

DNA translocation blockage, a general mechanism of cleavage site selection by type I restriction enzymes

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Type I restriction enzymes bind to a specific DNA sequence and subsequently translocate DNA past the complex to reach a non-specific cleavage site. We have examined several potential blocks to DNA translocation, such as positive supercoiling or a Holliday junction, for their ability to trigger DNA cleavage by type I restriction enzymes. Introduction of positive supercoiling into plasmid DNA did not have a significant effect on the rate of DNA cleavage by EcoAI endonuclease nor on the enzyme's ability to select cleavage sites randomly throughout the DNA molecule. Thus, positive supercoiling does not prevent DNA translocation. EcoR124II endonuclease cleaved DNA at Holliday junctions present on both linear and negatively supercoiled substrates. The latter substrate was cleaved by a single enzyme molecule at two sites, one on either side of the junction, consistent with a bi-directional translocation model. Linear DNA molecules with two recognition sites for endonucleases from different type I families were cut between the sites when both enzymes were added simultaneously but not when a single enzyme was added. We propose that type I restriction enzymes can track along a DNA substrate irrespective of its topology and cleave DNA at any barrier that is able to halt the translocation process.

Keywords: DNA supercoiling/DNA translocation/Holliday junction/type I restriction enzyme

Introduction

DNA translocation by proteins is a means to move along DNA and it is involved in a variety of processes such as replication (Yang *et al.*, 1989; Kong *et al.*, 1992), transcription (Liu and Wang, 1987), homologous recombination (Tsaneva *et al.*, 1992), DNA repair (Koo *et al.*, 1991; Allen *et al.*, 1997) and DNA restriction (Yuan *et al.*, 1980; Meisel *et al.*, 1995). Protein tracking along DNA can occur by various mechanisms. Some proteins such as the β subunit of DNA polymerase form clamps that slide freely along DNA (Kong *et al.*, 1992). Other proteins such as RNA polymerases or DNA helicases

track along the right-handed DNA double helix and can induce changes in the secondary and tertiary DNA structure (Droge, 1994).

For the type I restriction endonucleases, DNA translocation mediates communication between the recognition and cleavage sites. These enzymes recognize specific non-palindromic DNA sequences (e.g. GAGNNNNNNG-TCA where N is any nucleotide) but subsequently make a second contact with non-specific sequences near the recognition site and pull DNA through the complex in a reaction dependent on ATP hydrolysis. DNA cleavage occurs at undefined loci that may be several thousand base pairs away from the recognition site (Rosamond *et al.*, 1979; Yuan *et al.*, 1980; Endlich and Linn, 1985; Szczelkun *et al.*, 1996). Although type I restriction enzymes do not turn over in the cleavage reaction (Eskin and Linn, 1972a), the hydrolysis of ATP continues long after DNA degradation has ceased (Eskin and Linn, 1972b; Yuan *et al.*, 1972). In addition to restriction activity, the type I restriction enzymes exhibit an N6-adenine DNA methyltransferase activity at the recognition sequence, using S-adenosyl methionine (AdoMet) as the methyl donor (Burckhardt *et al.*, 1981).

The multifunctional properties of the type I restriction enzymes are reflected in their quaternary structure. All type I restriction enzymes are composed of three different subunits: HsdS, HsdM and HsdR (Meselson and Yuan, 1968; Eskin and Linn 1972a; Suri *et al.*, 1984; Price *et al.*, 1987). The subunit stoichiometry of the functional endonuclease is R₂M₂S₁ (Dryden *et al.*, 1997; Janscak *et al.*, 1998). The M₂S₁ component of this complex mediates its binding to the recognition sequence and can also function independently as a DNA methyltransferase (Taylor *et al.*, 1992; Dryden *et al.*, 1993; Janscak and Bickle, 1998). The HsdR subunit is essential for restriction. It contains a set of seven conserved amino acid sequence motifs typical of the helicase superfamily II that may be relevant to the ATP-dependent DNA translocation (Gorbalenya and Koonin, 1991; Murray *et al.*, 1993). Conservative changes in any of these motifs impair both restriction and ATPase activities (Webb *et al.*, 1996; Davies *et al.*, 1998). The HsdM subunit contains the catalytic site for DNA methylation as well as the binding site for the methyl donor and restriction cofactor AdoMet (Willcock *et al.*, 1994). The HsdS subunit determines DNA specificity. It contains two separate DNA-binding domains each recognizing one specific half of the recognition sequence (Fuller-Pace *et al.*, 1984; Gubler *et al.*, 1992).

Most type I restriction–modification systems characterized so far are from enterobacteria. Based on subunit complementation, DNA hybridization and antibody cross-reactivity experiments, these systems are grouped into four families (Murray *et al.*, 1982; Price *et al.*, 1987; Titheradge *et al.*, 1996). Members of the same family

can interchange individual subunits, but members from different families cannot. Although the genetic complementation is seen solely within a family, it is probable that all families share common reaction mechanisms for both DNA methylation and DNA restriction: (i) the predicted amino acid sequences of the products of all *hsdR* genes so far sequenced contain the helicase-like domain and a number of other short conserved regions that may be implicated in DNA restriction (Titheradge *et al.*, 1996); (ii) amino acid sequence comparison and tertiary structure modelling suggest a common structure for all type I DNA methyltransferases (Dryden *et al.*, 1995); and (iii) the biochemical properties of the enzymes studied are quite similar.

The mechanism by which type I restriction enzymes select cleavage sites is not clearly understood. The enzyme activity depends on the nature of the DNA substrate. Linear DNA molecules with a single recognition site are either refractory to cleavage (Rosamond *et al.*, 1979; Dreier *et al.*, 1996) or undergo limited cleavage at a very high excess of enzyme over DNA (Murray *et al.*, 1973; Szczelkun *et al.*, 1996). However, linear DNA molecules containing two or more sites are good substrates for cleavage. The cleavage of these molecules essentially occurs in the region between the recognition sites, and the enzyme shows a preference for cleavage roughly half way between the sites (Studier and Bandyopadhyay, 1988; Dreier *et al.*, 1996; Szczelkun *et al.*, 1997). Additional preferred cleavage sites located in the vicinity of the recognition sites are also observed with some enzymes (Szczelkun *et al.*, 1997). Varying the relative orientation of two asymmetric recognition sequences does not affect the cleavage (Dreier *et al.*, 1996; Szczelkun *et al.*, 1997). On the basis of these findings, a cooperative model has been proposed according to which a type I restriction enzyme bound to its recognition site translocates DNA towards itself simultaneously from both directions and DNA cleavage occurs at the site where two convergently translocating enzyme molecules meet (Studier and Bandyopadhyay, 1988; Dreier *et al.*, 1996). The cleavage of one-site substrates has been suggested to be a result of collision between an enzyme tracking from the recognition site and a second enzyme bound to a non-specific site (Studier and Bandyopadhyay, 1988).

In contrast to linear substrates, circular DNA molecules containing one recognition site are cleaved efficiently (Rosamond *et al.*, 1979; Dreier *et al.*, 1996; Janscak *et al.*, 1996). In one model, it is proposed that this susceptibility is a consequence of changes in DNA topology induced by enzyme tracking along the major or the minor groove of the DNA helix (Szczelkun *et al.*, 1996). It is hypothesized that the translocation of circular DNA past the enzyme molecule anchored to the recognition site leads to generation of overwound DNA in the contracting domain and underwound DNA in the expanding domain. The accumulation of torsionally stressed DNA ultimately would stall enzyme translocation and result in DNA cleavage (Szczelkun *et al.*, 1996).

To investigate further the link between DNA translocation and the cleavage event, we examined the action of type I restriction enzymes on DNA molecules that contained potential blocks to DNA translocation such as positive supercoils or Holliday junctions. We also investigated

interactions between two type I restriction enzymes from different families. The results from these experiments suggest that type I restriction enzymes can translocate DNA irrespective of its topological status and have the potential to cleave DNA at barriers that are able to halt the DNA translocation process.

Results

Cleavage of positively supercoiled DNA by *EcoAI*

A build up of positive supercoils in the contracting DNA loop has been proposed to be the trigger for DNA cleavage by type I restriction enzymes on circular substrates, by causing either a halt or a pause in the helix-tracking process, during which the enzyme cuts both DNA strands (Szczelkun *et al.*, 1996). To address this model, we have investigated cleavage of positively supercoiled plasmid DNA by *EcoAI* (IB family). If positive supercoiling presented a barrier for tracking, a positively supercoiled DNA substrate would be expected to be cleaved faster than a relaxed substrate, and cleavage sites would be located near the enzyme recognition site.

Positive supercoils were introduced into pJP25 DNA, a 2.87 kb plasmid containing a single *EcoAI* recognition site, as described in Materials and methods. We found that this DNA preparation was cleaved readily by *EcoAI* to linear DNA with an initial rate slightly lower than that observed with relaxed molecules, but slightly higher than the rate of cleavage of negatively supercoiled molecules (Figure 1). To determine the position of double-strand breaks introduced by *EcoAI* into the positively supercoiled, relaxed and negatively supercoiled substrates, the corresponding *EcoAI* linear products were digested with *XmnI*, which has a unique site in pJP25 located 970 bp away from the *EcoAI* site. All three subsequent *XmnI* digestions appeared as uniform smears of DNA on an agarose gel (Figure 2). This shows that *EcoAI* cleaved all three substrates at random positions throughout the DNA circle. Thus, it appears that neither positive nor negative supercoiling presents a block to DNA translocation by type I restriction enzymes. The observed differences in cleavage rates (Figure 1) may reflect different numbers of DNA supercoils in individual substrates since better resolution of DNA topoisomers on a long agarose gel (not shown) revealed that the positively supercoiled pJP25 molecules had a lower degree of supercoiling than the negatively supercoiled pJP25 molecules from HB101. These results agree with previously published data demonstrating that *EcoR124I* endonuclease activity increases with a decreased number of negative supercoils in a DNA substrate (Janscak *et al.*, 1996). Thus, it seems that both negative and positive supercoils reduce the rate of DNA cleavage by type I restriction enzymes relative to relaxed substrates.

Cleavage of DNA substrates containing a Holliday junction by *EcoR124II*

To examine the effect of physical blocks to DNA translocation on DNA cleavage by type I restriction enzymes, we used DNA molecules containing a Holliday junction generated *in vivo* by Xer site-specific recombination of pSD115, a 4.95 kb plasmid which carries two directly repeated *cer* recombination sites and a single site for the type IC restriction endonuclease *EcoR124II*. A stable

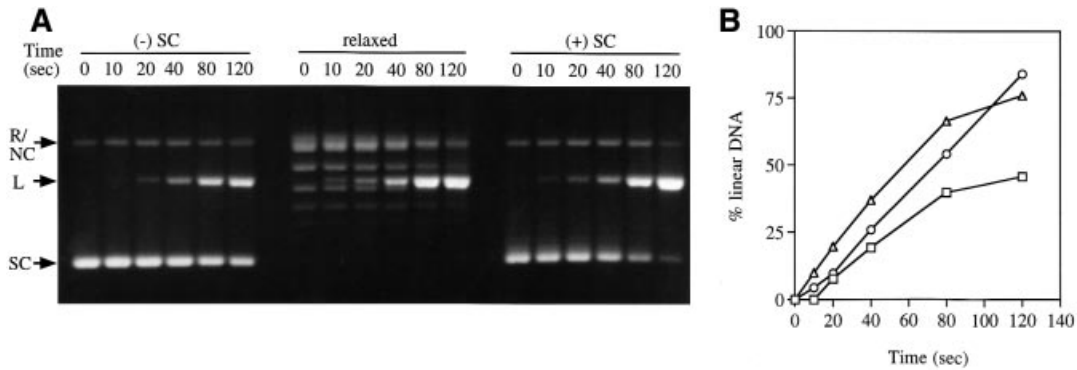


Fig. 1. Kinetics of DNA cleavage by *EcoAI* on positively supercoiled, relaxed and negatively supercoiled plasmid substrates. (A) *EcoAI* restriction assay. The negatively supercoiled (-SC) pJP25 DNA (single *EcoAI* site), isolated from *E. coli* HB101, was converted to the relaxed and positively supercoiled (+SC) forms, respectively, as described in Materials and methods. Cleavage reactions were carried out in buffer C at 37°C and contained 15 nM DNA and 15 nM *EcoAI* endonuclease. Aliquots were removed at the indicated times and analysed on a 0.9% agarose gel run in 0.5× TBE buffer at 1.5 V/cm for 12 h. DNA was visualized by ethidium bromide staining. The positions of supercoiled (SC), linear (L), relaxed (R) and nicked (NC) forms of plasmid DNA are marked on the left of the gel. The additional bands in the relaxed DNA lanes correspond to topoisomers with a low degree of negative supercoiling resulting from incomplete relaxation of pJP25. (B) Plot of the relative intensity of the linear DNA bands on the agarose gel shown in (A) against time for cleavage of positively supercoiled (○), relaxed (△) and negatively supercoiled (□) substrates by *EcoAI*. The gel was quantified by densitometric scanning. The relative intensity of linear DNA bands is expressed as a percentage of total DNA per lane.

intermediate of this reaction (figure-of-eight structure DNA) is composed of 2.6 and 2.35 kb circular duplexes joined by a Holliday junction that results from incomplete strand exchange at the *cer* sites (McCulloch *et al.*, 1994). The Holliday junction is confined to a region of ~290 bp of homology (*cer* sites) through which the junction can migrate spontaneously (McCulloch *et al.*, 1994). The pSD115 plasmid preparation from induced cells of *Escherichia coli* RM40 contains a mixture of several species: 4.95 kb (SC) substrate, non-catenated and catenated figure-of-eight intermediates, and the two final circular recombination products with sizes of 2.6 and 2.35 kb. On an agarose gel, the catenated figure-of-eight molecules co-migrate with 4.95 kb (SC) pSD115 DNA, while non-catenated molecules migrate slightly faster (Zerbib *et al.*, 1997). Cleavage of the figure-of-eight DNA molecules in one or both supercoiled domains with type II restriction enzyme(s) results in DNA structures which were easily separated from other pSD115 digestion products on an agarose gel (not shown) and could be purified and used as substrates for *EcoR124II*.

EcoRI cleavage of the figure-of-eight molecules (unique site in pSD115) resulted in an α -structure with the *EcoR124II* recognition site situated in the 2.35 kb supercoiled domain (Figure 3A). In contrast to the random location of cleavage sites on circular substrates, the α -structure was found to be cleaved by *EcoR124II* into two discrete DNA fragments with sizes similar to the sizes of the linearized forms of the final Xer recombination products which are 2.35 and 2.6 kb (Figure 3B, bands P₁ and P₂). This indicated that *EcoR124II* cleaved the 2.35 kb domain at two sites, one on either side of the Holliday junction, consistent with the bi-directional translocation model (Studier and Bandyopadhyay, 1988). The α -structure was refractory to cleavage by the *EcoR124I* endonuclease which has no recognition site in pSD115 (not shown). It is worth noting that the α -structure substrate gave two closely migrating bands on agarose gels run in the presence of ethidium bromide (Figure 3B). It was shown previously that superhelical torque induced by ethidium bromide

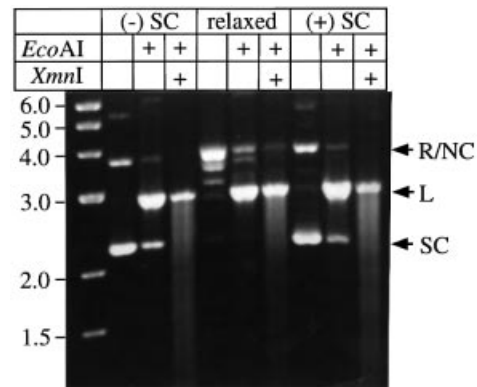


Fig. 2. Location of *EcoAI* cleavage sites on positively supercoiled, relaxed and negatively supercoiled DNA substrates. Negatively supercoiled (-SC) pJP25 DNA (a single *EcoAI* site), isolated from *E. coli* HB101, was converted to the relaxed and positively supercoiled (+SC) forms, as described in Materials and methods. The 20 μ l cleavage reactions were carried out in buffer C at 37°C and contained 15 nM DNA and 80 nM *EcoAI*. Following a 10 min incubation, the reactions were stopped by heating at 70°C for 15 min and divided into two aliquots. One aliquot was treated further with *XmnI* (unique site in pJP25) for 30 min. Samples were analysed by agarose gel electrophoresis as described in Figure 1. The presence of restriction enzymes in individual reactions is indicated by (+) above each lane. The positions of supercoiled (SC), linear (L), relaxed (R) and nicked (NC) forms of plasmid DNA are indicated on the right of the gel. The appearance of a uniform smear of DNA in the *XmnI* lanes indicates that *EcoAI* cleavage occurred at random positions throughout the DNA circle. Since *EcoAI* did not cleave either substrate completely, the subsequent *XmnI* digestions also contain the full-size linear form of pJP25.

binding to a circular DNA can force a Holliday junction to locate at either of two possible extreme positions within a region of homology (Yang *et al.*, 1998). For the α -structure, this would result in two species with different arm length ratios and hence slightly different mobilities. A time course of α -structure digestion by *EcoR124II* revealed a reaction with two intermediates being formed (Figure 3B). The first intermediate to appear was nicked α -structure (band I₁), which agrees with observations on

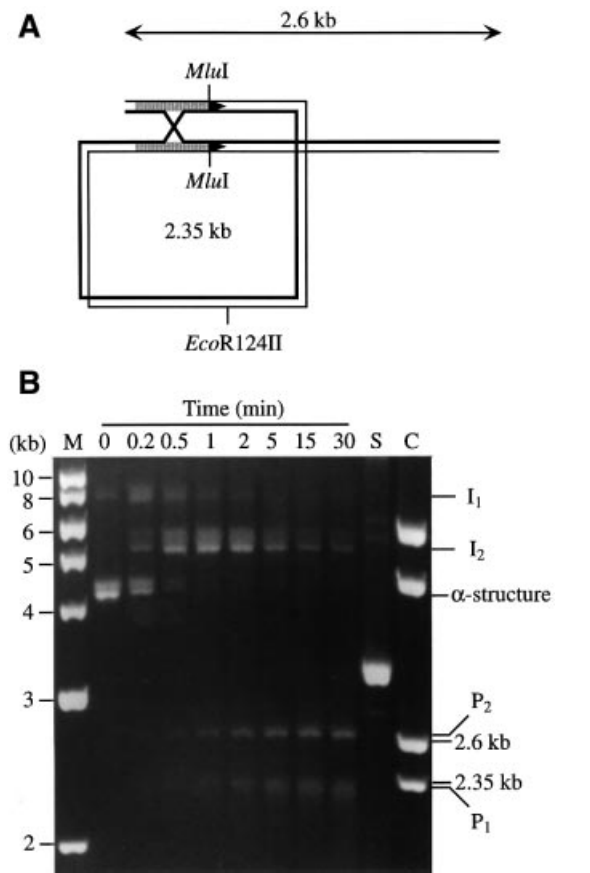


Fig. 3. *EcoR124II* cleavage of α -structure DNA containing a single *EcoR124II* site in the supercoiled domain. (A) Diagram of the α -structure resulting from *EcoRI* cleavage of the figure-of-eight molecules generated by Xer recombination of pSD115. The single *EcoR124II* recognition site is located in the 2.35 kb supercoiled domain. The sum of lengths of the two linear arms (2.6 kb) is indicated above the diagram. The 290 bp regions of homology (*cer*) are shown in grey. The orientation of the *cer* recombination sites is indicated by black arrowheads. For clarity, the diagram is not drawn to scale. The Holliday junction is drawn in parallel configuration. (B) *EcoR124II* restriction time course. Reactions were carried out at 37°C in buffer C and contained 10 nM DNA and 30 nM *EcoR124II*. Aliquots were removed at the indicated time points and analysed by electrophoresis on a 1% agarose gel run in 0.5× TBE buffer, containing ethidium bromide (0.5 µg/ml), at 2.5 V/cm for 12 h. The positions of the α -structure DNA, *EcoR124II* products (P_1 , P_2) and reaction intermediates (I_1 , I_2) are shown to the right of the gel. As a control, total plasmid DNA isolated from induced RM40/pSD115 was cleaved with *EcoRI* and *EcoRV* (lane C). This converted the Xer recombination products to their linear forms with sizes of 2.35 and 2.6 kb indicated to the right of the gel. Lane S contains the supercoiled form of pSD115 and lane M contains DNA size markers.

circular DNA substrates (Janscak *et al.*, 1996). In the second reaction step, a presumably branched DNA molecule was produced by a single double-strand break on one side of the junction (band I_2). Since the first double-strand break can occur on either side of the Holliday junction (bi-directional translocation) and anywhere within the region of homology (branch migration), the second reaction intermediate appeared as a broad band on agarose gel corresponding to a population of branched intermediates with different lengths of arms and therefore different electrophoretic mobilities. A higher intensity at the bottom of the smeared band suggested a preferred position for cleavage. Finally, the branched structure was

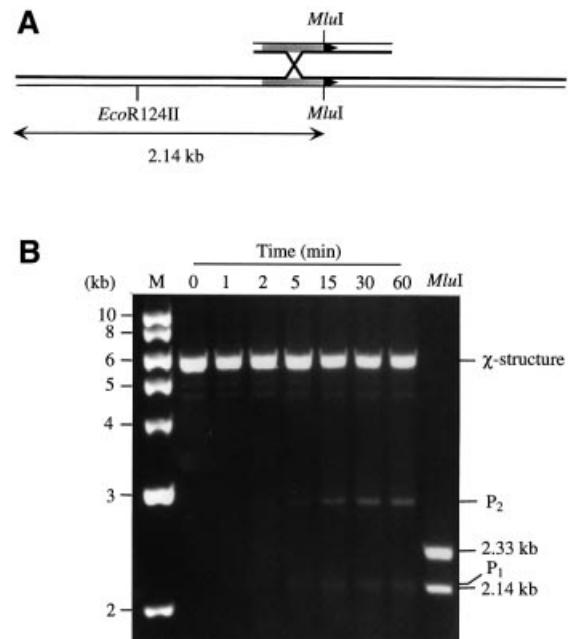


Fig. 4. Cleavage of χ -structure DNA by *EcoR124II*. (A) Diagram of the χ -structure resulting from *EcoRI*–*EcoRV* digestion of the figure-of-eight molecules generated by Xer recombination of pSD115. The 290 bp regions of homology (*cer*) are shown in grey. The orientation of the *cer* recombination sites is indicated by black arrowheads. The unique *EcoR124II* recognition site is located in the 2.14 kb arm. For clarity, the diagram is not drawn to scale. The Holliday junction is drawn in parallel configuration. (B) *EcoR124II* restriction time course. Reactions were carried out and analysed as described in Figure 3. Reaction time points are indicated above each lane. The positions of the χ -structure and the two *EcoR124II* products (P_1 , P_2) are indicated on the right of the gel. Two additional, faint bands appear on the gel. The faster migrating band corresponds to the *EcoRI*–*EcoRV* fragment of pSD115 that was not completely removed by gel isolation of the χ -substrate (not cleaved by *EcoR124II*). The slower migrating band could correspond to a variant of the χ -structure with the branch point located at a different position. As a control, the χ -structure was cleaved with *MluI* (lane indicated by *MluI*). The positions of the 2.14 and 2.33 kb linear products of the control reaction, resulting from dissociation of the originally produced χ -structure due to spontaneous branch migration, are indicated on the right of the gel. The other two fragments produced by *MluI* run off the gel. Lane M contains DNA size markers.

resolved to the two linear products by a cleavage on the other side of the junction, perhaps by introduction of another double-strand break or just by nicking the continuous strand.

The figure-of-eight DNA molecules were found to be cleaved by *EcoR124II* at the same positions as the α -structure when assayed for cleavage in the mixture with the other DNA species produced by Xer recombination (not shown).

To investigate whether *EcoR124II* endonuclease can cut DNA at a Holliday junction present on linear substrates, the figure-of-eight DNA molecules were treated with *EcoRI* and *EcoRV* (unique sites in pSD115) to produce a χ -structure with the *EcoR124II* site located in one arm (Figure 4A). In contrast to a linear DNA with a single recognition site, this DNA substrate was cleaved by *EcoR124II* to result in two discrete DNA fragments (Figure 4B, bands P_1 and P_2). The sizes of these fragments and their restriction digest profiles (not shown) suggested that the cleavage of the χ -structure occurred at the Holliday junction, thereby releasing the arm containing

the *EcoR124II* site. Interestingly, this fragment was approximately the same size as the 2.14 kb linear fragment produced from cleavage of the χ -structure with *MluI*, which has a single recognition site at the distal end of the *cer* sites (Figure 4A). This suggests that *EcoR124II* promoted branch migration to the end of the region of 290 bp homology and then introduced a double-strand break at the site where the further branch migration was blocked by DNA heterology. In addition, the rate of cleavage of the χ -structure by *EcoR124II* was much lower than that of the α -structure with an *EcoR124II* site in the supercoiled domain (Figure 3B). This could mean that the Holliday junction may not completely block DNA translocation by *EcoR124II* and some fraction of enzyme molecules could track across the junction via the continuous strand and dissociate from non-specific DNA when they encounter the DNA end.

Cooperation between two type I restriction enzymes from different families in cleavage of linear DNA

The experiments with Holliday junction substrates demonstrated that a physical block to DNA translocation can trigger DNA cleavage by a type I restriction enzyme. This suggests that cleavage of linear DNA with two recognition sites does not occur through specific protein-protein contacts between two translocating enzyme molecules. Instead, prevention of DNA translocation by collision between the two translocating enzymes is the trigger for DNA cleavage. We tested this prediction by examining the consequence of convergent DNA translocation by two type I restriction enzymes from different families, which show little amino acid homology. Linear DNA substrates containing a single site for each enzyme were used. Such DNA substrates are refractory to cleavage if only one of the enzymes is present. It should be noted that cooperation between *EcoR124II* and *EcoDXXI*, two type IC family enzymes, in DNA cleavage was demonstrated previously (Dreier *et al.*, 1996). However, the subunits of these enzymes show a high level of amino acid identity and are also interchangeable.

We investigated combinations of *EcoKI* (IA family) with either *EcoAI* (IB family) or *EcoR124I* (IC family). Plasmids pJP25 (*EcoKI* + *EcoAI*) and pJP39 (*EcoKI* + *EcoR124I*) were cut by the type II restriction enzyme *AlwNI* to produce 2.9 kb linear substrates with type I recognition sites in tail-to-tail orientation (Figure 5A). The distance between the sites in both preparations was ~0.9 kb. DNA substrates were treated for 10 min with a 7-fold molar excess of appropriate enzymes, and the appearance of cleavage products was monitored by agarose gel electrophoresis (Figure 5B). Under these reaction conditions, neither substrate was digested if only one of the corresponding enzymes was present. In contrast, when both enzymes were added, DNA cleavage occurred. The cleavage produced by the *EcoKI*-*EcoAI* mixture was more efficient than that produced by the *EcoKI*-*EcoR124I* mixture (Figure 5B). In both cases, agarose gels revealed not only the smear of heterologous DNA fragments from random cleavage events, but also a series of discrete bands within the DNA smear, indicating preferred cleavage sites (Figure 5B). To identify the discrete products, the gels were scanned by densitometry (Figure 5C). The sizes of

individual fragments were determined from the position of the corresponding peaks on the *x*-axis of the densitogram relative to DNA size markers. This allowed us to determine approximate boundaries for the major region of cleavage. For the *EcoKI*-*EcoAI* mixture, the sizes of discrete fragments suggested that the majority of cleavage events occurred within the region starting half way between the sites and ending at the *EcoKI* site. For the *EcoKI*-*EcoR124I* mixture, the majority of cleavage events occurred in a short region near the *EcoR124I* site.

Discussion

Type I restriction enzymes specifically bind to their DNA recognition sites, but cleave DNA at variable distances from their recognition sites. The enzyme molecule reaches its non-specific cleavage site by translocation of DNA via a secondary contact site while remaining fixed to the recognition site.

The mechanism by which a type I restriction enzyme selects its cleavage site has not been identified unequivocally. In one model for cleavage site selection, DNA cleavage occurs at the site where two convergently translocating enzyme molecules meet (Studier and Bandyopadhyay, 1988). According to this model, cooperation between the two enzyme molecules is required for DNA cleavage. Another model invokes as the cleavage inducer changes in DNA topology which arise on circular substrates as a consequence of enzyme tracking along a groove of the DNA helix (Szczelkun *et al.*, 1996). On a closed circular DNA, the DNA passage past a protein fixed to another site will result in rotation of the DNA about its helical axis to generate positive supercoils in the contracting DNA loop and negative supercoils in the expanding DNA loop (Ostrand *et al.*, 1990). Since the binding of a type I restriction enzyme to DNA is thought to initially create a relatively small expanding loop, it has been postulated that DNA in the expanding loop needs to be nicked prior to tracking to accommodate the ensuing reduction in twist. However, the corresponding increase in twist in the contracting DNA loop will generate a topological barrier that may be the trigger for DNA cleavage, by causing either a halt or a pause in the translocation process which permits the enzyme to cleave both DNA strands (Szczelkun *et al.*, 1996). This model argues that two convergently translocating enzymes can never meet on circular DNA molecules due to generation of the topological barrier. For tracking on linear DNA, the increase in twist in the contracting domain can be dissipated by the free rotation of the DNA ends and tracking enzyme will cleave when it stalls due to a collision with another enzyme molecule (Szczelkun *et al.*, 1996). In our experiments, the introduction of positive supercoiling into a circular plasmid containing a single *EcoAI* recognition site had no significant effect on the rate of DNA cleavage by *EcoAI* (relative to the relaxed form of the plasmid) nor on the enzyme's ability to select a cleavage site throughout the DNA molecule (Figures 1 and 2). In contrast, positively supercoiled DNA did prevent incision by the *E. coli* Uvr(A)BC enzyme, a DNA repair system in which the UvrAB helicase is proposed to displace a damaged DNA site into a small, negatively supercoiled domain that is the substrate for UvrC incision (Koo *et al.*, 1991; Kovalsky *et al.*, 1996). The fact that an increase in the plasmid linking

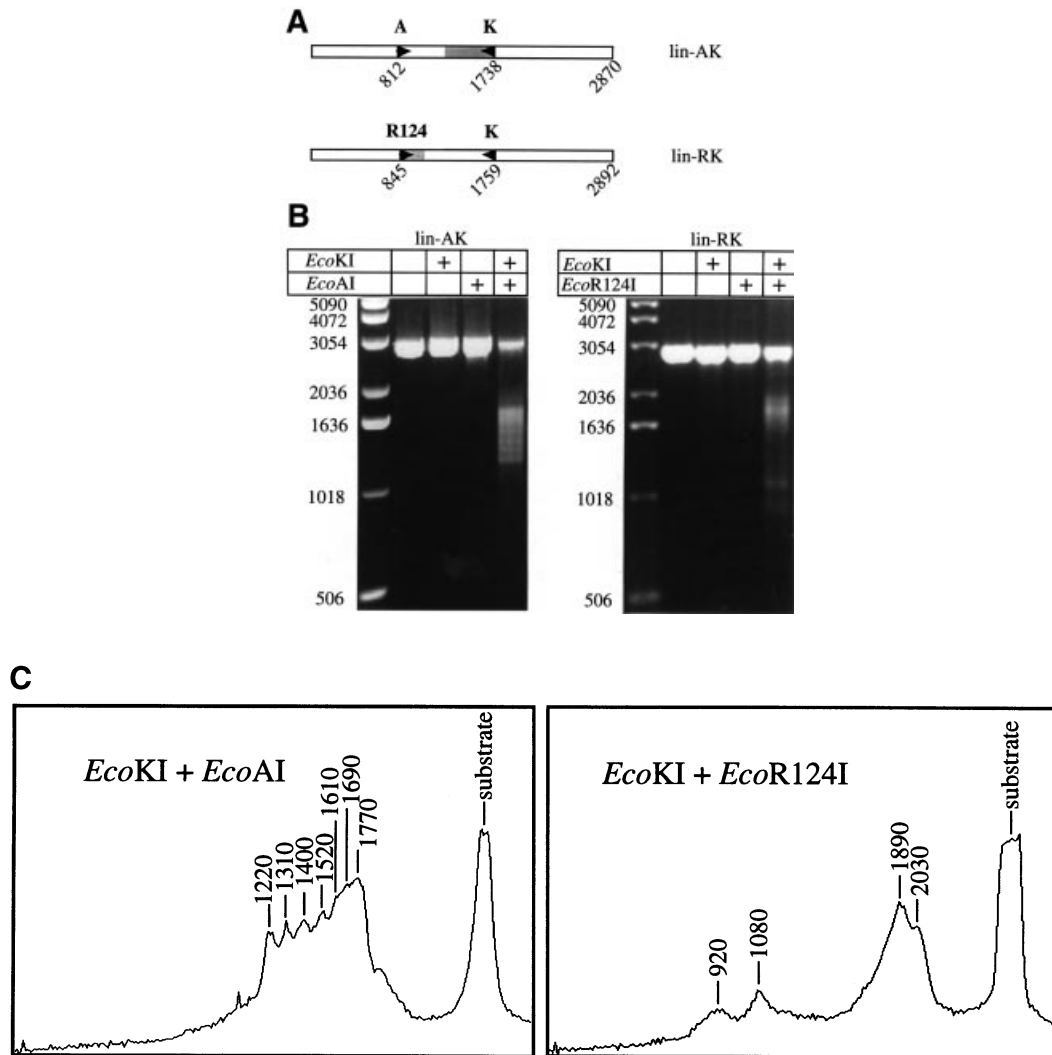


Fig. 5. Cooperation between two type I restriction enzymes from different families in cleavage of linear DNA. (A) Diagrams of DNA substrates. Plasmids pJP25 and pJP39 were cleaved with *AlwNI* to produce substrates lin-AK and lin-RK, respectively. DNA is represented as a thin rectangle. The positions and orientations of asymmetric *EcoKI* (K), *EcoAI* (A) and *EcoR124I* (R124) recognition sites are shown by filled arrowheads. The numbering of the sites refers to the position of the first base pair of the recognition sequence. (B) Restriction assay. Reactions were carried out at 37°C in buffer C. The enzymes (100 nM) were added to the appropriate DNA substrate (14 nM) individually or in combination. Following an 8 min incubation, aliquots were analysed by electrophoresis on a 1% agarose gel run in 0.5× TBE buffer, containing ethidium bromide (0.5 µg/ml), at 3 V/cm for 4 h. (C) Densitometric scans of the gels shown in (B). The sizes of fragments which correspond to major peaks of the densitometric traces are indicated. The regions encompassing the preferred cleavage sites on individual substrates are shown on the DNA representations in (A) as grey boxes.

number did not increase the rate of *EcoAI* cleavage suggests that positive supercoiling does not provide the barrier to DNA translocation that leads to DNA cleavage. The possibility that the initial DNA topology was dissipated by a nicking event occurring prior to translocation is unlikely since no accumulation of large amounts of nicked circular DNA intermediate was observed in the early stages of *EcoAI* reaction (Figure 1). Nicked circular DNA intermediates have been observed during time course digestions of negatively supercoiled plasmids by the type I restriction enzymes *EcoBI* or *EcoR124I* (Adler and Nathans, 1973; Janscak *et al.*, 1996); however, this nicking activity may rather reflect a sequential cleavage of the two DNA strands at the site of cleavage.

Supporting evidence suggesting that cleavage of circular DNA by type I restriction enzymes is not triggered by a build up of torsional stress in the contracting

DNA loop comes from cleavage experiments performed on a 2.35 kb supercoiled DNA domain linked by a Holliday junction (Figure 3A). The *EcoR124II* endonuclease, which had a single recognition site in the supercoiled domain, exclusively cleaved the DNA circle at two sites, one on either side of the junction (Figure 3B). The distances between the *EcoR124II* site and the Holliday junction in this α -structure are 1240 bp in one direction and 1110 bp in the other; these distances between the *EcoR124II* site and the Holliday junction are relative to the *MluI* sites located at the end of the homologous region along which the junction can migrate spontaneously (Figure 3A). If the translocation process was blocked by changes in DNA topology, *EcoR124II* could not reach the Holliday junction and it would cleave DNA within the regions between the junction and the enzyme recognition site.

If cleavage of circular substrates is not triggered by a

topological barrier to translocation, what elicits cleavage on circular DNA with a single recognition site? One possibility is that the two HsdR subunits of a specifically bound enzyme translocate the DNA in opposite directions and DNA cleavage is triggered when the entire circle has been translocated, as also proposed by Studier and Bandyopadhyay (1988). This view of bi-directional translocation is strongly supported by the fact that *EcoR124II* makes two double-stranded cleavages on the α -structure substrate containing a single *EcoR124II* recognition site in the circular region (Figure 3). The random location of cleavage sites which is observed on circular substrates could be explained by the assumption that initiation of translocation in one direction is delayed. This possibility may be supported by electron microscopic studies of *EcoKI* and *EcoBI* which revealed formation of intermediates with only one extruding loop (Yuan *et al.*, 1980; Endlich and Linn, 1985).

Do our data fit the helix-tracking model despite the fact that type I restriction enzymes can translocate a circular DNA substrate irrespective of its topology? One possible explanation could be that the enzyme complex has an ability to relieve the superhelical tension produced by the translocation along a groove of DNA helix. Some support for this idea comes from electron microscopic studies with *EcoKI* and *EcoBI*, which demonstrated formation of both twisted and relaxed loop intermediates (Rosamond *et al.*, 1979; Yuan *et al.*, 1980; Endlich and Linn, 1985). An alternative interpretation of our results is that the enzyme tracking is not restricted to a groove and the DNA passes from the contracting loop to the expanding loop without being twisted, so that the initial substrate supercoiling is distributed equally between the two domains. Formation of DNA loops with no superhelical turns by a translocation mechanism coupled to ATP hydrolysis was observed with MutS dimer which is involved in *E.coli* methyl-directed mismatch repair (Allen *et al.*, 1997). We do not exclude the possibility that DNA supercoiling affects the rate of DNA translocation. In fact, we detected slightly reduced rates of *EcoAI* cleavage of both negatively and positively supercoiled substrates relative to relaxed substrate (Figure 1). It appears that the higher the degree of supercoiling, the lower the rate of *EcoAI* cleavage. This correlates with previously published results for the *EcoR124I* endonuclease (Janscak *et al.*, 1996).

The data presented here are not in full agreement with the model of Studier and Bandyopadhyay (1988), because we show that DNA cleavage by *EcoR124II* endonuclease does not require cooperative interactions between two enzyme complexes. Our experiments demonstrated that DNA cleavage can be elicited by a collision of *EcoR124II* endonuclease with a Holliday junction present in either linear (χ -structure) or circular (α -structure) substrates containing a single *EcoR124II* site (Figures 3 and 4). It should be noted that the ability of type I restriction enzymes to cleave DNA at Holliday junctions was suggested previously by cleavage of a DNA cruciform structure in a crude extract from *EcoKI*⁺ cells (Taylor and Smith, 1990). In our experiments, the α -structure was found to be a much better substrate for *EcoR124II* than the χ -structure. It is possible that a Holliday junction on linear DNA causes only a pause in *EcoR124II* translocation; the enzyme may have some ability to track across a

Holliday junction, perhaps by following the continuous DNA strand. During the transient pause, only a fraction of tracking enzyme molecules will introduce a double-strand break. In contrast, our model would suggest that the α -structure substrate presents an insurmountable barrier to further enzyme movement due to the combination of a discontinuous DNA structure and bi-directional translocation by a single enzyme molecule. Enzyme stalling would allow the HsdR subunits to activate enzyme cleavage domains for DNA cleavage on both sides of the junction.

We have also demonstrated that DNA cleavage can result from convergent translocation of two type I restriction enzymes from different families. These results imply that no protein-protein interactions between the colliding enzyme molecules are required for cleavage (Figure 5). DNA cleavage induced by a collision of two identical enzymes gives a cleavage site pattern that is symmetrical relative to the midpoint between the recognition sites (Studier and Bandyopadhyay, 1988; Szczelkun *et al.*, 1997). However, combination of two enzymes from different families, especially that of *EcoKI* and *EcoR124I*, resulted in a shift of the position of cleavage sites towards one of the recognition sites (Figure 5). This may be due to the different enzymes having different rates of movement along DNA or different initial DNA-binding rates. Such differences may also account for the reduced DNA cleavage efficiencies observed in these reactions, since the faster translocating enzyme could prevent the slower one from initiating DNA translocation.

Based on the above results, it appears that a single molecule of type I restriction enzyme can cleave DNA at any physical barrier that is able to cause a halt or pause in the translocation process. We have shown previously that a Lac repressor bound to linear DNA with a single *EcoR124II* recognition site did not trigger DNA cleavage by *EcoR124II*. These results suggested that non-covalently bound proteins can be displaced from DNA by a tracking type I restriction enzyme molecule (Dreier *et al.*, 1996). Therefore, it seems that only a subset of physical blocks (Holliday junction or oppositely translocating enzyme molecule) can stop DNA translocation by type I restriction enzymes. Furthermore, a nick placed between two *EcoR124I* sites biased the restriction reaction such that cleavage preferentially occurred at or in the vicinity of the nick, suggesting that a DNA nick also may cause a pause in DNA translocation (Dreier *et al.*, 1996). However, linear DNA containing a single *EcoR124II* site and a site-specific nick was not cleaved, suggesting that the pause caused by a nick is not sufficiently long to trigger DNA cleavage (Dreier *et al.*, 1996).

We have adapted the collision model for DNA cleavage proposed by Studier and Bandyopadhyay (1998) to accommodate our observations (Figure 6). Our model argues against specific cooperation between two enzyme molecules in DNA cleavage and postulates that DNA translocation blockage is the only requirement for DNA cleavage to occur. The question arises: how can a single type I endonuclease molecule elicit double-stranded cleavage as seen on the Holliday junction substrates if the two HsdR subunits of the enzyme complex contact DNA on opposite sides of the recognition sequence? In our model, we favour the possibility that the HsdR subunit has the ability to cleave both DNA strands. A single endonuclease domain

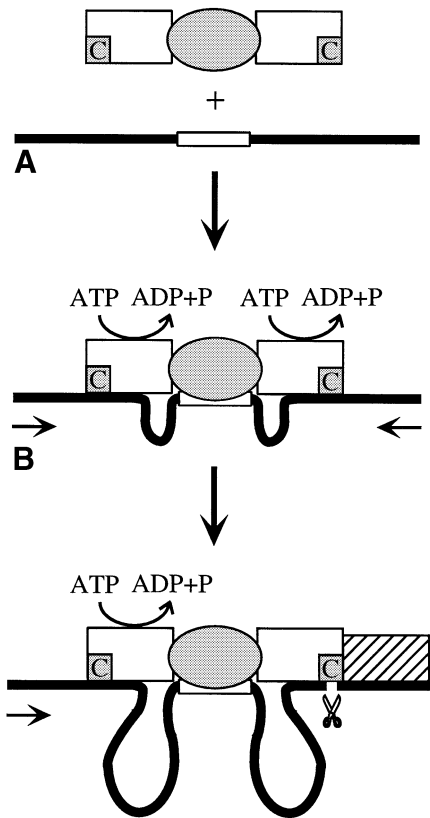


Fig. 6. A model for DNA cleavage by type I restriction enzymes. DNA is shown as a continuous line, with an open box representing a recognition site for a type I restriction enzyme. The multimeric enzyme complex is composed of the methylase M_2S_1 (grey oval) which mediates binding to the recognition site, and two HsdR subunits (open box). The catalytic centre for DNA cleavage in HsdR is shown schematically as a grey box marked by C. (A) The enzyme binds to its unmethylated recognition site and subsequently translocates non-specific DNA from both directions (indicated by arrows) past the HsdR subunits, irrespective of DNA topology. This process is fuelled by ATP hydrolysis. (B) When one of the HsdR subunits encounters a barrier that can halt the translocation process (hatched box), it introduces a double-strand break in the DNA substrate at the site of the collision (indicated by scissors) while the other HsdR subunit can continue in translocation. Collision of HsdR subunits of two convergently tracking enzymes would result in introduction of two double-strand breaks in close proximity to each other. The model can be also modified for uni-directional translocation.

is implicated in double-strand breakage in a model for the multisubunit restriction enzyme *BcgI*, a member of a separate class of restriction enzymes (Kong, 1998). *BcgI* resembles type I enzymes in terms of domain structure and mode of DNA interaction, except for the absence of the DEAD-box region and DNA translocation activity which correlates with its ability to cleave DNA at a fixed distance on both sides of the recognition sequence (Kong *et al.*, 1993; Kong, 1998).

In conclusion, the results presented here suggest that barriers that halt DNA translocation by type I restriction enzymes have the potential to elicit DNA cleavage. The Holliday junction recombination intermediate can serve as just such a barrier. Thus it appears that in addition to restriction of foreign DNA, type I restriction enzymes may play other roles in the cell, including participation in special types of recombination of foreign DNA as recently proposed by Kusano *et al.* (1997).

Materials and methods

Proteins

EcoKI endonuclease was isolated from *E. coli* C3-6/pVMC3 as described previously (Weiserova *et al.*, 1993). The *EcoR124I* and *EcoR124II* endonucleases were reconstituted from separate preparations of the corresponding methylase and HsdR subunit. The *EcoR124I* and *EcoR124II* methylases were purified from *E. coli* DH5 α (Woodcock *et al.*, 1989) transformed with plasmids pMG1 and pMG2, respectively, as described previously (Gubler and Bickle, 1991). The *EcoR124I* and *EcoR124II* systems have identical *hsdR* genes. The HsdR subunit was produced from the plasmid pMG3 (Gubler and Bickle, 1991) in *E. coli* DH5 α and purified as described by Janscak *et al.* (1998). The endonucleases were reconstituted immediately before use by mixing 6 mol of HsdR per mol of methylase. The *EcoAI* endonuclease was also reconstituted before use by mixing purified HsdR, HsdM and HsdS subunits in a molar ratio of 6:2:1. The *EcoAI* subunits were produced in *E. coli* BL21(DE3) (Studier *et al.*, 1990) from the plasmids pJP22 (HsdR), pJP23 (HsdM) and pJP26 (HsdS), respectively, and purified as described elsewhere (Janscak and Bickle, 1998). The excess HsdR used for all reconstitutions is needed due to instability of the $R_2M_2S_1$ cleavage-competent complex of these enzymes (Suri *et al.*, 1984; Janscak *et al.*, 1998). The final concentration of reconstituted endonucleases was taken as the input concentration of the methylase for *EcoR124I* and *EcoR124II* or the input concentration of HsdS for *EcoAI*. Type II restriction enzymes were purchased from New England Biolabs (Beverly, MA). The archaeal histone-like protein HMfB used for preparation of positively supercoiled DNA was purified from BL21(DE3)/pKS323 as described by Starich *et al.* (1996). Wheat germ topoisomerase I was purchased from Promega (Madison, WI).

Preparation of DNA substrates

All plasmid DNA purifications were carried out using Qiagen Maxiprep kit (Qiagen). DNA concentration was estimated from UV absorbance at 260 nm. The construction of the plasmid pJP25 (contains single sites for both *EcoAI* and *EcoKI*) is described elsewhere (Janscak and Bickle, 1998). The plasmid pJP39 (single site for both *EcoR124I* and *EcoKI*) was constructed by introduction of the *KpnI-SalI* fragment from pDRM-1R (Janscak *et al.*, 1996), containing one *EcoR124I* site, into the corresponding sites in pTZ19R (Pharmacia). Both plasmid substrates were isolated from *E. coli* HB101 (Sambrook *et al.*, 1989) and, if required, cut with appropriate type II restriction enzymes to produce full-length linear substrates.

Positively supercoiled plasmid pJP25 DNA was prepared by treatment of negatively supercoiled pJP25 DNA isolated from *E. coli* HB101 with wheat germ topoisomerase I and the archaeal histone HMfB essentially as described previously (LaMarr *et al.*, 1997). Binding of HMfB to a circular DNA followed by relaxation of unrestrained supercoils by topoisomerase I and subsequent removal of all proteins results in highly positively supercoiled DNA (LaMarr *et al.*, 1997). Relaxed pJP25 DNA was prepared according to the same procedure except that HMfB was not added. As a control, a population of negatively supercoiled pJP25 molecules were also subjected to the same treatment except that storage buffers were added instead of topoisomerase I and HMfB.

The α - and χ -structure DNA substrates containing a Holliday junction were prepared by cutting figure-of-eight DNA intermediates of Xer-mediated site-specific recombination of the plasmid pSD115 (McCulloch *et al.*, 1994) with appropriate type II restriction enzymes. The figure-of-eight DNA molecules were generated in *E. coli* RM40 which contains the Xer recombination system tightly regulated by Lac repressor (McCulloch *et al.*, 1994). The RM40/pSD115 strain was grown and induced as described by McCulloch *et al.* (1994). Following restriction enzyme digestion of isolated pSD115 DNA, the α - and χ -structure DNAs were separated from the other DNA species on a 0.9% agarose gel run in TAE buffer (40 mM Tris-acetate buffer pH 8.3, 1 mM EDTA, 0.5 μ g/ml ethidium bromide) at 3 V/cm for 3 h, excised and purified using Qiaquick Gel Extraction Kit (Qiagen).

Restriction reactions

All DNA substrates were digested with appropriate type I restriction enzymes at 37°C in buffer C (50 mM Tris-HCl pH 8.0, 10 mM MgCl₂, 25 mM NaCl, 1 mM dithiothreitol, 0.2 mM AdoMet, 5 mM ATP). Reactions were started by the addition of ATP and stopped by the addition of 0.5 vol of stop solution [150 mM EDTA, 0.5% (w/v) SDS, 30% (v/v) glycerol, 0.4 mg/ml bromophenol blue]. For subsequent cleavage with type II restriction enzymes, reactions were terminated by

heating at 70°C for 15 min. Enzyme and DNA concentrations used for individual substrates are indicated in the figure legends. Reaction products were resolved on agarose gels run in TBE buffer (45 mM Tris–borate pH 8.3, 1 mM EDTA) under the conditions described in the figure legends. DNA was visualized by ethidium bromide staining. If required, agarose gel images were digitized and quantified using NIH Image 1.61 software.

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References

- Adler,S.P. and Nathans,D. (1973) Studies of SV40 DNA: V. Conversion of circular to linear SV40 DNA by restriction endonuclease from *Escherichia coli* B. *Biochim. Biophys. Acta*, **199**, 177–188.
- Allen,D.J., Makhov,A., Grilley,M., Taylor,J., Thresher,R., Modrich,P. and Griffith,J.D. (1997) MutS mediates heteroduplex loop formation by a translocation mechanism. *EMBO J.*, **16**, 4467–4476.
- Burckhardt,J., Weisemann,J. and Yuan,R. (1981) Characterization of the DNA methylase activity of the restriction enzyme from *Escherichia coli* K. *J. Biol. Chem.*, **256**, 4024–4032.
- Davies,G.P., Powell,L.M., Webb,J.L., Cooper,L.P. and Murray,N.E. (1998) EcoKI with an amino acid substitution in any one of seven DEAD-box motifs has impaired ATPase and endonuclease activities. *Nucleic Acids Res.*, **26**, 4828–4836.
- Dreier,J., MacWilliams,M.P. and Bickle,T.A. (1996) DNA cleavage by the type IC restriction–modification enzyme EcoR124II. *J. Mol. Biol.*, **264**, 722–733.
- Droge,P. (1994) Protein tracking-induced supercoiling of DNA: a tool to regulate DNA transactions *in vivo*? *BioEssays*, **16**, 91–99.
- Dryden,D.T.F., Cooper,L.P. and Murray,N.E. (1993) Purification and characterization of the methyltransferase from the type I restriction and modification system of *Escherichia coli* K12. *J. Biol. Chem.*, **268**, 13228–13236.
- Dryden,D.T.F., Sturrock,S.S. and Winter,M. (1995) Structural modelling of a type I DNA methyltransferase. *Nature Struct. Biol.*, **2**, 632–635.
- Dryden,D.T.F., Cooper,L.P., Thorpe,P.H. and Byron,O. (1997) The *in vitro* assembly of the EcoKI type I DNA restriction/modification enzyme and its *in vivo* implications. *Biochemistry*, **36**, 1065–1076.
- Endlich,B. and Linn,S. (1985) The DNA restriction endonuclease of *Escherichia coli* B. I. Studies of the DNA translocation and the ATPase activities. *J. Biol. Chem.*, **260**, 5720–5728.
- Eskin,B. and Linn,S. (1972a) The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. II. Purification, subunit structure, and catalytic properties of the restriction endonuclease. *J. Biol. Chem.*, **247**, 6183–6191.
- Eskin,B. and Linn,S. (1972b) The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. III. Studies of the restriction adenosine triphosphatase. *J. Biol. Chem.*, **247**, 6192–6196.
- Fuller-Pace,F.V., Bullas,L.R., Delius,H. and Murray,N.E. (1984) Genetic recombination can generate altered restriction specificity. *Proc. Natl Acad. Sci. USA*, **81**, 6095–6099.
- Gorbalenya,A.E. and Koonin,E.V. (1991) Endonuclease (R) subunits of type-I and type-III restriction–modification enzymes contain a helicase-like domain. *FEBS Lett.*, **291**, 277–281.
- Gubler,M. and Bickle,T.A. (1991) Increased protein flexibility leads to promiscuous protein–DNA interactions in type IC restriction–modification systems. *EMBO J.*, **10**, 951–957.
- Gubler,M., Braguglia,D., Meyer,J., Piekarczyk,A. and Bickle,T.A. (1992) Recombination of constant and variable modules alters DNA sequence recognition by type IC restriction–modification enzymes. *EMBO J.*, **11**, 233–240.
- Janscak,P. and Bickle,T.A. (1998) The DNA recognition subunit of the type IB restriction–modification enzyme EcoAI tolerates circular permutations of its polypeptide chain. *J. Mol. Biol.*, **284**, 937–948.
- Janscak,P., Abadjieva,A. and Firman,K. (1996) The type I restriction endonuclease R.EcoR124I: over-production and biochemical properties. *J. Mol. Biol.*, **257**, 977–991.
- Janscak,P., Dryden,D.T.F. and Firman,K. (1998) Analysis of the subunit assembly of the type IC restriction–modification enzyme EcoR124I. *Nucleic Acids Res.*, **26**, 4439–4445.
- Koo,H.S., Claassen,L., Grossman,L. and Liu,L.F. (1991) ATP-dependent partitioning of the DNA template into supercoiled domains by *Escherichia coli* UvrAB. *Proc. Natl Acad. Sci. USA*, **88**, 1212–1216.
- Kong,H. (1998) Analyzing the functional organization of a novel restriction modification system, the BcgI system. *J. Mol. Biol.*, **279**, 823–832.
- Kong,H., Morgan,R.D., Maunus,R.E. and Schildkraut,I. (1993) A unique restriction endonuclease, BcgI, from *Bacillus coagulans*. *Nucleic Acids Res.*, **21**, 987–991.
- Kong,X.P., Onrust,R., O'Donnell,M. and Kuriyan,J. (1992) Three-dimensional structure of the beta subunit of *E.coli* DNA polymerase III holoenzyme: a sliding DNA clamp. *Cell*, **69**, 425–437.
- Kovalsky,O.I., Grossman,L. and Ahn,B. (1996) The topodynamics of incision of UV-irradiated covalently closed DNA by the *Escherichia coli* Uvr(A)BC endonuclease. *J. Biol. Chem.*, **271**, 33236–33241.
- Kusano,K., Sakagami,K., Yokochi,T., Naito,T., Tokinaga,Y., Euda,E. and Kobayashi,I. (1997) A new type of illegitimate recombination is dependent on restriction and homologous interaction. *J. Bacteriol.*, **179**, 5380–5390.
- LaMarr,W.A., Sandman,K.M., Reeve,J.N. and Dedon,P.C. (1997) Large scale preparation of positively supercoiled DNA using the archaeal histone HMfB. *Nucleic Acids Res.*, **25**, 1660–1661.
- Liu,L.F. and Wang,J.C. (1987) Supercoiling of the DNA template during transcription. *Proc. Natl Acad. Sci. USA*, **84**, 7024–7027.
- McCulloch,R., Coggins,L.W., Colloms,S.D. and Sherratt,D.J. (1994) Xer-mediated site-specific recombination at *cer* generates Holliday junctions *in vivo*. *EMBO J.*, **13**, 1844–1855.
- Meisel,A., Mackeldanz,P., Bickle,T.A., Kruger,D.H. and Schroeder,C. (1995) Type III restriction endonucleases translocate DNA in a reaction driven by recognition site-specific ATP hydrolysis. *EMBO J.*, **14**, 2958–2966.
- Meselson,M. and Yuan,R. (1968) DNA restriction enzyme from *E.coli*. *Nature*, **217**, 1110–1114.
- Murray,N.E., Batten,P.L. and Murray,K. (1973) Restriction of bacteriophage λ by *Escherichia coli* K. *J. Mol. Biol.*, **81**, 395–407.
- Murray,N.E., Gough,J.A., Suri,B. and Bickle,T.A. (1982) Structural homologies among type I restriction–modification systems. *EMBO J.*, **1**, 535–539.
- Murray,N.E., Daniel,A.S., Cowan,G.M. and Sharp,P.M. (1993) Conservation of motifs within the unusually variable polypeptide sequences of type I restriction and modification enzymes. *Mol. Microbiol.*, **9**, 133–143.
- Ostrander,E.A., Benedetti,P. and Wang,J.C. (1990) Template supercoiling by a chimera of yeast GAL4 protein and phage T7 RNA polymerase. *Science*, **249**, 1261–1265.
- Price,C., Pripl,T. and Bickle,T.A. (1987) EcoR124 and EcoR124/3: the first members of a new family of type I restriction and modification systems. *Eur. J. Biochem.*, **167**, 111–115.
- Rosamond,J., Endlich,B. and Linn,S. (1979) Electron microscopic studies of the mechanism of action of the restriction enzyme of *Escherichia coli* B. *J. Mol. Biol.*, **129**, 619–635.
- Sambrook,J.C., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Starich,M.R., Sandman,K., Reeve,J.N. and Summers,M.F. (1996) NMR structure of HMfB from the hyperthermophile, *Methanothermobacter feravidus*, confirms that this archaeal protein is a histone. *J. Mol. Biol.*, **255**, 187–203.
- Studier,F.W. and Bandyopadhyay,P.K. (1988) Model for how type I restriction enzymes select cleavage sites in DNA. *Proc. Natl Acad. Sci. USA*, **85**, 4677–4681.
- Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.*, **185**, 60–89.
- Suri,B., Shephard,J.C.W. and Bickle,T.A. (1984) The EcoA restriction and modification system of *Escherichia coli* 15T-: enzyme structure and DNA recognition sequence. *EMBO J.*, **3**, 575–579.
- Szczelkun,M.D., Dillingham,M.S., Janscak,P., Firman,K. and Halford,S.E. (1996) Repercussions of DNA tracking by the type IC restriction endonuclease EcoR124I on linear, circular and catenated substrates. *EMBO J.*, **15**, 6335–6347.
- Szczelkun,M.D., Janscak,P., Firman,K. and Halford,S.E. (1997) Selection of non-specific DNA cleavage sites by the type IC restriction endonuclease EcoR124I. *J. Mol. Biol.*, **271**, 112–123.

- Taylor,A.F. and Smith,G.R. (1990) Action of RecBCD enzyme on cruciform DNA. *J. Mol. Biol.*, **211**, 117–134.
- Taylor,I., Patel,J., Firman,K. and Kneale,G.G. (1992) Purification and biochemical characterisation of the *EcoR124* type I modification methylase. *Nucleic Acids Res.*, **20**, 179–186.
- Titheradge,A.J.B., Ternent,D. and Murray,N.E. (1996) A third family of allelic *hsd* genes in *Salmonella enterica*: sequence comparisons with related proteins identify conserved regions implicated in restriction of DNA. *Mol. Microbiol.*, **22**, 437–447.
- Tsaneva,I.R., Muller,B. and West,S.C. (1992) ATP-dependent branch migration of Holliday junctions promoted by the RuvA and RuvB proteins of *E.coli*. *Cell*, **69**, 1171–1180.
- Webb,J.L., King,G., Ternent,D., Titheradge,A.J. and Murray,N.E. (1996) Restriction by *EcoKI* is enhanced by cooperative interactions between target sequences and is dependent on DEAD box motifs. *EMBO J.*, **15**, 2003–2009.
- Weiserova,M., Janscak,P., Benada,O., Hubacek,J., Zinkevich,V.E., Glover,S.W. and Firman,K. (1993) Cloning, production and characterisation of wild type and mutant forms of the R.*EcoK* endonucleases. *Nucleic Acids Res.*, **21**, 373–379.
- Willcock,D.F., Dryden,D.T.F. and Murray,N.E. (1994) A mutational analysis of the two motifs common to adenine methyltransferases. *EMBO J.*, **13**, 3902–3908.
- Woodcock,D.M., Crowther,P.J., Doherty,J., Jefferson,S., DeCruz,E., Noyer-Weidner,M., Smith,S.S., Michael,M.Z. and Graham,M.W. (1989) Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants. *Nucleic Acids Res.*, **17**, 3469–3478.
- Yang,L., Jessee,C.B., Lau,K., Zhang,H. and Liu,L.F. (1989) Template supercoiling during ATP-dependent DNA helix tracking: studies with simian virus 40 large tumor antigen. *Proc. Natl Acad. Sci. USA*, **86**, 6121–6125.
- Yang,X., Vologodskii,A.V., Liu,B., Kemper,B. and Seeman,N.C. (1998) Torsional control of double-stranded DNA branch migration. *Biopolymers*, **45**, 69–83.
- Yuan,R., Heywood,J. and Meselson,M. (1972) ATP hydrolysis by restriction endonuclease from *E.coli* K. *Nature*, **240**, 42–43.
- Yuan,R., Hamilton,D.L. and Burckhardt,J. (1980) DNA translocation by the restriction enzyme from *E.coli* K. *Cell*, **20**, 237–244.
- Zerbib,D., Colloms,S.D., Sherratt,D.J. and West,S.C. (1997) Effect of DNA topology on Holliday junction resolution by *Escherichia coli* RuvC and bacteriophage T7 endonuclease I. *J. Mol. Biol.*, **270**, 663–673.

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