊖) decreased or, conversely, slightly (⊕) or strongly (⊕) enhanced. DNase I footprinting is shown in **lanes 5−16**. Reactions contained increasing amounts of either R1721 (**lane 9**, 13 ng; **lane 8**, 25 ng; **lane 7**, 63 ng; **lane 6**, 125 ng; **lane 5**, 500 ng) or R21 (**lane 11**, 5 ng; **lane 12**, 13 ng; **lane 13**, 25 ng; **lane 14**, 50 ng; **lane 15**, 250 ng; **lane 16**, 500 ng) or no resolvase (−R, **lane 10**).

resolvase proteins encoded by Tn*21* and Tn*1721* have been purified (Halford *et al.*, 1985; Rogowsky and Schmitt, 1985). Both proteins catalysed *in vitro* site-specific recombination between directly repeated *res* sites from either Tn*21* or Tn*1721* but were inactive on a Tn*3* co-integrate. Hence we are now in a position to characterize the interactions between these resolvases and the *res* sites of Tn*21* and Tn*1721*.

Results

Scope of the experiments

Previous experiments have located the *res* site of Tn*1721* to within 200 bp adjacent to the 5′ end of the *tnpR* gene (Altenbuchner and Schmitt, 1983). The cross-over point on Tn*1721* (and on Tn*501*) was confined to a stretch of 11 nucleotides, 161−172 bp upstream of the *tnpR* translational start, and the DNA sequence within this region shows dyad symmetry around an axis 171 bp from *tnpR*: it was suggested that the cross-over occurs at this centre of symmetry (Rogowsky and Schmitt, 1984). Further characterization of the extent and fine structure of *res* on Tn*1721* and also Tn*21* is now possible: the resolution functions on these two transposons are interchangeable yet their sequences have diverged substantially (Diver *et al.*, 1983; Rogowsky and Schmitt, 1984). Hence regions that remain conserved between these elements may contain functional sites and three such regions of 30−40 bp are found upstream of *tnpR* Grindley *et al.* (1985) speculated that the equivalent three regions in Tn*501* may constitute its *res* site and our data on Tn*21* and Tn*1721* presented below confirms much of their speculation.

The susceptibility of end-labelled DNA fragments to DNase

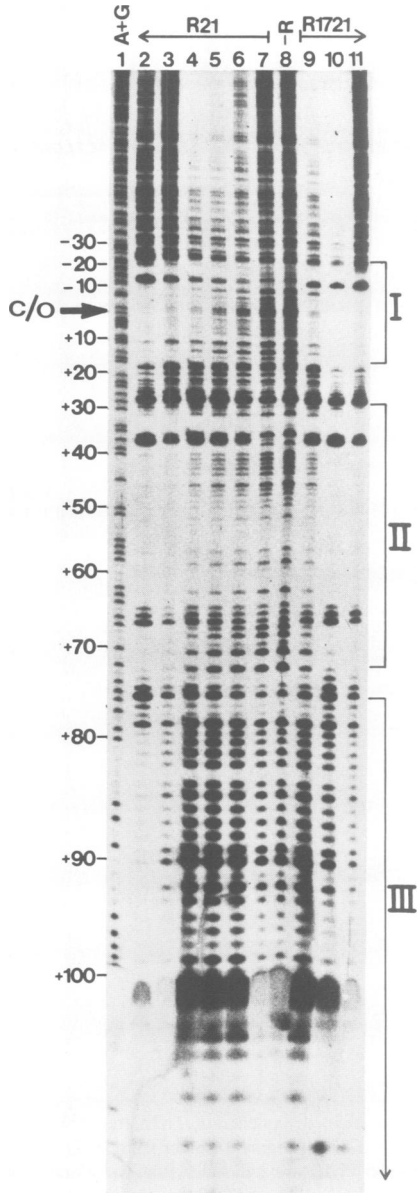

Fig. 4. DNase I footprinting of Tn*21 res* DNA. All lanes contain the Tn*21* *Eco*RI-*Bam*HI fragment 5′-labelled at the *Eco*RI site (see Figure 1). A+G sequence marker, numbering of sequence ladder and of protected sites corresponds to Figure 2. Reactions contained increasing amounts of either R21 (**lane 7**, 0.5 ng; **lane 6**, 5 ng; **lane 5**, 13 ng; **lane 4**, 25 ng; **lane 3**, 50 ng; **lane 2**, 500 ng) or R1721 (**lane 9**, 25 ng; **lane 10**, 250 ng; **lane 11**, 500 ng) or no resolvase (−R, **lane 8**).

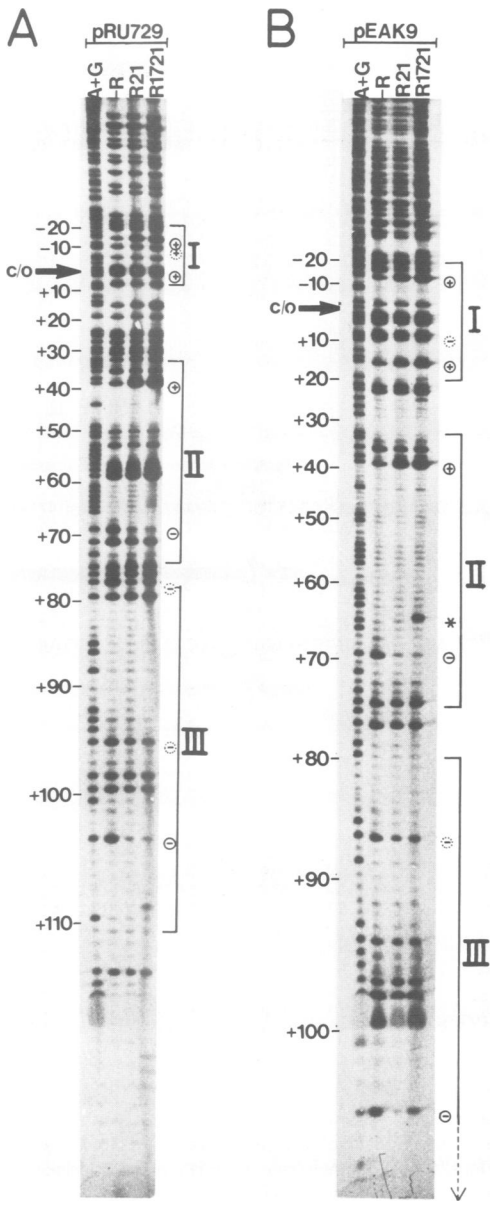

Fig. 5. G protection of Tn*21 res* (pRU729) and Tn*21 res* (pEAK9) DNA by resolvases R21 and R1721. (**A**) All lanes contain the Tn*1721* *Eco*RI-*Bam*HI fragment A 5′ end-labelled at the *Eco*RI site as shown in Figure 1. (**B**) All lanes contain the Tn*21* *Eco*RI-*Bam*HI fragment 5′ end-labelled at the *Eco*RI site. The A+G sequence marker, numbering of the sequence ladder and designation of sites are as in Figures 2 and 4, respectively. The methylation reactions contained either no resolvase (−R), 500 ng of R21, or 500 ng of R1721. Decreased or enhanced G methylation is indicated at the margin as in Figure 3. The asterisk (*) marks an adenosine that was enhanced only in the R1721-protected Tn*21* fragment (see Discussion).

I ('footprinting'; Galas and Schmitz, 1978) or to guanine methylation by dimethyl sulphate ('G protection'; Siebenlist *et al.*, 1980), in the presence of a protein bound to the DNA, are methods that have been used extensively in the analysis of DNA-protein interactions. These techniques were applied here to identify the sites within *res* which bind to the resolvases from Tn*21* and Tn*1721* (abbreviated to R21 and R1721, respectively).

The DNA fragments used in these studies are shown in Figure 1. Plasmid pRU729 (Rogowsky, 1985) contains two Tn*1721 res* sites in repeat order on a 1.15-kb *Eco*RI fragment. The *Eco*RI sites generated by Tn*1725* insertions (Altenbuchner *et al.*, 1983) were used for 5′ end labelling of the plus and minus strands; the two *res* sites were then separated by *Pst*I cleavage to yield the end-labelled subfragments A and B (Figure 1). Plasmid pEAK9 contains a complete Tn*21 res* sequence on a 0.5-kb *Eco*RI-*Bam*HI fragment; as indicated, this DNA was 5′ end-labelled only at the minus strand. The purified proteins, R21 and R1721, used for protection experiments were obtained as described previously (Halford *et al.*, 1985; Rogowsky and Schmitt, 1985).

Footprinting

Figure 2 and Figure 3 (lanes 5−16) show the footprinting of resolvase on two DNA fragments (A and B in Figure 1) that contain *res* from Tn*1721*: fragment A (Figure 2) carries the 5′ end label in the complementary strand to fragment B (Figure 3). Compared with the limited DNase I digests of the fragments in the

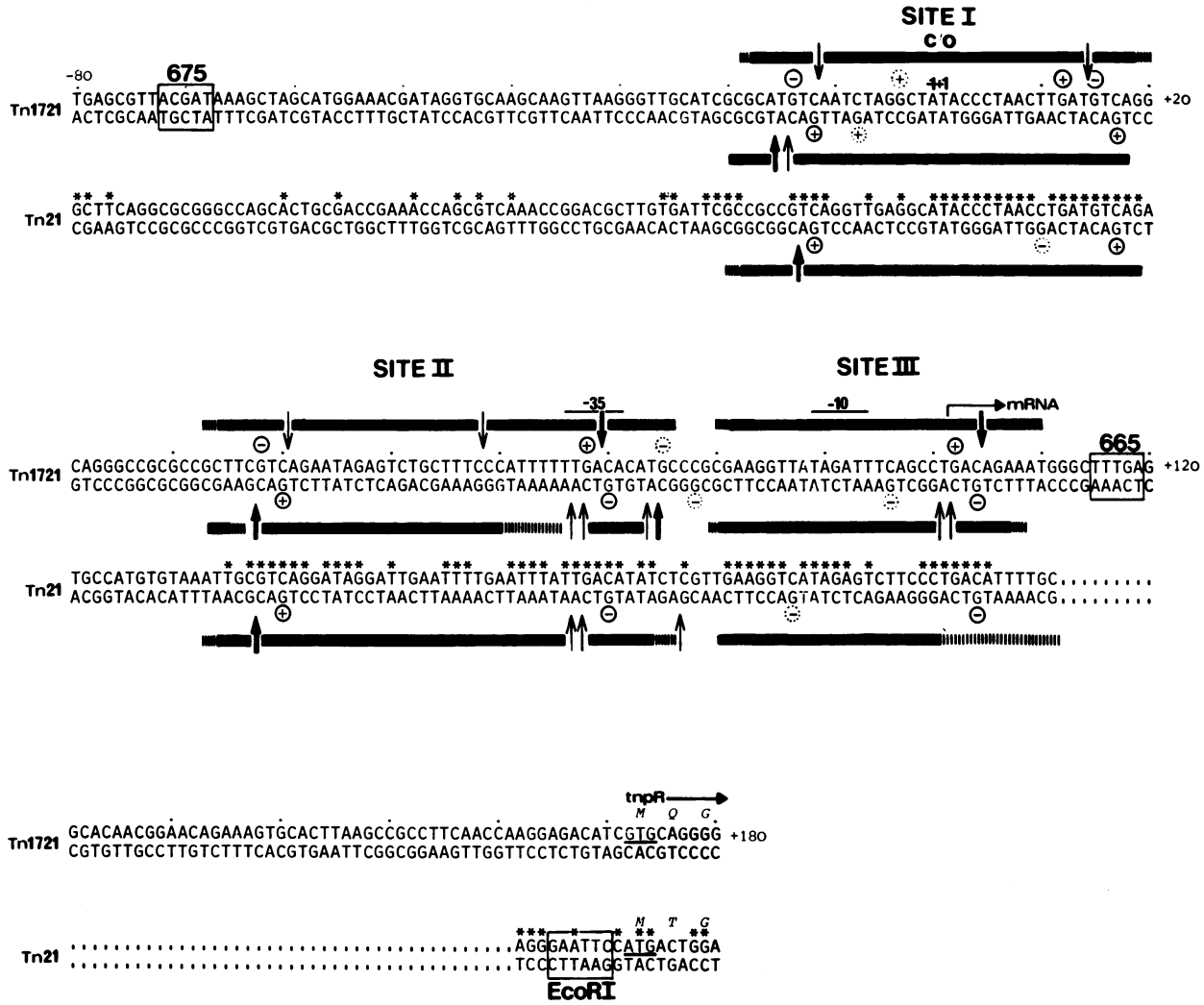

Fig. 6. Sites protected by resolvase. The DNA sequence of Tn*1721* upstream of the *tnpR* gene and the aligned sequence of Tn*21* are shown with numbering from the cross-over point (c/o); asterisks denote homologies between the two sequences, dots stand for a 50-bp segment absent from Tn*21* (Diver *et al.*, 1983). The *tnpR* promoter, transcription initiation and translation starts (with amino acids in single letter code) are indicated (Rogowsky and Schmitt, 1985). The positions of Tn*1725* insertions 665 and 675 on Tn*1721* (boxes indicate 5-bp direct repeats) and an *Eco*RI site on Tn*21* are shown (see Figure 1). Bars indicate protection from DNase I, striped portions mark positions that are resistant to DNase I in the unprotected control, so that protection at these sites remains undetermined. Arrows show sites of slight (↑) or enhanced (↟) DNase I cleavage within an otherwise protected region; these assignments were made after inspection of several gels of the type shown in Figures 2–4. The three sites protected by resolvase are designated as in the γδ/Tn*3* system (Grindley *et al.*, 1982). As indicated, the methylation of guanine within these sites may be slightly (⊖) or strongly (⊖) decreased or, conversely, slightly (⊕) or strongly (⊕) enhanced. Unmarked G residues exhibited no differences from the unprotected control.

absence of resolvase (the '−R' lanes), discrete regions of the DNA were protected against DNase I by either R21 or R1721. Both proteins created the same pattern. Thus it appears that R21 and R1721 can bind to specific sites at Tn*1721 res* on linear DNA fragments, even though site-specific recombination by these resolvases *in vitro* requires circular superhelical DNA (Halford *et al.*, 1985; Rogowsky and Schmitt, 1985). Three protected regions were noted: a 38-bp sequence around the cross-over point was named site I in analogy with γδ/Tn*3 res* (Grindley *et al.*, 1982) and two other protected regions closer to *tnpR* were designated site II (43 bp) and site III (30 bp), respectively. However, differing amounts of resolvase were required to achieve the same degree of protection at each site. For example, with 25 ng protein (Figure 2, lanes 5 and 9; Figure 3, lanes 8 and 13), site I was well protected but site III hardly protected at all: equal protection of site III needed 250 ng of protein (Figure 2, lanes 3 and 10; Figure 3, lane 15). This data suggests that the binding of either R21 or R1721 to *res* DNA from Tn*1721* is tighter at site I than at site III: site II appears to display an intermediate affinity.

The footprinting pattern of R21 and R1721 obtained on one labelled strand of Tn*21 res* DNA (Figure 4) was in close agreement with the pattern on the corresponding strand of Tn*1721 res* DNA (Figure 2). Both resolvases bound to three sites on Tn*21* DNA that are congruent with those identified on Tn*1721*. Again, differential affinity was observed, with site I on Tn*21 res* being protected from DNase I by less resolvase than was required at site III.

The above footprinting experiments did not always define precisely the transition from protected to unprotected nucleotides. First, DNase I did not cleave all phosphodiester bonds in the DNA with equal probability, as seen in the '−R' controls (e.g., Figure 2, lane 6). Certain bonds were refractory to cleavage and it thus could not be determined whether or not they were pro-

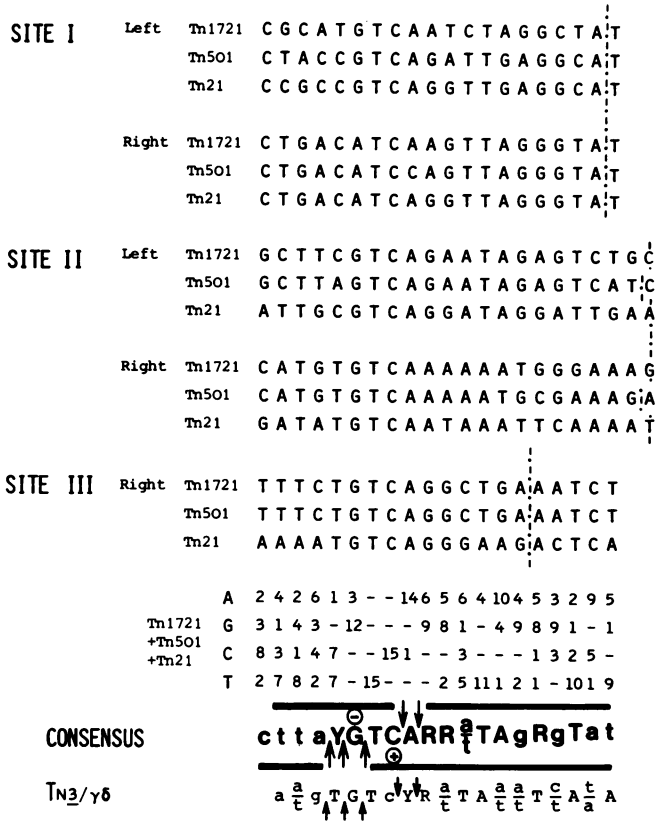

Fig. 7. Sequence homology between resolvase-protected half-sites of Tn*1721*, Tn*501* and Tn*21*. The sequence of each half-site is written as a single strand from 5′ to 3′ starting at the outer edge of the protected region towards the symmetry-axis and by aligning the fully conserved T residues at position 7. The centres of symmetry are indicated by dash-points. Below the sequences the frequencies of occurrence of each base at each position are shown: this summary excludes the left half of site III. The consensus sequence for a half-site was derived; capital letters indicate highly conserved bases and lower case letters weaker conserved bases; R and Y denote purines and pyrimidines respectively. G-protection ⊖ and G methylation ⊕ are marked as in Figure 6; symbols above the consensus sequence relate to the DNA strand shown, symbols below relate to the complementary strand. For comparison, the aligned consensus sequence for protected half-sites from the γδ/Tn*3* system (Grindley *et al.*, 1982) is shown below.

tected. Second, the high proportion of the DNA fragments protected by resolvase altered the length distribution of the DNase I digestion products. Third, certain bonds in an otherwise protected region were cleaved with normal or even increased efficiency. These phosphodiester linkages must remain accessible to DNase I while resolvase protects the surrounding DNA, and the enhanced cleavage at certain nucleotides can be accounted for by the conformation of the DNA at these locations changing as it binds to protein (Drew and Travers, 1984). In the equivalent strand of the DNA from Tn*1721* and Tn*21* (Figures 2 and 4), the protected regions yielded similar patterns of bonds with enhanced cleavage but a rather different pattern was observed in the complementary strand (Figure 3). However, in both cases, the sensitive bonds were clustered towards the ends of the binding site.

G-protection

The susceptibility of guanines in end-labelled fragments of DNA, that contain *res* from Tn*1721* or Tn*21*, to methylation by dimethyl sulphate was measured in the absence and in the presence of either R21 or R1721 (Figures 3 and 5). The data in Figures 3 (lanes 1–3) and 5a report on the complementary strands of Tn*1721* *res* and that in Figure 5B on the minus strand from Tn*21* *res* (Figure 1). In all three experiments neither R21 nor R1721 created any change in the methylation of guanines outside the regions protected from DNase I nor could any change be detected at more than half of the G residues within these three sites. However, certain guanines were either weakly or strongly protected by both R21 and R1721, whereas the methylation of other guanines was either weakly or strongly enhanced: these are indicated at the margin of the sequence ladders in Figures 3 and 5.

In G-protection experiments (Siebenlist *et al.*, 1980) reduced methylation of a guanine can arise from a direct interaction between the protein and that particular G residue whereas enhanced methylation has been assigned to the environment of the guanine becoming more hydrophobic in the presence of the protein (Simpson, 1982): the local configuration of the DNA may also affect these changes (Frederick *et al.*, 1984). In the reactions described here, major changes in methylation, either positive or negative, occurred at homologous positions in the sequences of Tn*1721* and Tn*21*. Differences between them were only seen where the two sequences diverge, and these differences pertain only to weak effects. Hence the interactions between resolvase and *res* must be similar on Tn*21* and Tn*1721*, with the conserved sequences playing the major role. Moreover, the G residues whose methylation is strongly affected by resolvase are clustered towards the ends of the sites protected from DNase I, in close proximity to the phosphodiester bonds within those binding sites where sensitivity to DNase I is enhanced.

Discussion

The structure of res

The experimental data in Figures 2–5 is summarized in Figure 6. Within 200 bp upstream of their *tnpR* genes, the DNA of Tn*1721* and Tn*21* both contain three sites (I, II and III) that bind resolvase, either R21 or R1721. The sites vary in length: site I is ~38 bp while sites II and III are ~43 and 30 bp, respectively. Sites I and II are separated by a spacer of 13 bp and II and III by a spacer of 4 bp. All three sites exhibit a certain degree of dyad symmetry. Between Tn*1721* and Tn*21*, the DNA sequences within the sites are largely conserved whereas the spacers have conserved lengths but no sequence homology. As R21 and R1721 are functionaly interchangeable (Halford *et al.*, 1985; Rogowsky and Schmitt, 1985), the specificity of these two resolvases must be defined by the sites themselves and their relative locations.

On Tn*1721*, the locations of the three binding sites for resolvase fit in with the dual functions of the protein at *res*, both site-specific recombination and genetic regulation (Altenbuchner and Schmitt, 1983). For site-specific recombination on Tn*1721*, the cross-over between the two *res* sites in the co-integrate has been located to a stretch of 11 bp (Rogowsky and Schmitt, 1984): this sequence is found within site I where it spans the axis of dyad symmetry. The critical role of site I in the recombination reaction was further demonstrated by experiments (not shown here) which employed a combination of a single site I from Tn*1721* and a complete *res* sequence as a co-integrate analogue. This substrate was resolved in *tnpR in vivo* if site I was oriented in direct repeat with *res* (Rogowsky, 1985). Figure 6 also notes the transcriptional start of *tnpR* on Tn*1721* that was identified by S1 mapping (Rogowsky and Schmitt, 1985) and the −10 and −35 sequences that appear to constitute the promotor for *tnpR*. The −10 sequence is within site III and the −35 is within site II. Hence, the binding of resolvase to these sites would presumably interfere with the binding of RNA polymerase and thus repress the expression of *tnpR*.

The functions required for the resolution of co-integrates in the Tn*21* subgroup of transposable elements are not interchangeable with those from γδ or Tn*3* (Diver *et al.*, 1983). Yet the overall structure of *res* observed in these studies on Tn*21* and Tn*1721*, three binding sites for resolvases on the DNA adjacent to the 5′ end of *tnpR* with the cross-over in the site furthest from *tnpR*, is similar to that described previously with γδ and Tn*3* (Grindley *et al.*, 1982; Kitts *et al.*, 1983). Sites I and II on Tn*21*/Tn*1721* are slightly longer than their counterparts on γδ/Tn*3*, and the spacer between I and II correspondingly shorter, but the distance between the centres of symmetry at sites I and II (53 bp) is the same for both groups of elements. Site III has the same length in Tn*21*/Tn*1721* and γδ/Tn*3* but is separated from site II by fewer nucleotides in the latter group. In addition, the phsophodiester bonds whose cleavage by DNase I is enhanced by the binding of resolvase are found at equivalent positions on Tn*21*/Tn*1721* (Figure 6) and on γδ/Tn*3* (Grindley *et al.*, 1982; Kitts *et al.*, 1983). Hence, the arrangement of the complex between *res* DNA and the cognate resolvase must be very similar on both groups of elements. As this arrangement has now been elucidated with two functionally distinct sets of Tn*3*-like elements, it seems probable that this structure is an intrinsic feature of *res* required for all of the activities of resolvase.

The differences between *res* of γδ/Tn*3* and that of Tn*21*/Tn*1721* are in the fine structure of each site (described below) and in the relative affinities of the cognate resolvases for sites I, II and III. On Tn*21* and Tn*1721*, more of either R21 or R1721 was required to protect site III from DNase I than site I (Figures 2–4). In contrast, the resolvases from γδ and Tn*3* protected each site of their *res* sequences equally well (Grindley *et al.*, 1982; Kitts *et al.*, 1983) and it was suggested that these proteins bound cooperatively to their DNA (Abdel-Meguid *et al.*, 1984). However, a proteolytic fragment of γδ resolvase, its C-terminal domain, bound independently and with differing affinities to each site of γδ *res* (Abdel-Meguid *et al.*, 1984).

A consensus sequence

The individual sites from *res* on Tn*21* and Tn*1721* show dyad symmetry and thus each can be considered as two half-sites, a left-hand (*tnpR*-distal) and a right-hand (*tnpR*-proximal). The 5′–3′ sequences are shown in Figure 7: one half of each site is from one strand of the DNA while the second, symmetrically related, half is from the complementary strand. (For reasons given below, the left hand of site III was excluded from this consideration.)

Figure 7 also includes the homologous sequences from the transposon Tn*501*, taken from Diver *et al.* (1983): we have no experimental data on the interactions *in vitro* between resolvase and Tn*501* DNA, but the resolution functions of Tn*501* and Tn*1721* are virtually identical (Diver *et al.*, 1983; Rogowsky and Schmitt, 1985). All 15 sequences, five half sites from three transposons, were aligned with reference to a completely conserved T residue found at the 7th position from the 5′ edge of each protected region. A consensus sequence was then derived from the frequencies of each base at each position in both left and right halves of sites I and II and the right half of site III (Figure 7).

The consensus sequence in Figure 7 falls into three sections. The first four positions, at the outer edges of the protected regions, show little (if any) preference for a particular base. There follows a region of six bases:

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      –    ↓
5′-YG TCAR-3′
3′-RCA G TY-5′
      ↑    +
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which is conserved virtually intact among all half sites. At all half sites except for the left half of site III (Figure 6), this region yields a very similar pattern of both DNase I-sensitive bonds (noted by arrows) and guanines whose methylation by dimethyl sulphate is enhanced or reduced (marked + or −, respectively). The remainder of the consensus sequence, close to the symmetry axis, shows a marked but not total preference for one base (or two) in each position. The specificity of resolvase appears to be for either purine at some positions or for A-T base pairs at other points. Similar degeneracies in DNA sequence recognition are also observed with certain class II restriction enzymes (Rosenberg and Green, 1982).

The consensus sequence for a half-site at *res* from Tn*21*, Tn*501* and Tn*1721* shows some similarities to that from γδ and Tn*3*, reproduced from Grindley *et al.* (1982) in Figure 7. Both contain the sequence YGTC followed by R(a/t)TA, but these motifs are separated by one base in the γδ/Tn*3* consensus and by two bases in that for the Tn*21* group. The section of each half-site in γδ *res* that is primarily responsible for the specificity of the DNA-protein interaction includes both of these motifs (Abdel-Meguid *et al.*, 1984). Hence, even though there are other differences between the two consensus sequences, the spacing of these motifs could play a major role in the discrimination of *res*-resolvase interactions.

DNA-protein complexes

The only aggregation state of R*21* that retains recombinational activity in solution is the dimeric protein (Halford *et al.*, 1985). The DNA sequences at sites I, II and III all show some dyad symmetry between left and right halves (Figure 6). Thus it seems likely that, at each site, one half of the DNA will bind to one subunit of a resolvase dimer. However, the dimeric form of R*21* (or R*1721*) has a mol. wt. of 42 000 (Halford *et al.*, 1985): a globular protein of this size would be ~50 Å in diameter (Zipper and Kratky, 1977). Yet the lengths of protected DNA at sites I and II in *res* from either Tn*21* or Tn*1721* are 129 Å and 146 Å, respectively (assuming the B form of DNA with 3.4 Å between base pairs). One model that can account for contact between these lengths of DNA and one small protein could be wrapping of the DNA around the protein, in the manner observed with nucleosomes (Klug *et al.*, 1980). The pattern of DNase I-sensitive sites, with a 5-bp stagger between one strand and the other, is consistent with only one face of the DNA being protected by the protein.

Site III at *res* from Tn*1721* and Tn*21* differs in several respects from sites I and II. Firstly, it is much shorter than the previous sites. This results in conserved elements from each half of site III being closer together. Secondly, the binding of resolvase yields characteristic patterns of guanine methylation and DNase I-sensitive sites in both halves of sites I and II, and also the right but not the left half of site III (Figure 6). Hence, the structure of the DNA-protein complex at site III, particularly its left half, cannot be like that at the other sites of *res*. The left half was therefore omitted from the derivation of the consensus (Figure 7).

In all of the experiments reported here (with one exception noted in Figure 5), the same results were obtained with R21 and

R1721 regardless of whether the *res* sequence was from Tn*21* or Tn*1721*. The amino acid sequences of R21 and R1721 differ at 34 out of 186 positions (Diver *et al.*, 1983; Rogowsky and Schmitt, 1985). Twelve of these substitutions are among the 45 amino acids from the C terminus which, by analogy with γδ resolvase (Abdel-Meguid *et al.*, 1984), is likely to form a separate domain in the protein structure (Halford *et al.*, 1985). The C-terminal domain of γδ resolvase interacts specifically with *res* DNA (Abdel-Meguid *et al.*, 1984). It is improbable that non-conservative substitutions within this region, such as proline 163 in R1721 for glutamine in R21 can be at sites in contact with the DNA. A speculation that might account for the conserved motifs in the consensus sequence from the Tn*21*/Tn*501*/Tn*1721* group being separated by one more nucleotide than in the consensus from γδ/Tn*3* (Figure 7) is that this is due to three extra amino acids in R21 or R1721 (positions 158 – 160) which have no counterparts in the resolvases from γδ or Tn*3*.

Materials and methods

Enzymes and chemicals

Preparations of resolvase from Tn*21* and Tn*1721* were described by Halford *et al.* (1985) and Rogowsky and Schmitt (1985), *Afl*II was a gift of N.L.Brown, *Eco*RI was prepared by S.H. Other restriction endonucleases, T4 DNA ligase, alkaline phosphatase, polynucleotide kinase and DNase I (grade I) were purchased from Boehringer (Mannheim, FRG). DNase I was dissolved in 10 mM Tris-HCl (pH 8), 10 mM $MgCl_2$, stored in aliquots at −20°C and diluted 1:10 in the same buffer before use. Dimethyl sulphate (Gold Label) was purchased from Aldrich Europe (Nettetal, FRG) and agarose from Bethesda Research Laboratories (Gaithersburg, MD).

Plasmids and 5′ end labeling of DNA fragments

Plasmid pRU729 contains two Tn*1721 res* sites (Rogowsky, 1985) and plasmid pEAK9 contains two Tn*21 res* sites (Halford *et al.*, 1985). The DNA fragments used for protection experiments are shown in Figure 1. They were 5′ end-labelled using polynucleotide kinase and [γ-^{32}P]ATP (Johnson and Walseth, 1979).

DNase I footprinting experiments

The assay used for footprinting was a modification of the method described by Galas and Schmitz (1978). For the binding reaction, the 5′ end-labelled DNA fragment and 3 μg calf thymus DNA was dissolved in 20 μl of 45 mM Tris-HCl (pH 7.5), 15 mM $MgCl_2$, 1.5 mM dithioerithrol and mixed with 10 μl of a solution containing resolvase at a concentration between 0.5 and 50 μg/ml in R buffer [10 mM K_2HPO_4, pH 7.5, 1 mM EDTA, 400 mM KCl, 33% (v/v) ethandiol]. The mixtures were incubated at 37°C for 20 min, then shifted to 20°C and equilibrated for 5 min. DNase I was added to 1 μg/ml and, after 3 min, digestion was stopped by the addition of 30 μl of 20 mM EDTA. After adding 3 μg calf thymus DNA, the DNA in the reaction was ethanol-precipitated, washed in ethanol and re-dissolved in loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanol and bromphenol blue). After boiling for 1 min, samples were analyzed by electrophoresis on 8% or 10% polyacrylamide gels in 90 mM Tris-borate (pH 8.5), 2.5 mM EDTA, 7 M urea.

Guanine protection experiments

The methylation of guanine residues by dimethyl sulphate in the presence of resolvase was tested according to Sienbenlist *et al.* (1980). For resolvase binding, the 5′ end-labelled DNA fragment was dissolved in 90 μl of a modified dimethyl sulphate buffer [Maxam and Gilbert, 1980; 50 mM sodium cacodylate (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA, 100 mM KCl] and mixed with 10 μl of R buffer containing resolvase at a concentration of 50 μg/ml. The mixture was incubated at 37°C for 20 min, shifted to 0°C, equilibrated for 5 min and, upon addition of 0.5 μl dimethyl sulphate, incubated at 20°C for 5 min. All subsequent steps were those of the G reaction described by Maxam and Gilbert (1980). The resulting DNA fragments were analyzed by electrophoresis on denaturing 8% or 10% polyacrylamide gels and autoradiography using FUJI-RX film.

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