The EMBO Journal vol.6 no.3 pp.815–821, 1987

Contacts between γδ resolvase and the γδ *res* site

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Communicated by P.van de Putte

We have investigated the interaction between resolvase and the *res* site of the transposon γδ by methylation and ethylation interference experiments. We have examined the effect of these DNA modifications both on binding and resolution *in vitro*. Major groove methylations within a 9 bp sequence that borders each site inhibit binding of resolvase to that site. Ethylation of certain phosphates within, and adjacent to, this border sequence inhibits binding. Together, these interference points define a contact region, present at all three *res* sites. *In vitro* resolution is inhibited only by modifications within site I. Inhibition of resolution by methylation of adenines at the center of site I suggests that minor groove contacts near the crossover may be required for resolution activity.

Key words: ethylation interference/methylation interference/protein–DNA interaction/site-specific recombination/transposon γδ

Introduction

The γδ resolvase is a 21 000-dalton protein encoded by the transposable element γδ, a member of the Tn*3* family of bacterial transposons (for reviews, see Grindley and Reed, 1985; Heffron, 1983). Resolvase is both a site-specific recombinational protein and a repressor. It interacts with a site named *res* which lies within the region between the divergently transcribed *tnpA* and *tnpR* genes of the γδ transposon. This interaction has two roles. First, when two copies of *res* are present in the same orientation on a single replicon (as in a cointegrate, the intermediate of γδ transposition) resolvase catalyzes a site-specific, reciprocal recombination between them. This results in formation of two circular products, each with a single *res* site. Second, binding to *res* negatively regulates transcription of both the *tnpA* (transposase) and *tnpR* (resolvase) genes.

The resolution reaction has been accomplished *in vitro* (Reed, 1981). Its only requirements are resolvase, Mg^{2+}, and a supercoiled DNA substrate containing two identically oriented *res* sites. Resolvase protects three binding sites within *res* from nuclease digestion (Grindley *et al.*, 1982; Kitts *et al.*, 1983). Each site consists of inverted repeats of a 9 bp segment [with the consensus sequence TGTCYNNTA (Y = pyrimidine, N = any base)] separated by a variable spacer of 7, 10 or 16 bp. Site I, which has a 10 bp spacer, contains the recombinational crossover point; all three sites are required for efficient recombination (Grindley *et al.*, 1982; Kitts *et al.*, 1983; Wells and Grindley, 1984).

Two types of interaction may be envisaged to occur at *res*. First, resolvase must recognize and bind to each site. This recognition is required both for repression as well as for resolution and can take place at each of the three binding sites. Second, resolvase must distinguish site I from the other two binding sites and interact specifically with the nucleotides in its center to mediate recombination. Details of the interaction between a protein and its DNA recognition site can be learned by determining the positions of methylated purines and ethylated phosphates that interfere with the formation of specific protein–DNA complexes (Siebenlist and Gilbert, 1980). We have used such interference experiments to probe the resolvase–*res* interaction. Contacts important for binding were determined by analysis of resolvase–DNA complexes; additional interactions required specifically for the recombinational process were investigated by analysis of the products of resolution *in vitro*.

Results

Inhibition of binding by methylation of purines

Treatment of duplex DNA with dimethyl sulfate results in methylation of the N7 position of guanine (in the major groove) and the N3 position of adenine (in the minor groove). By using a DNA substrate methylated to a limited extent we can determine positions at which methylation of a ring nitrogen will interfere with binding, either through loss of an important major or minor groove contact point or by steric inhibition of the resolvase recognition process (Siebenlist and Gilbert, 1980).

We have found that resolvase–DNA complexes can be separated readily from non-complexed DNA by electrophoresis on 5% polyacrylamide gels (Fried and Crothers, 1981; Hatfull and Grindley, 1986). Using this separation technique we have analyzed complexes formed with partially methylated DNA restriction fragments that contain each of the individual binding sites. As can be seen from Figure 1, methylated G's at specific positions within each site strongly inhibit complex formation. These G's are at positions −13 and +10 on the 'top' strand of site I (see Figure 2A), and −11, −10 and +13 on the 'bottom' strand; at positions 38 and 67 on the top strand and 40 and 69 on the bottom strand of site II; and at positions 77, 94 and 95 on the top strand and 79, 80 and 99 on the bottom strand of site III. All these G residues occur within the conserved 9 bp segment found at each half site. The inhibitory effects of these methylations indicate that resolvase interacts specifically with bases in the major groove of the DNA in these segments.

In the same experiments methylation of several A residues was also found to inhibit resolvase binding at all three sites, although not as completely as those observed with methylated G's (Figure 1). The most inhibitory methylations of A's cluster at the inside ends of the 9 bp segments and the adjacent portions of the spacer (see Figure 2A). These inhibitions may result either from steric interference with a minor groove location of the resolvase polypeptide, or from the introduction of a local positive charge with the methylation of the ring nitrogen. Note that methylations within the cluster of A's at the center of site II (the site with the largest spacer) have no inhibitory effect (Figure 1).

Inhibition of binding by ethylation of phosphates

Ethylation of the phosphate backbone of DNA with ethylnitrosourea removes a negative charge and imposes a potential steric

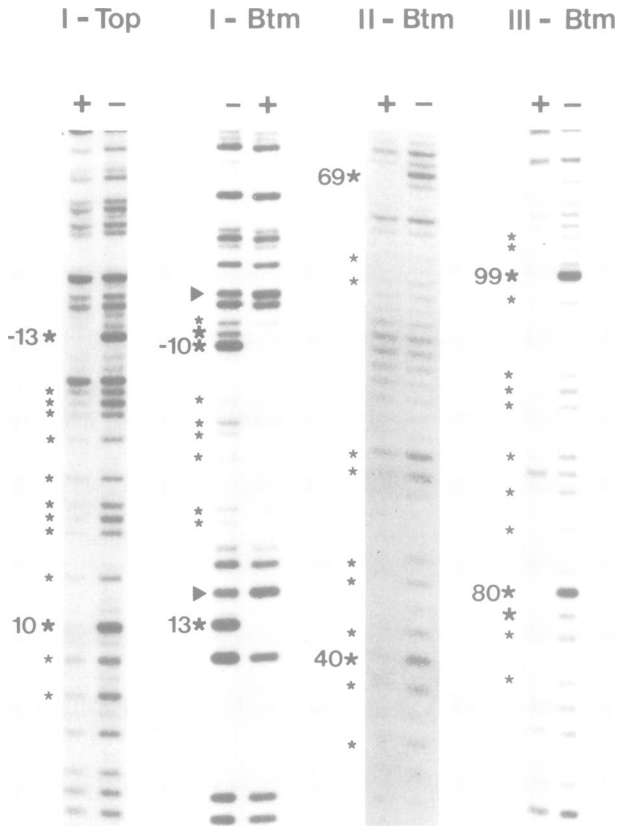

Fig. 1. Inhibition of resolvase binding by purine methylation. Autoradiograms show the distribution of methylated purines in resolvase-complexed (+) and free (−) DNA. Data are shown from both strands (Top and Btm) of site I and the bottom strand (Btm, as shown in Figure 2A) of sites II and III. Positions at which a methylated purine inhibits strongly (★), weakly (⋆), or enhances (►) complex formation are indicated.

block, and can be used to probe phosphates that come in close contact with a DNA binding protein (Siebenlist and Gilbert, 1980). We have analyzed complexes between resolvase and partially ethylated DNA of site I (both strands) and of site III (bottom strand); data for site I are shown in Figure 3. There are seven phosphate residues within each half site that strongly inhibit resolvase binding (see Figure 2A). They map to the same positions relative to the 9 bp segment in the three cases analyzed. In Figure 2B these positions are shown superimposed on the consensus sequence for the binding segments, along with the methylation data.

Inhibition of resolution in vitro

The experiments outlined above indicate contacts between resolvase and its DNA binding sites that are required for binding. If additional resolvase−DNA contacts are required for the recombination reaction they should become apparent if the binding assay is replaced by resolution *in vitro*. The strategy used to analyze interference with *in vitro* resolution is outlined in Figure 4. Superhelical DNA of the cointegrate analog pRW16 was methylated by treatment with DMS or ethylated with ethylnitrosourea and subjected to an *in vitro* resolution assay. The resolved products were separated from unresolved starting material by restriction with *Eco*RI and agarose gel electrophoresis. Purified fragments were end-labeled, digested with *Acc*I, and cleaved at modified positions. Resolved and unresolved samples were run on sequencing gels and autoradiographed (Figure 5, A−D).

Only modifications within site I interfered with resolution (Figure 5). Methylation of G's at positions −13 and −10 in the left half of site I and +13 in the right half strongly inhibited the reaction, those at −11 and +10 inhibited more weakly. As expected, these results indicate that the symmetric major groove interactions required for resolvase binding to site I are necessary for resolution activity as well. More significantly, methylation of certain

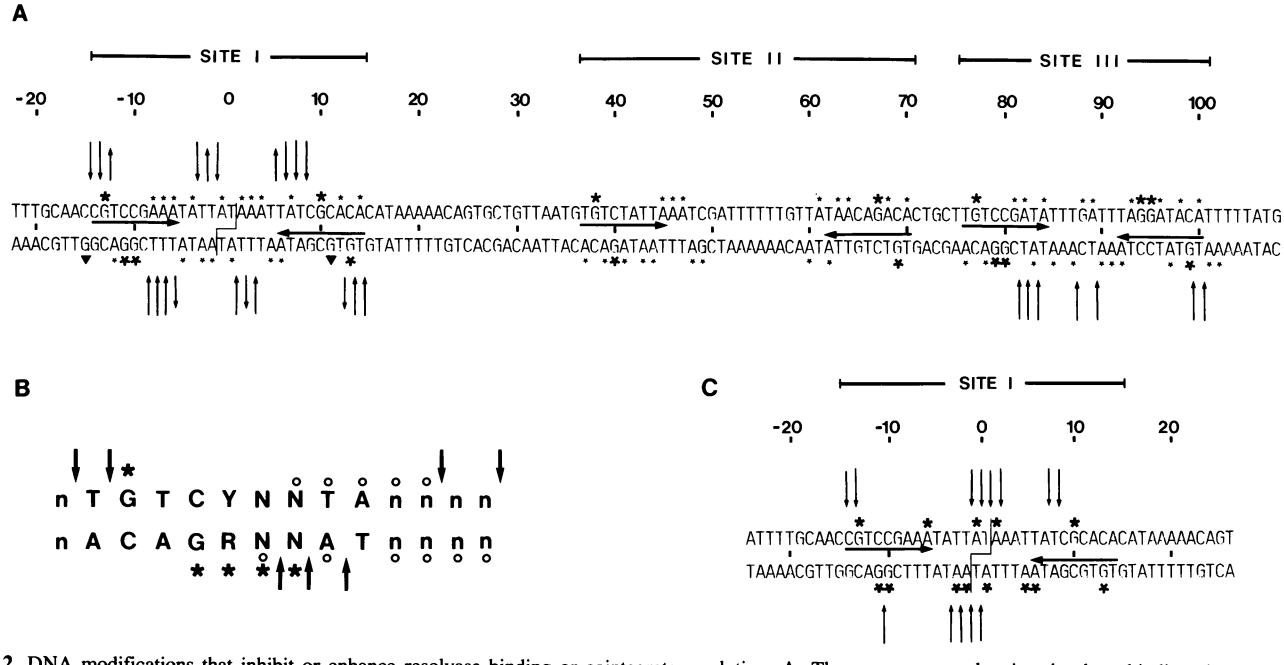

Fig. 2. DNA modifications that inhibit or enhance resolvase binding or cointegrate resolution. **A**. The *res* sequence showing the three binding sites and modifications that affect binding of resolvase. Symbols above and below DNA sequence indicate purines that, when methylated, inhibit strongly (★) or weakly (⋆), or enhance (▼) resolvase binding. Vertical arrows (↑ ↓) point towards the positions of ethylated phosphates that inhibit binding and away from those that enhance binding; the ethylation data were obtained only for site I (both strands) and site III (bottom strand). **B**. A consensus of inhibitory modifications displayed relative to the 9 bp consensus sequence. R = purine, Y = pyrimidine, N or n = any base (n is used for positions outside the 9 bp conserved segment). Shown are: (↓) inhibitory ethylated phosphates, (★) major groove inhibitions (i.e. inhibitory methylations when a guanine occurs in this position), and (○) minor groove inhibitions (i.e. inhibitory methylations when an adenine occurs in this position). **C**. The sequence of site I showing modifications that inhibit resolution. The symbols are the same as those used in panel A.

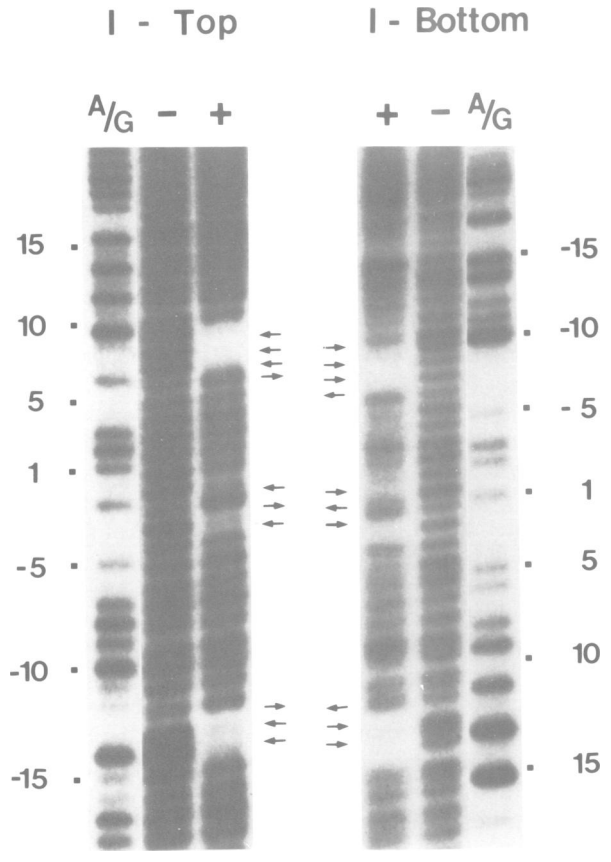

Fig. 3. Inhibition of resolvase binding to site I by ethylation of phosphates. Autoradiograms display the positions of ethylated phosphates in resolvase complexes (+) or free DNA (−). The data are for site I top and bottom strands (as indicated) labeled at their 5′ ends. Marker lanes (A/G) show a standard Maxam−Gilbert A+G reaction. Each number indicates the phosphate located on the 5′ side of the corresponding base. Arrows indicate phosphates that, when ethylated, interfere with (pointed towards the band) or enhance (pointed away from the band) binding.

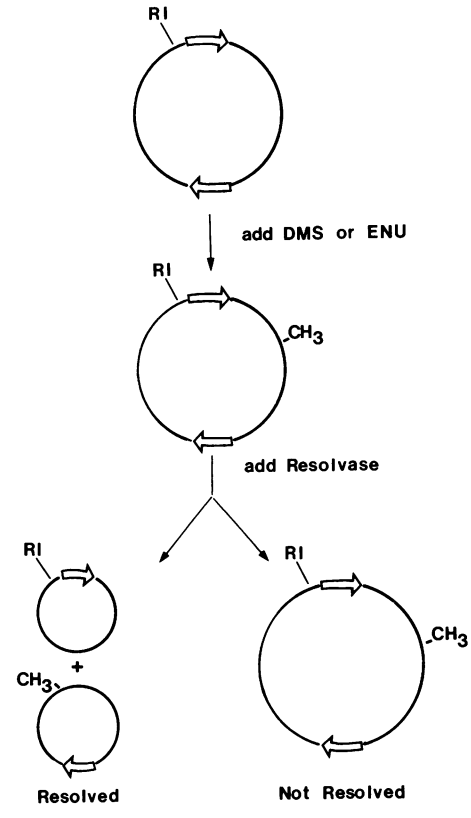

Fig. 4. Scheme for studying inhibition of resolution *in vitro*. Chemical modification of supercoiled cointegrate substrate DNA is symbolized by $-CH_3$. The diagram shows the fate of a single cointegrate in a population of molecules. When modification of the substrate occurs at a residue that is important for resolution activity, the efficiency of resolution will be reduced. For further details, see text.

A residues near the center of site I also inhibited resolution. These A's are at positions −6, −1 and +2 on the top strand and −3, −2, +1, +5 and +6 on the bottom strand (Figure 2C). The inhibition of resolution appears not to be simply a reflection of the mild inhibition of resolvase binding; for example, although methylations of positions 2, 3 and 4 on the top strand are about equally inhibitory to binding (Figure 1), only the position 2 methylation inhibits resolution (Figure 5A).

Phosphate ethylations within site I that interfered with binding inhibited resolution only weakly. However, additional resolution-specific inhibitory ethylations were observed at phosphates 5′ to positions −1, +1, 2 and 3 on the top strand and −1, −2, −3, −4 and −11 on the bottom strand (Figures 5C,D and 2C).

Figure 5E shows the results of using a mildly depurinated co-integrate as a substrate for *in vitro* resolution. The absence of a base at most purine positions within site I inhibited resolution. On the bottom strand the strongest effects were seen at positions −12, −11, −10, −5, −3, −2, +1, 5 and 13; only modest inhibition was observed through loss of purines at 6, 8, 9 and 11 (all in the binding sequence in the right half of site I). These results stress the importance of the central spacer region in site I for the recombination event.

Analysis of the res−resolvase complex by methylation

When incubated with increasing concentrations of resolvase, a DNA fragment containing a complete *res* site forms a series of complexes of decreasing electrophoretic mobility (Figure 6A; Hatfull and Grindley, 1986). Inhibition of binding by G-methylation can be used to identify the sites bound within a given complex. We have analyzed those complexes between resolvase and methylated *res* DNA which had the greatest and the least electrophoretic mobility (Figure 6B). In the most rapidly migrating complex, the intensity of bands corresponding to certain methylated G's within sites I and II was diminished by about 50% (positions −10, 13, 40 and 69); bands within site III were unaffected (positions 79, 80 and 99; Figure 6B, lanes 3 and 6). Since methylation of, for example, G's at −10 and +13 in site I or at 40 and 69 in site II completely inhibits binding to these respective sites (see Figure 1), we infer that this complex contains *res* DNA with a single binding site occupied, with site I filled in about half of the molecules and site II filled in the remainder. Confirmation that the fastest migrating complex contains just one active unit of resolvase (presumably a dimer) has been obtained in studies of a mutant resolvase that, by gel assay, is defective in binding to site I. In this case the equivalent complex contained mutant resolvase bound exclusively to site II (Hatfull and Grindley, 1986).

Analysis of the complex with the lowest electrophoretic mobility showed that, in addition to the G bands in sites I and II, the important G's in site III (79, 80 and 99) were also substantially diminished (compare Figure 6B, lanes 1 and 3, and lanes 4 and 6). This indicated that all three binding sites were occupied by resolvase in this complex.

Discussion

The *res* sites of γδ and other Tn*3* related transposons are complex regions that contain three resolvase binding sites within a 120 bp segment (Grindley *et al.*, 1982; Kitts *et al.*, 1983; Rogowsky *et al.*, 1985). The initial footprinting studies both with intact resolvase and with the small C-terminal domain (Abdel-Meguid *et al.*, 1984) had suggested two unusual features of *res*. First, each individual binding site differed in size as a result of a variable spacer (7, 10 or 16 bp) separating the two inverted copies of a conserved 'binding sequence'. Second, in contrast to other complex regulatory regions (such as O_LP_L and O_RP_R of bacteriophage λ; Ptashne *et al.*, 1980) the individual binding sites were irregularly spaced relative to one another (with 53 bp separating the centers of sites I and II, and 34.5 bp between the centers of sites II and III). The data described in this paper considerably strengthen these conclusions, identify many of the contacts between resolvase and its binding sites, and allow us to position with accuracy the DNA binding domain on each half of the three binding sites (Figure 7).

The region of DNA contacted by a single DNA binding domain of resolvase is unusually large. Figure 7 shows a two-dimensional projection of the γδ *res* DNA helix, indicating the positions of the 9 bp inverted repeats and the guanine and phosphate contacts. Within each half-site these phosphates cluster in an area (stippled in Figure 7) that covers about one-third of the circumference around the helix and includes one adjacent major and minor groove of the DNA (about 14 bp). The conserved 9 bp segment lies within the outer portion of this area that spans the major groove; it is here that all the guanine contacts are found. These outer major groove contacts are probably with a helix-turn-helix structure proposed to be within the C-terminal domain of resolvase (Abdel-Meguid *et al.*, 1984; Grindley *et al.*, 1985); such a structure has been demonstrated crystallographically for several other well characterized regulatory proteins (for a review, see Pabo and Sauer, 1984). The binding domains of these other proteins, however, recognize short sequences (typically 6–8 bp) and their phosphate contacts are generally confined to both sides of a single major groove (although CAP interacts with the sugar-phosphate backbone across the minor groove to the outside of each binding site; see Simpson, 1980). In the case of resolvase, additional contacts are made across the inner minor groove of each binding site. Furthermore, preliminary studies with site I mutants of *res* indicate that the bases important for binding extend at least from positions −4 through −14 and +4 through +14 (our unpublished data). Additional DNA binding interactions with this inner region must be made either by another portion of the C-terminal domain or, given the very small size of the domain, by part of the N-terminal domain. [Although a pattern of phosphate contacts similar to those of site I is observed with the AraC protein, which interacts with its regulatory sequence, *AraI*, over approximately 40 bp (Hendrickson and Schleif, 1985), AraC appears to make contacts in all three consecutive major grooves and does not seem to possess homology to the DNA binding domains of other repressors. Unfortunately, in the case of site I of *res*, the central major groove contains no G residues, making it invisible to DMS methylation.]

The similarities in the inhibition data between the various half-

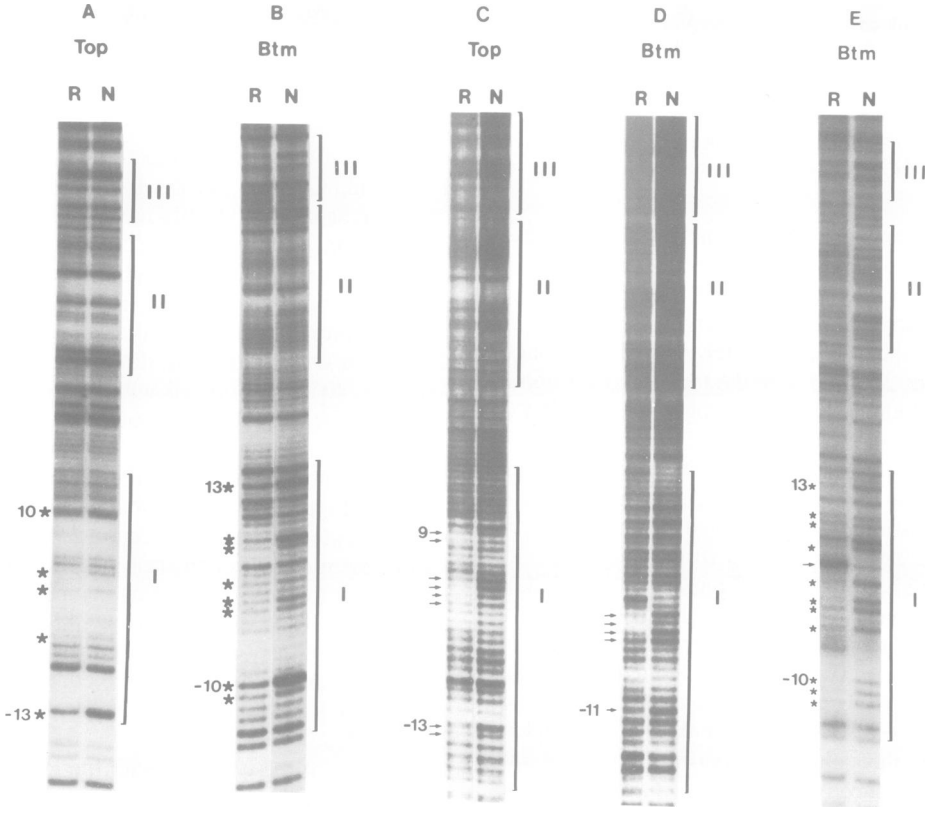

Fig. 5. Inhibition of resolution *in vitro* by DNA modifications. **Panels A** and **B** show the results of purine methylations in the resolved population (R) and the starting material (N) on the top (5′ end labeled) and bottom strand (3′ end labeled); asterisks mark the positions of inhibitory modifications. **C** and **D** show results of phosphate ethylation; arrows mark positions of inhibitory ethylation. **Panel E** shows results of depurinating the substrate DNA; asterisks mark positions where removal of a base interferes with resolution. The arrow in **panel E** indicates position of a resolvase cleavage.

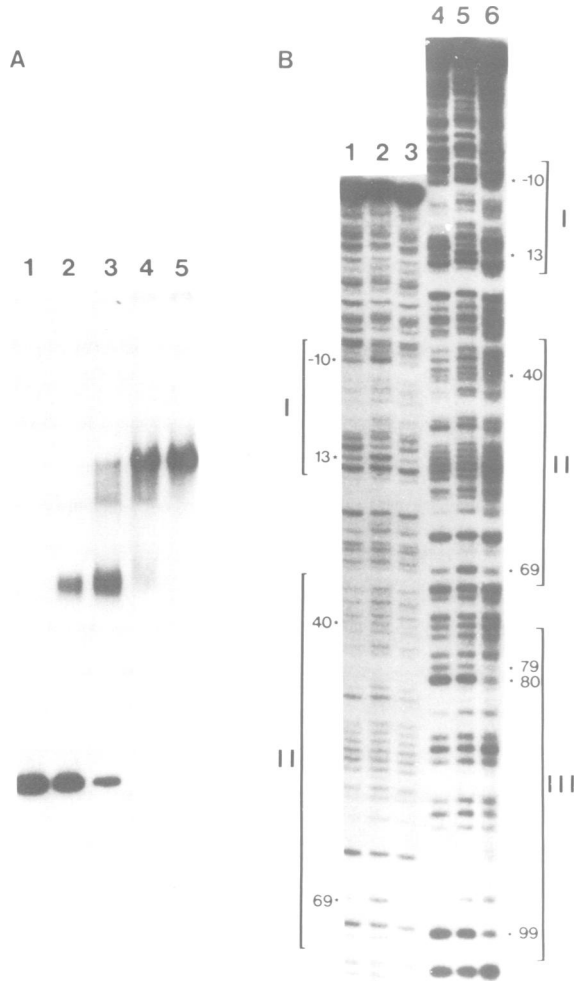

Fig. 6. Resolvase–*res* complex formation. **A**. Autoradiogram of polyacrylamide gel showing resolvase–*res* complex formation with increasing concentrations of resolvase. DNA fragments containing all three sites were 3′ end labeled. The reaction in **lane 1** contained no resolvase; those in **lanes 2–5** contained resolvase at approximately 0.3, 1, 3 and 10 μg/ml respectively. **B**. Autoradiogram showing the methylation patterns of the DNA in the fastest migrating complex (**lanes 1** and **4**), the slowest complex (**lanes 3** and **6**) and free DNA (**lanes 2** and **5**). DNA fragments were 3′ end labeled; inhibitions shown are on the bottom strand in Figure 2A. Bands (corresponding to methylated G residues) whose absence indicates binding of resolvase at a particular site are marked with asterisks. **Lanes 1–3** are longer runs of the samples in **lanes 4–6**.

sites suggest that each 9 bp segment interacts with the DNA binding domain of resolvase in essentially the same manner regardless of the length of the spacer that separates the two halves of each binding site. The variability in spacer length makes the binding sites remarkably different from one another. As drawn in Figure 7 (which assumes a linear B-DNA conformation), the two C-terminal domains of a dimer at site I bind to the back of the helix with an unoccupied major groove between them. To reach the crossover site at the front of the helix, each N-terminal domain must wrap around the DNA. At sites II and III the two C-terminal domains of each resolvase dimer are displaced angularly from one another with monomer units at the bottom and top of the helix at site II, and at the front and back of the helix at site III. It is clear from the figure that, to accommodate the different geometries at each binding site, either a dimer of resolvase must have a highly extendable, flexible structure or the DNA itself must be distorted (untwisted or bent). Flexibility, at least of the C-terminal (DNA-binding) domain relative to the N-terminal (dimerization and catalytic) domain, is suggested by the crystallographic data (Abdel-Meguid *et al.*, 1984). In addition, analysis of the behavior of resolvase–DNA complexes on polyacrylamide gels had provided evidence that binding of resolvase induces bending of each recognition site (J.Salvo and N.D.F.Grindley, in preparation).

By using resolution *in vitro* in addition to a binding assay to study the inhibitory effects of nucleotide modifications, we hoped to identify resolvase–DNA contact points that are important for recombination but play no significant role in the binding interaction. Modifications could interfere specifically with cointegrate resolution in any of three ways: by inhibiting a necessary resolvase–DNA interaction, by inhibiting a DNA–DNA interaction, or by preventing formation of a recombinogenic structure that may depend on local base sequence and integrity. The only DNA–DNA interaction that is known to occur during resolution is the formation of the 2 bp heteroduplex joint when the left half of site I from one *res* is recombined with the right half of site I from the other *res* (Reed and Grindley, 1981). The positive charge introduced by methylation of the ring nitrogen of adenine potentially could affect the base stacking and hydrogen bonding properties of the modified base. Thus, within and immediately adjacent to the heteroduplex region, adenine methylations may interfere with DNA–DNA interactions. Similarly, recent work has demonstrated that resolvase bends site I DNA, inducing a localized distortion (perhaps a kink) right at the crossover point (Hatfull *et al.*, 1987; J.Salvo and N.D.F.Grindley, in prepara-

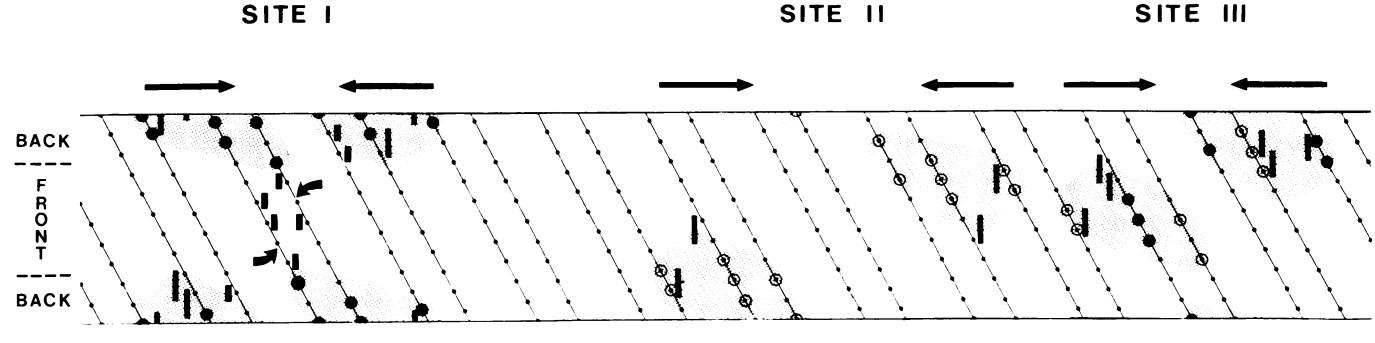

Fig. 7. Summary of resolvase contacts. The spatial arrangement of contact points is displayed on a planar projection of a DNA helix containing the *res* site. Shown are: ▌, guanines that strongly interfere with resolvase binding; ▮, adenine contacts that, along with the guanines at site I, interfere with resolution; ●, phosphates that interfere with binding; and ○, presumed phosphate contacts in sites II and III. The 9 bp inverted repeats at each site are shown with the horizonal arrows. The stippled shape superimposed on the contact points represents the C-terminal DNA binding domain of resolvase. The crossover point at the center of site I is marked with arrows.

tion). We cannot yet assess whether formation of this structural distortion depends on local base interactions that may be inhibited by adenine methylation. Despite our uncertainties concerning the effects of methylated adenine on DNA structure, the observed inhibition of resolution by N^3-methyl adenines at positions clustered around the crossover point (but, in some cases, outside the region of heteroduplex) suggests that resolvase may interact with the minor groove of the DNA in this region. The phosphate ethylation data suggest that there are contacts between the N-terminal catalytic domain of resolvase and the crossover region which are vital for recombination but not for binding of resolvase (see Figure 2C).

When the effects of DNA alkylations were assayed by resolution *in vitro*, we found that modifications only within site I were inhibitory. Previous results, however, have shown that binding of resolvase to all three sites of *res* is required for efficient resolution. This apparent paradox probably reflects the different requirements of the two assays; for the binding assay a rather stable complex is required, whereas only a transient complex may be needed for resolution. This is illustrated by the properties of a mutant resolvase (serine 173 to glycine) which has lost repressor activity, has substantially reduced affinity for *res in vitro*, yet retains recombinational activity (G.Hatfull and N.D.F.Grindley, unpublished results). In the alkylation experiments a single modification of an important position is likely to severely weaken the binding of resolvase but not eliminate it completely. Since sites II and III play an ancillary role, weaker binding to one of them should allow transient complex formation and therefore have little effect on recombination even though detection of stable complexes may be dramatically reduced.

We have used the inhibition of resolvase binding by guanine methylation to determine which sites are occupied in the intermediate complexes formed between resolvase and a complete *res* site. We find that the initial, fastest migrating, complex contains *res* with either site I or site II occupied in approximately equal proportions; there appears to be no significant interaction with site III in this complex (see also Hatfull and Grindley, 1986). The final, slowest migrating, complex has resolvase bound at all three sites. The complex that presumably contains *res* with two dimers of resolvase does not accumulate in sufficient quantities to allow easy identification; however, from the clear preference for binding sites I and II rather than III, it seems likely that site III would generally be left unoccupied. That this complex does not accumulate significantly but is chased rapidly into a fully occupied *res* complex suggests that binding to the third site may be to some extent cooperative.

Materials and methods

γδ res plasmids and preparation of DNA substrates

pRW16 (Grindley *et al.*, 1982) and pRW80 (= *resΔ101R* × *resΔ101R*; Wells and Grindley, 1984), γδ cointegrate analogs containing two complete *res* sites, have been described previously. pRW33 is the original *resΔ24R* plasmid (Wells and Grindley, 1984) in which one of the two *res* sites contains only site I. pRW115 contains a single copy of site II (*res* sequences 27−81) and pRW118 contains the right half of site II and a complete copy of site III (*res* sequences 49−100); details of both these constructions will be published elsewhere. DNA fragments containing each of the three individual binding sites, or all three sites together were obtained by appropriate restriction digest of pRW33, pRW115, pRW118 and pRW80. Fragments were labeled at their 5′ end using T4 polynucleotide kinase and [γ-^{32}P]ATP or at their 3′ end using DNA polymerase I (Klenow fragment) and [α-^{32}P]dATP.

Methylation and ethylation interference experiments

Methylation and ethylation interference experiments were done as described by Siebenlist and Gilbert (1980). For methylations, 0.5−1 μg of DNA fragments or 1−5 μg of supercoiled plasmid DNA were treated with 50 mM dimethyl sulfate in a buffer containing 50 mM sodium cacodylate, pH 8.0/10 mM $MgCl_2$/1 mM EDTA in a total volume of 200 μl. After 3 min at 20°C reactions were stopped by addition of 50 μl of 1 M β-mercaptoethanol/1.5 M sodium acetate/1 mM EDTA. Samples were precipitated several times by addition of 3 volumes of ethanol, rinsed with 70% ethanol and dried. Methylated DNA was then subjected to either the binding or resolution assay outlined below.

For ethylations, 1−5 μg of DNA fragments or supercoiled plasmid DNA were resuspended in 100 μl of 50 mM sodium cacodylate, pH 8.0 and mixed with an equal volume of ethanol saturated with ethylnitrosourea. After 1 h at 50°C, the reaction was stopped by addition of 20 μl of 3 M sodium acetate and 200 μl of 95% ethanol, and precipitated at −20°C. The DNA fragments were ethanol precipitated three more times, rinsed with 70% ethanol and dried. Ethylated DNA was then subjected to either the binding or resolution assay described below.

Depurination interference experiment

1−5 μg of supercoiled DNA was depurinated by incubating with 0.1 M pyridinium formate, pH 2.0 in a volume of 40 μl for 60 min at 20°C, according to the A+G reaction described by Maxam and Gilbert (1980). Samples were lyophilized, rinsed and lyophilized again. Depurinated DNA was then subjected to the resolution assay as described below.

In vitro resolution assay

Resolution of modified superhelical pRW16 was carried out using purified resolvase as described (Reed, 1981). The extent of completion of the reaction was assayed after 1 h by digestion with *Eco*RI and agarose gel electrophoresis. After 50% of the starting material was converted into product, the entire reaction was digested with *Eco*RI and end-labeled using either polynucleotide kinase for 5′ labeling or DNA polymerase I (Klenow fragment) for 3′ labeling. Secondary digestion with *Acc*I was carried out at 37°C overnight and *Eco*RI−*Acc*I fragments representing resolved and non-resolved species were separated by agarose gel electrophoresis. DNA fragments were identified by autoradiography, electroeluted from gel slices, and purified using DE52 cellulose chromatography. Samples were ethanol precipitated, phenol extracted, ether extracted, and re-precipitated with ethanol.

DNA fragments were cleaved at methylated or ethylated bases by resuspending pellets in 20 μl of 10 mM sodium phosphate pH 7.0/1 mM EDTA, heating at 90°C for 15 min, adding 1 μl of 1.5 mM NaOH/1 mM EDTA, and incubating for an additional 30 min at 90°C (Maxam and Gilbert, 1980). Samples were mixed with 2 volumes of formamide/10 mM EDTA/0.1% xylene cyanol and bromophenol blue, and were analyzed by electrophoresis through 8% polyacrylamide gels (acrylamide:bis-acrylamide, 20:1) in 50 mM Tris−borate pH 8.3/1 mM EDTA/8 M urea.

Resolvase binding assay

Resolvase−DNA complexes were formed and separated from uncomplexed DNA as described by Hatfull and Grindley (1986). End-labeled and modified DNA fragments were mixed with resolvase in 10 μl reactions containing 20 mM Tris−HCl, pH 7.5/100 mM NaCl/10 mM $MgCl_2$/1 mM dithiothreitol/1 μg of sonicated calf thymus DNA. Reactions were incubated at 37°C for 5 min and at room temperature for 5 min. To separate protein-bound fragments from unbound, samples were loaded on a 5% polyacrylamide gel in 100 mM Tris−borate pH 8.3/1 mM EDTA using the method described by Fried and Crothers (1981). DNA fragments were identified by autoradiography, eluted from gel slices, treated with proteinase K (1 mg/ml) for 20 min at 37°C, phenol extracted, ether extracted, and ethanol precipitated. Fragments were then cleaved at modified positions exactly as described in the *in vitro* resolution assay.

Acknowledgements

We thank Graham Hatfull for advice and assistance with the techniques and Alan Weiner for suggesting the depurination experiment. We also thank Kim Mowry, Larry Wiater, Joe Salvo and Graham Hatfull for helpful discussions and comments on the manuscript. This work was supported by grant GM 28470 from the National Institutes of Health.

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Received on November 26, 1986