The Effects of Central Asymmetry on the Propagation of Palindromic DNA in Bacteriophage λ Are Consistent With Cruciform Extrusion *in Vivo*

Alison F. Chalker,[1] Ewa A. Okely, Angus Davison and David R. F. Leach

Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland

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ABSTRACT

The propagation of λ phages carrying long perfect palindromes has been compared with that of phages carrying imperfect palindromes with small regions of central asymmetry. The perfect palindromes confer a more deleterious phenotype than those with central asymmetry and the severity of the phenotype declines with the length of asymmetry in the range from 0 to 27 base pairs. These results argue that a center-dependent reaction is involved in the phenotypic effects of palindromic DNA sequences, consistent with the idea that cruciform extrusion occurs *in vivo*.

DNA palindromes longer than 100–150 base pairs (bp) cannot be propagated in wild-type *Escherichia coli* and replicons carrying such sequences suffer two fates. They are very poorly replicated leading to the phenotype described as inviability or the palindromes they contain are partially or completely deleted, a phenotype denoted instability (COLLINS 1981; LILLEY 1981; COLLINS, VOLCKAERT and NEVERS 1982; HAGAN and WARREN 1982; MIZUUCHI, MIZUUCHI and GELLERT 1982; HAGAN and WARREN 1983; LEACH and STAHL 1983; LEACH and LINDSEY 1986; SHURVINTON, STAHL and STAHL 1987; LINDSEY and LEACH 1989). These two fates are interrelated since replicons that have deleted their palindromes will have a selective advantage over their parents. However, it is not yet known how, or indeed whether, the mechanism of inviability is related to the mechanism of instability.

The propagation of palindromic sequences is also affected by the host genotype. It was initially demonstrated (LEACH and STAHL 1983) that a λ phage carrying a long palindrome could plate on *recBC sbcB* hosts which were subsequently shown to harbor an additional mutation in the *sbcC* gene (LLOYD and BUCKMAN 1985). Further work demonstrated that it was the *sbcC* mutation that specifically relieved the inviability of this phage (CHALKER, LEACH and LLOYD 1988). We now know that *sbcC* is part of a two gene system (the second gene has been named *sbcD*) implicated in the inhibition of propagation of palindromic DNA and the cosuppression of recombination deficiency of *recBC* mutants (LLOYD and BUCKMAN 1985; GIBSON, LEACH and LLOYD 1992). In addition to these specific interactions, mutations that improve the replication or packaging of λ contribute indirectly to its ability to propagate palindromes (CHALKER, LEACH and LLOYD 1988; CHALKER 1990). In the case of *red gam* phage, mutations in *recB* or *recC* perform this function by permitting rolling-circle replication (see ENQUIST and SKALKA 1975) and mutations in *recD* do so, both by permitting rolling circle replication and by stimulating recombination (CHAUDHURY and SMITH 1984; AMUNDSEN *et al.* 1986; BIEK and COHEN 1986). These indirect effects have been discussed previously (CHALKER, LEACH and LLOYD 1988). Finally, it has been shown that the *gam* gene of λ facilitates the propagation of palindromes (LEACH, LINDSEY and OKELY 1987; KULKARNI and STAHL 1989) and that this effect occurs via a novel mechanism in addition to the known inhibition of RecBCD by Gam since it can be observed in a *recBCD* deletion mutant (KULKARNI and STAHL 1989). The latter authors suggest that Gam may work by inactivating the SbcC protein.

The question of whether palindromic DNA sequences can adopt cruciform structures *in vivo* has been difficult to answer conclusively. Initially, work suggested that the palindromes tested could not be detected in a cruciform conformation (COUREY and WANG 1983; SINDEN, BROYLES and PETTIJOHN 1983). These studies however used palindromes which were short (68 and 66 bp) and did not cause a severe phenotype *in vivo*. It might therefore be predicted that they would not reside predominantly in a cruciform conformation. More recently, different assays and the use of specifically designed palindromes with AT rich centers, coupled to the use of physiological conditions expected to favor extrusion, have permitted the detection of cruciform structures inside cells (PANAYATATOS and FONTAINE 1987; MCCLELLAN *et al.* 1990; DAYN *et al.* 1991; ZHENG *et al.* 1991; SINDEN *et al.* 1991). The work reported below adds support

[1] Present address: Smith Kline Beecham, Brockham Park, Betchworth, Surrey RH3 7AJ, England.

to the idea that cruciforms can form *in vivo* and extends the evidence to normal physiological conditions and to palindromes that do not contain particularly AT-rich centers. Since such palindromes have been shown to have high kinetic barriers to cruciform extrusion *in vitro* (COUREY and WANG 1983, 1988; GELLERT, O'DEA and MIZUUCHI 1983; MURCHIE and LILLEY 1987) it seems likely that their extrusion *in vivo* is catalyzed.

MATERIALS AND METHODS

Bacterial strains: The bacterial strains used are derivatives of AB1157 F⁻ DEL(*gpt-proA*)*62 argE3 his-4 leu-6 thr-1 ara-14 galK2 lacY1 xyl-5 thi-1 supE44 rpsL31 tsx33* and were obtained from R. G. LLOYD.

Bacteriophage strains: The palindrome containing phages are all derived from DRL116, λ *pal spi6 cI*857, as shown in Figure 1. Initially, DRL133 was constructed by cleavage of DRL116 with *Sst*I and religation to delete the central 109 bp. The central *Sst*I site of DRL133 was then used for the insertion of oligonucleotide sequences obtained from the Oswel DNA service (T. BROWN, Department of Chemistry) to construct DRL134, AD1 and AD2. To facilitate the isolation and identification of the desired clones, the oligonucleotides used introduced a new restriction target sequence and inactivated the *Sst*I site. The oligonucleotides were not phosphorylated. This should prevent the insertion of multimers and no multimers were detected in any of the constructions. DRL137 was constructed by insertion of the polylinker sequence of the plasmid pMTL24 (CHAMBERS *et al.* 1988) into the *Sst*I site of DRL133, and DRL148 was made by cleavage of DRL137 with *Sal*I and religation. All manipulations were by standard methods, as outlined in SAMBROOK, FRITSCH and MANIATIS (1989).

Bacteriophage plating: Overnight cultures of cells were diluted 1:10 into L broth with 5 mM $MgSO_4$ and 0.2% maltose and grown for 2 hr at 37°. An equal volume of 10 mM Tris-HCl (pH 8), 10 mM $MgSO_4$ was then added. Aliquots of 0.25 ml of these plating cells were then incubated for 15 min at room temperature with an appropriate dilution of phage and poured onto Baltimore Biological Laboratories (BBL) plates in 2 ml of BBL top agar. The relative efficiency of plating on different hosts was calculated as:

$$\frac{(\textit{pal}\text{ phage on strain } x)/[\textit{pal}\text{ phage on JC7623 } (\textit{recBC sbcBC})]}{(\text{DRL112 on strain } x)/[\text{DRL112 on JC7623 } (\textit{recBC sbcBC})]}$$

DRL 112, λ Δ*B spi*6 *cI*857, is an isogenic palindrome free phage used to normalize the plating efficiencies on various hosts for minor differences not attributable to the palindromes.

Recovery of supercoiled DNA and its analysis: The methods used were as described in LEACH and LINDSEY (1986) with the following modifications. Infection was allowed to proceed for 10 min before DNA extraction. Two successive CsCl gradients instead of one were employed to ensure minimal contamination of supercoils with linear and relaxed DNA. Densitometry of bands was performed on a Shimadzu CS-930 densitometer. The control, palindrome-free, phage used in these experiments was MMS659, λ Δ*b*1453 *cI*857, obtained from F. STAHL.

EXPERIMENTAL RESULTS

A set of palindromes with different central sequences: λ Phages carrying palindromes that differ only in their central sequence were constructed. These phages were derived from DRL116, that has previously been used to study the behavior of palindrome bearing phage (CHALKER, LEACH and LLOYD 1988). All of the phages carry the same 446 bp (2 × 223 bp) inverted repeat but have different central sequences that vary in their degree of symmetry. Four of the phages have perfect centers and three have imperfections at or near the center, 8, 15 and 27 bp in length. These palindromes are depicted in Figure 1.

Plating behavior of palindrome phage: A subset of the above phages (DRL116, DRL133, DRL134, DRL137 and DRL148) was initially assessed for plating on strains of *E. coli* known to affect the propagation of DNA palindromes. The results of this experiment are shown in Table 1. It can be seen that the efficiency of plating (eop) of the different phages varied considerably and that the phages with perfect palindromes showed a lower eop than those with imperfect centers. This poor plating was particularly marked for phages DRL133 and 134. DRL148, which also has a perfect center, gave an eop intermediate between this pair and the phages with imperfect centers. Of these DRL137, with a 27-bp (off-set) asymmetry, plated with more facility than DRL116, with a 15-bp center asymmetry, as shown using N2362 and N2692, the least permissive hosts. We were in fact able to define a hierarchy of plating which is as follows (the number of base pairs of asymmetry is shown in brackets):

$$\text{DRL137 (27)} > \text{DRL116 (15)} > \text{DRL148 (0)} > \text{DRL133 (0), DRL134 (0).}$$

This is a hierarchy that applies across the set of host strains tested which argues that it is independent of the host genotype.

Recovery of supercoiled DNA: In order to determine whether the effect, observed at the level of plating, was reflected by an effect at the DNA level, experiments were performed to determine the recovery of supercoiled DNA. Previous work had shown that the recovery of supercoiled DNA of the phage with the 15-bp asymmetry at the center of the palindrome (DRL116) was affected by the *sbcC* genotype of the host (CHALKER, LEACH and LLOYD 1988). We now confirm that, after a period of 15 min postinjection, recovery of DRL116 DNA in supercoiled form is close to 100% from an *sbcC* mutant but reduced to 30% when purified from a wild-type host. However, when DRL134, one of the phage with a perfect palindrome conferring the severe phenotype was tested, recovery from an *sbcC* mutant was reduced to 30%

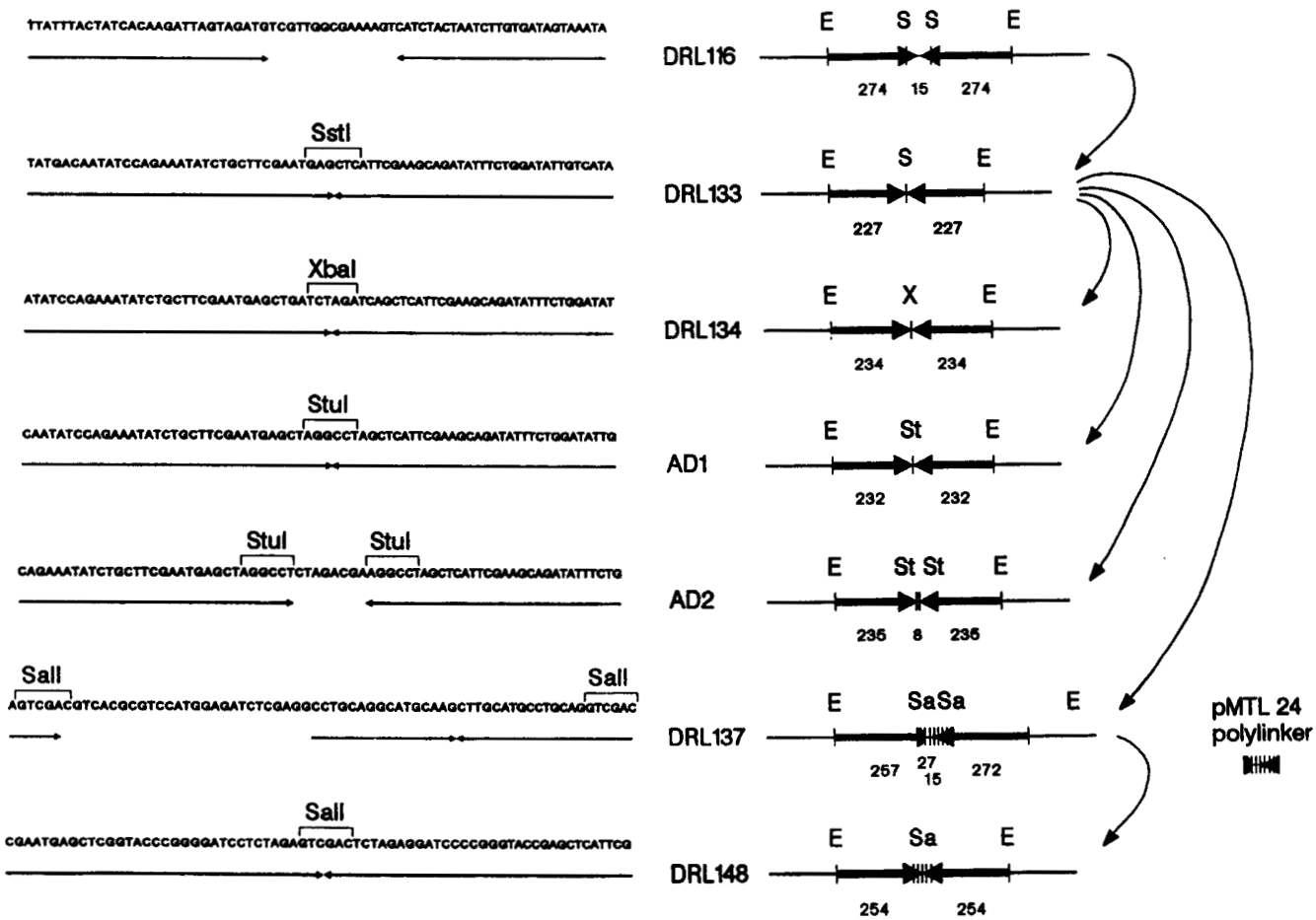

FIGURE 1.—The structure and derivation of the palindromes studied. The restriction sites involved in the construction of the palindromes are indicated as follows: E (*Eco*RI), S (*Sst*I), Sa (*Sal*I), St (*Stu*I), X (*Xba*I). The numbers of base pairs in the arms and (if asymmetric) the centers of the palindromes are listed under each representation. DRL133, DRL134, AD1 and DRL148 are perfect palindromes, whereas DRL116, AD2 and DRL137 are imperfect. AD2 has an asymmetric center of 8 bp, DRL116 has an asymmetric center of 15 bp and DRL137 has an asymmetry of 27 bp which is located 15 bp from the center of the palindrome. The left hand side of the figure depicts the central 70 bp of each of the palindromes.

and that from wild type to 2%. The behavior of DRL148, the phage with a perfect palindrome conferring an intermediate plating phenotype, was also intermediate at the DNA level. It showed a recovery of 90% from an *sbcC* mutant and 6% from wild type.

Plating behavior of a phage with an 8-bp asymmetry: To extend these observations to a palindrome with a shorter asymmetry, AD2, a phage with an 8-bp central insertion, was constructed and its behavior was compared with that of an isogenic phage with a perfect palindrome (AD1). The control phage with the perfect palindrome behaved similarly to phages DRL133 and DRL134, but AD2 showed a phenotype intermediate between that conferred by the perfect palindromes and the palindrome with a 15-bp asymmetry. These results have been incorporated into Table 1.

DISCUSSION

A center-dependent pathway exists *in vivo*: *In vitro*, it has been shown that the central sequence dictates the cruciform extrusion rate for palindromes of average base composition (MURCHIE and LILLEY 1987; COUREY and WANG 1988; ZHENG and SINDEN 1988). However, *in vivo*, the effects of central sequence have been less well documented. The experiments of WARREN and GREEN (1985) demonstrated that inviability could not be overcome unless large insertions of 50 bp or more were made at the center of the palindrome. They argued, therefore, that the structure responsible for inviability was generated by a mechanism involving interaction of the arms of the palindrome and was independent of the center. By contrast, our results demonstrate that when more sensitive assays are used, effects of much smaller changes in the central DNA sequence can be detected. In agreement with WARREN and GREEN, all six phages studied here are unable to plate on wild-type hosts. We can, however, see a marked effect of central asymmetry even in wild-type hosts when we look at the recovery of supercoiled DNA. Effects are also

TABLE 1

Plating behavior of palindrome-containing phages on mutant *E. coli* hosts

	E. coli strain and genotype	λ Strain DRL137	DRL116	AD2	DRL148	DRL133 DRL134 AD1
N2361	*rec*$^+$ *sbcC*$^+$	−	−	−	−	−
N2362	*recB21*	±[a]	−	−	−	−
N2692	*recD1009* *recA269::Tn10*	+[b]	−	−	−	−
N2678	*recD1009*	+	+	±[c]	±[c]	−
N2364	*sbcC201*	+	+	+	+	±[d]
N2365	*recB21* *sbcC201*	+	+	+	+	+
N2680	*recD1009* *sbcC201*	+	+	+	+	+

+ indicates an eop of $>10^{-1}$, − indicates an eop of $<10^{-2}$. Certain combinations of phage and bacteria are on the borderline between plating and not plating and the precise eop in these situations is very sensitive to minor variations in environmental conditions, such as the thickness of the plates, the age of the plates, the batch of agar, and the temperature of incubation. Platings were therefore done under standard conditions but in some cases it has still been necessary to report the observed range of eop that was observed in repeated experiments.

[a] eop of 10^{-1}–10^{-2} (small plaques).

[b] eop of $>10^{-1}$ (small plaques).

[c] eop of 10^{-1}–10^{-2} (very small plaques).

[d] eop of 1–10^{-2} (very small plaques).

evident when we examine plating behavior on mutant hosts. This suggests that a center-dependent pathway for phenotypic effects of palindromes does exist in both wild-type and mutant *E. coli*.

We have shown that palindromes carrying small central asymmetries confer a less severe phenotype than do perfect palindromes and the severity of the phenotype correlates inversely with the length of asymmetry. Thus a central asymmetry of only 8 bp (AD2) clearly alleviates the severe phenotype caused by the perfect palindromes in AD1, DRL133 and DRL134. *In vitro* work has indicated that cruciform extrusion of perfect palindromes requires denaturation of the central 8–10 bp (MURCHIE and LILLEY 1987; COUREY and WANG 1988) and that most cruciform loops include 4 bases (GOUGH, SULLIVAN and LILLEY 1986). That a difference can be detected *in vivo* between perfect palindromes and a palindrome with 8 bases of asymmetry argues strongly for a center-dependent pathway of extrusion. It is interesting that one of the perfect palindromes (DRL148) confers an intermediate phenotype, similar to that of the palindrome with the 8 bp asymmetry and less severe than those of the other perfect palindromes. It is true that the center of DRL148 is slightly more G/C rich than those of the other perfect palindromes. However, there are other features of the DRL148 structure which may share responsibility for its more moderate phenotype. The central region carries several palindromic recognition sequences for restriction enzymes which might abortively attempt to nucleate extrusion and thus inhibit extrusion from the central sequence; additionally, there is a sequence 5′-CCCGGGG-3′, 13-bp on either side of the center, which may interfere with extrusion. Finally, perhaps the central sequence itself is inherently less prone to initiate extrusion. Further work is required to determine why DRL148 behaves differently from the other perfect palindromes.

If cruciform extrusion occurs *in vivo*, it must be catalyzed: It has previously been demonstrated that cruciform extrusion *in vitro* occurs so slowly, in normal DNA sequences under physiological conditions, that no *in vivo* effects would be expected (COUREY and WANG 1983, 1988; GELLERT, O'DEA and MIZUUCHI 1983; MURCHIE and LILLEY 1987). This kinetic argument against cruciform extrusion *in vivo* has been supported by several studies in which cruciform structures have not been detected *in vivo* (COUREY and WANG 1983; SINDEN, BROYLES and PETTIJOHN 1983). Recent work, however, has revealed that under conditions of elevated superhelical density, palindromes that have central sequences favoring cruciform extrusion *in vitro* can extrude such structures *in vivo* (MCCLELLAN *et al.* 1990; DAYN *et al.* 1991; ZHENG *et al.* 1991; SINDEN *et al.* 1991). In addition, the deletion frequencies of certain palindromes argue for a pathway involving a cruciform intermediate (SINDEN *et al.* 1991). Nevertheless, the sum of these observations has been taken to argue against significant extrusion of palindromes of *average* base composition under *normal* physiological conditions.

Our results are, however, most easily explained if cruciform extrusion does occur *in vivo* in perfect palindromes with centers of normal base composition and even in palindromes with asymmetric centers of 8, 15 and 27 bp. In order to reconcile the *in vitro* data with our results we must suggest that cruciform extrusion is catalyzed *in vivo*. If so, why have previous studies *in vivo* required unusual palindromes and/or unusual physiological conditions to detect extrusion? We suggest that the relevant difference between the palindromes studied here and those studied previously is length. The palindromes employed in the present work are between 446 and 571 bp in length, whereas those used by other workers had been kept shorter than 150 bp to avoid the propagation problems of the longer sequences. It is, however, this phenotype of inviability that is likely to correlate with the accumulation of cruciform structures *in vivo*. We propose therefore that (as for palindromes *in vitro*) the center affects the kinetics of cruciform extrusion in a catalyzed reaction, but the length of the palindrome is critical in determining the lifetime of the cruciform structure.

In vitro, cruciform extrusion occurs either via the C-type or the S-type pathway (LILLEY 1985). The C-type pathway involves a large denaturation bubble which is favored by highly A/T-rich sequences at low salt concentrations and is therefore unlikely to relate to extrusion *in vivo*. The S-type pathway proceeds via a small central denaturation bubble which folds into a protocruciform that in turn extrudes to the full cruciform by branch migration. The transition state, which is probably the protocruciform, is thought to be stabilized by salt (SULLIVAN and LILLEY 1987). If the pathway to cruciform extrusion *in vivo* is related to S-type extrusion *in vitro*, we predict that specific base-pair changes that alter the rate of *in vitro* extrusion will have predictable effects on the phenotype of palindrome containing phage *in vivo*. Experiments to test this prediction are under way.

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LITERATURE CITED

AMUNDSEN, S. K., A. F. TAYLOR, A. M. CHAUDHURY and G. R. SMITH, 1986 *recD*: the gene for an essential third subunit of exonuclease V. Proc. Natl. Acad. Sci. USA **83:** 5558–5562.

BIEK, D. P., and S. N. COHEN, 1986 Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:** 594–603.

CHALKER, A. F., 1990 *SbcC* and palindrome-mediated inviability in *Escherichia coli*. Ph.D. Thesis, University of Edinburgh.

CHALKER, A. F., D. R. F. LEACH and R. G. LLOYD, 1988 *Escherichia coli sbcC* mutants permit stable propagation of replicons containing a long DNA palindrome. Gene **71:** 201–205.

CHAMBERS, S. P., S. E. PRIOR, D. A. BARSTOW and N. P. MINTON, 1988 The pMTL *nic* cloning vectors. I. Improved pUC polylinker regions to facilitate the use of sonicated DNA for nucleotide sequencing. Gene **68:** 139–149.

CHAUDHURY, A. M., and G. R. SMITH, 1984 A new class of *Escherichia coli recBC* mutants: implications for the role of RecBC enzyme in homologous recombination. Proc. Natl. Acad. Sci. USA **81:** 7850–7854.

COLLINS, J., 1981 The instability of palindromic DNA. Cold Spring Harbor Symp. Quant. Biol. **45:** 409–416.

COLLINS, J., G. VOLCKAERT and P. NEVERS, 1982 Precise and nearly-precise excision of the symmetrical inverted repeats of Tn*5*: common features of *recA*-independent deletion events in *E. coli*. Gene **19:** 139–146.

COUREY, A. J., and J. C. WANG, 1983 Cruciform formation in a negatively supercoiled DNA may be kinetically forbidden under physiological conditions. Cell **33:** 817–829.

COUREY, A. J., and J. C. WANG, 1988 Influence of DNA sequence and supercoiling on the process of cruciform formation. J. Mol. Biol. **202:** 35–43.

DAYN, A., S. MALKHOSYAN, D. DUZHY, V. LYAMICHEV, Y. PANCHENKO and S. MIRKIN, 1991 Formation of $(dA\text{-}dT)_n$ cruciforms in *Escherichia coli* cells under different environmental conditions. J. Bacteriol. **173:** 2658–2664.

ENQUIST, L. W., and A. SKALKA, 1975 Replication of bacteriophage lambda DNA dependent on the function of host and viral genes. I. Interaction of *red gam* and *rec*. J. Mol. Biol. **75:** 185–212.

GELLERT, M., M. H. O'DEA and K. MIZUUCHI, 1983 Slow cruciform transitions in palindromic DNA. Proc. Natl. Acad. Sci. USA **80:** 5545–5549.

GIBSON, F. P., D. R. F. LEACH and R. G. LLOYD, 1992 Identification of *sbcD* mutations as cosuppressors of *recBC* that allow propagation of DNA palindromes in *Escherichia coli* K12. J. Bacteriol. **174:** 1222–1228.

GOUGH, W. G., K. M. SULLIVAN and D. M. J. LILLEY, 1986 The structure of cruciforms in supercoiled DNA: probing the single-stranded character of nucleotide bases with bisulphite. EMBO J. **5:** 191–196.

HAGAN, C. E., and G. J. WARREN, 1982 Lethality of palindromic DNA and its use in selection of recombinant plasmids. Gene **19:** 147–151.

HAGAN, C. E., and G. J. WARREN, 1983 Viability of palindromic DNA is restored by deletions occurring at low but variable frequency in plasmids of *Escherichia coli*. Gene **24:** 317–326.

KULKARNI, S. K., and F. W. STAHL, 1989 Interaction between the *sbcC* gene of *Escherichia coli* and the *gam* gene of phage lambda. Genetics **123:** 249–253.

LEACH, D. R. F., and J. C. LINDSEY, 1986 *In vivo* loss of supercoiled DNA carrying a palindromic sequence. Mol. Gen. Genet. **204:** 322–327.

LEACH, D. R. F., J. C. LINDSEY and E. A. OKELY, 1987 Genome interactions which influence DNA palindrome mediated instability and inviability in *Escherichia coli*. J. Cell Sci. Suppl. **7:** 33–40.

LEACH, D. R. F., and F. STAHL, 1983 Viability of lambda phages carrying a perfect palindrome in the absence of recombination nucleases. Nature **305:** 448–451.

LILLEY, D. M. J., 1981 *In vivo* consequences of plasmid topology. Nature **292:** 380–382.

LILLEY, D. M. J., 1985 The kinetic properties of cruciform extrusion are determined by DNA base-sequence. Nucleic Acids Res. **13:** 1443–1465.

LINDSEY, J. C., and D. R. F. LEACH, 1989 Slow replication of palindrome-containing DNA. J. Mol. Biol. **206:** 779–782.

LLOYD, R. G., and C. BUCKMAN, 1985 Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. J. Bacteriol. **164:** 836–844.

MCCLELLAN, J. A., P. BOUBLIKOVA, E. PALECEK and D. LILLEY, 1990 Superhelical torsion in cellular DNA responds directly to environmental and genetic factors. Proc. Natl. Acad. Sci. USA **87:** 8373–8377.

MIZUUCHI, K., M. MIZUUCHI and M. GELLERT, 1982 Cruciform structures in palindromic DNA are favoured by DNA supercoiling. J. Mol. Biol. **156:** 229–244.

MURCHIE, A. I. H., and D. M. J. LILLEY, 1987 The mechanism of cruciform formation in supercoiled DNA: initial opening of central basepairs in salt dependent reaction. Nucleic Acids Res. **15:** 9641–9654.

PANAYATATOS N., and A. FONTAINE, 1987 A native cruciform DNA structure probed in bacteria by recombinant T7 endonuclease. J. Biol. Chem. **262:** 11364–11368.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS Editors, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SHURVINTON, C. E., M. M. STAHL and F. W. STAHL, 1987 Large palindromes in the λ phage genome are preserved in a rec^+ host by inhibiting DNA replication. Proc. Natl. Acad. Sci. USA **84:** 1624–1628.

SINDEN, R. R., S. BROYLES and D. PETTIJOHN, 1983 Perfect palindromic *lac* operator DNA sequence exists as a cruciform structure in supercoiled DNA *in vitro* but not *in vivo*. Proc. Natl. Acad. Sci. USA. **80:** 1797–1801.

SINDEN, R. R., G. ZHENG, R. G. BRANKAMP and K. N. ALLEN,

1991 On the deletion of inverted repeated DNA in *Escherichia coli*: effects of length, thermal stability and cruciform formation *in vivo*. Genetics **129:** 991–1005.

SULLIVAN, K. M., and D. M. J. LILLEY, 1987 Influence of cation size and charge on the extrusion of a salt-dependent cruciform. J. Mol. Biol. **193:** 397–404.

WARREN, G. J., and R. L. GREEN, 1985 Comparison of physical and genetic properties of palindromic DNA sequences. J. Bacteriol. **161:** 1103–1111.

ZHENG, G., and R. R. SINDEN, 1988 Effects of base composition at the center of inverted repeated DNA sequences on cruciform transitions in DNA. J. Biol. Chem. **263:** 5356–5361.

ZHENG, G., T. KOCHEL, R. W. HOEPFNER, S. E. TIMMONS and R. R. SINDEN, 1991 Torsionally tuned cruciform and Z-DNA probes for measuring unrestrained supercoiling at specific sites in DNA of living cells. J. Mol. Biol. **221:** 107–129.

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