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Resolution of synthetic Holliday junctions in DNA by an endonuclease activity from calf thymus

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Extracts of calf thymus have been fractionated to reveal a nuclease activity that specifically cleaves model Holliday junctions *in vitro*. The products of cleavage are unbranched linear duplex DNA molecules. Using synthetic four-way junctions, we show that the major sites of cutting are diametrically opposed, at sites one nucleotide from the base of the junction. Other types of four-way junctions, including pseudo-cruciform structures and cruciforms extruded from supercoiled plasmids, are also cleaved by the nuclease. The M_r of the partially purified activity, determined by gel filtration, is ~75 000. The calf thymus enzyme provides the first example of an endonuclease from a higher eukaryote that acts specifically on branch points in DNA, and indicates that junction-resolving proteins are normal constituents of somatic cells.

Key words: cruciform DNA/DNA repair/recombination intermediates/T4 endonuclease VII

Introduction

Resolution of a Holliday junction is an essential step during genetic recombination and the recombinational repair of DNA damage. A number of enzymes from prokaryotic viruses and lower eukaryotes have been isolated that cleave synthetic junctions *in vitro* (de Massy *et al.*, 1984; Kemper *et al.*, 1984; Symington and Kolodner, 1985; West and Korner, 1985; Jensch *et al.*, 1989). Studies with the most characterized enzyme, T4 endonuclease VII (Kemper and Garabett, 1981; Lilley and Kemper, 1984), indicate that cleavage involves the introduction of nicks into two strands of like polarity, at sites diametrically opposed across the junction (for reviews see West 1989, 1990). The resulting nicked duplex products may subsequently be acted upon by DNA ligase (Mizuuchi *et al.*, 1982). The action of junction-resolving nucleases has been extensively studied using a series of model substrates including (i) cruciform structures extruded from supercoiled plasmids (Lilley and Kemper, 1984; de Massy *et al.*, 1987; Parsons and West, 1988), (ii) pseudo-cruciform structures containing inverted repeats flanked by complementary sequences (Gough and Lilley, 1985), and (iii) synthetic four-way junctions made by annealing four oligonucleotides (Churchill *et al.*, 1988; Duckett *et al.*, 1988; Mueller *et al.*, 1988; Parsons *et al.*, 1990). These nucleases are also capable of resolving recombination intermediates made *in vivo* (Mizuuchi *et al.*, 1982) or *in vitro* by *Escherichia coli* RecA protein (Müller *et al.*, 1990).

Attempts to isolate junction-resolving activities from higher organisms have been largely unsuccessful. Two approaches have been attempted. Synthetic four-way junctions have been used to isolate proteins that bind specifically to junction DNA. Two proteins have been described, one from rat liver (Bianchi, 1988; Bianchi *et al.*, 1989) and the other from human cells grown in culture (Elborough and West, 1988). Unfortunately, neither protein has been shown to cleave junctions *in vitro*. In the other approach, the enzymatic resolution of junction DNA was investigated. It was shown that junctions were processed by HeLa cell extracts (Waldman and Liskay, 1988), and by an activity from placental tissue (Jayaseelan and Shanmugam, 1988). However, neither report presented evidence that resolution occurred by a specific nucleolytic incision event close to the base of the junction.

In the present work, calf thymus extracts have been fractionated to reveal an activity that cleaves a variety of synthetic four-way junctions into unbranched linear duplex DNA. Cleavage occurs by specific endonucleolytic cleavage in strands that are diametrically opposed at the junction point, in a reaction analogous to that catalysed by the phage-encoded junction-resolving proteins. The work therefore provides the first demonstration of a junction-specific nucleolytic activity from mammalian cells.

Results

Cleavage of synthetic four-way junctions by fractionated calf thymus extracts

The DNA substrate and assay used to detect cleavage are shown schematically in Figure 1A. The junction was composed of four oligonucleotides that were annealed to form an X-structure. Similar structures have been used in studies of junction geometry and the nucleolytic activity of the phage-encoded junction-resolving proteins (Kallenbach *et al.*, 1983; Duckett *et al.*, 1988; Mueller *et al.*, 1988; Cooper and Hagerman, 1989; Parsons *et al.*, 1990). Cleavage of the junction results in the formation of linear duplex DNA (Figure 1A). The substrates and products of the reaction can be easily identified by polyacrylamide gel electrophoresis.

In an attempt to detect a mammalian Holliday junction-resolving protein, we fractionated calf thymus extracts as described in Materials and methods. Fractions eluting from a phosphocellulose column were assayed for the ability to cleave junction DNA into linear duplex DNA (Figure 1B). An exonuclease activity, which removed the 5′-^{32}P-end label from the substrate eluted in fractions 30–45. The properties of this activity are similar to those described for mammalian DNase IV, a 5′–3′ exonuclease (Lindahl *et al.*, 1969a; Ishimi *et al.*, 1988). In other fractions, we observed the elution of an activity (fractions 50–65) that appeared to cleave junction DNA to produce a band that co-migrated

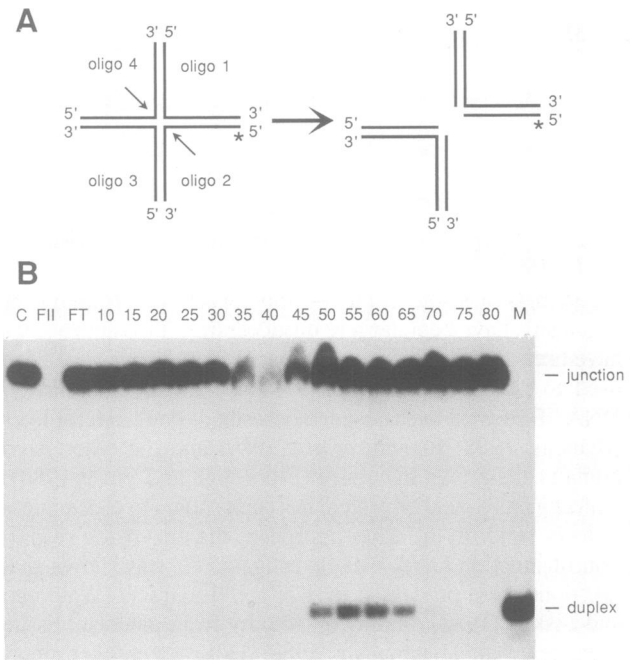

Fig. 1. Cleavage of junction DNA by fractionated calf thymus extract. (**A**) Diagram indicating the production of linear duplex DNA by endonucleolytic cleavage of the junction. The 5′-^{32}P-end label is indicated with an asterisk. (**B**) Junction DNA was incubated with 2 μl of the indicated fractions for 60 min, as described in Materials and methods. DNA products were analysed by neutral PAGE. Lane C, no protein; lane FII, fraction II; lane FT, column flow through; lanes 10–80, fractions eluting from phosphocellulose with 0–500 mM KCl gradient (see Materials and methods); lane M, linear duplex marker. Fractions showing junction-cleaving activity (50–65) were pooled (fraction III).

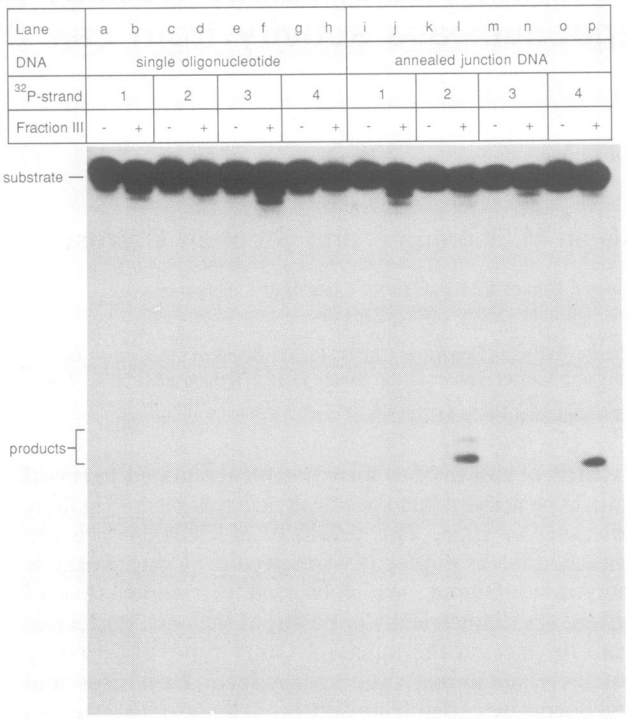

Fig. 2. Specific cleavage of junction DNA by calf thymus endonuclease by nicking of strands 2 and 4. 5′-^{32}P-labelled single-stranded oligonucleotides (lanes a–h) or junction DNA molecules (lanes i–p) were incubated in the absence (−) or presence (+) of fraction III (0.31 μg) for 30 min. The DNA concentration was 0.3 μM. Samples were analysed by denaturing PAGE. The four junctions were 5′-^{32}P-end labelled on the indicated strand.

with a linear duplex DNA marker (Figure 1B). These fractions were recovered for further study (fraction III).

Characterization of the cleavage reaction

Since the production of a linear duplex product is indicative of enzymatic resolution of the four-way junction, four identical junctions were prepared in which one strand (1, 2, 3 or 4) was 5′-^{32}P-end labelled. These junctions were used to determine whether the duplex product resulted from endonucleolytic cleavage. The four junctions were treated with fraction III and the DNA products were denatured for analysis by electrophoresis through a polyacrylamide gel containing 7 M urea (Figure 2, lanes i–p). Although little or no cleavage could be detected in strands 1 and 3 (lanes j and n), we observed the production of fragments from strands 2 and 4 (lanes l and p). In strand 2, there were two cuts (one major and one minor), and in strand 4 there was a single major cleavage site. When the individual oligonucleotides that comprise the junction were ^{32}P-end labelled and treated with fraction III, we failed to observe endonucleolytic cleavage (Figure 2, lanes b, d, f and h). In all reactions treated with fraction III we observed a low level of contaminating 3′–5′ exonuclease.

An aliquot of fraction III was further purified by gel filtration through a Superose 12 FPLC column equilibrated with R buffer containing 0.5 M KCl. In comparison with molecular weight standards, the junction-cleaving activity eluted as a protein with a M_r of ~75 000 (Figure 3). The activity (fraction IV) was essentially free of both 5′–3′ and 3′–5′ exonuclease. Since fractions III and IV produced identical cleavage products as determined by electrophoresis through denaturing polyacrylamide gels (data not shown), the more concentrated fraction (fraction III) was used for the following experiments.

The cleavage sites in each strand were mapped precisely using denaturing polyacrylamide gel electrophoresis (Figure 4). The cleavage products were flanked by sequence ladders produced by chemical degradation of 5-^{32}P-end labelled oligonucleotides (Maxam and Gilbert, 1980). The results, summarized in Figure 5A, show that the calf thymus activity cleaved the DNA predominantly in one orientation by cutting phosphodiester bonds close to the junction point in strands 2 and 4. The major cleavage sites were diametrically opposed and were located one nucleotide to the 3′-side of the base of the junction. The minor cleavage site in strand 2 was located two nucleotides from the junction point. Minor sites of cleavage in the opposite orientation (as indicated in strands 1 and 3) were observed only on over-exposed autoradiographs (data not shown). For comparison, the cleavage sites produced by T4 endonuclease VII are indicated (Figure 5B) (Parsons *et al.*, 1990).

Requirements for cleavage

Resolution of junction DNA by calf thymus endonuclease was dependent on the presence of Mg^{2+}, and was optimal at pH 6.7 (Table I). The activity was undetectable in a reaction mixture containing 200 mM NaCl, and was inactivated by boiling or treatment with proteinase K–SDS. The presence of 50 mM potassium glutamate had a stimulatory

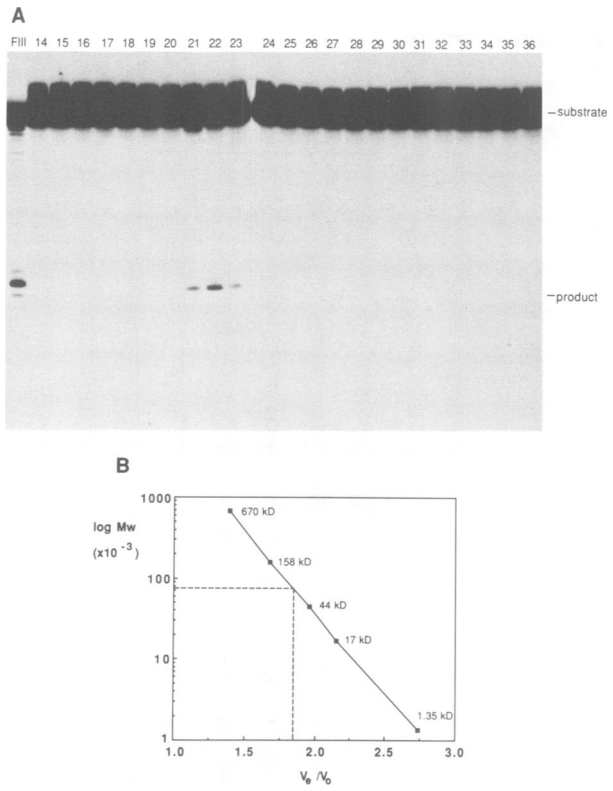

Fig. 3. Superose 12 FPLC gel filtration of the calf thymus endonuclease. The column was run as described in Materials and methods. (**A**) An aliquot (1 μl) of each fraction was incubated with junction DNA (^{32}P-end labelled on strand 2) and the products were assayed by denaturing PAGE. Lane FIII, fraction III as applied to column; lanes 14–36, fractions eluted from column. (**B**) The elution volume (V_e) of the activity (fraction IV), as detected in the autoradiograph above, was compared with thyroglobulin (670 kd), γ-globulin (158 kd), ovalbumin (44 kd), myoglobin (17 kd) and vitamin B12 (1.35 kd) mol. wt standards.

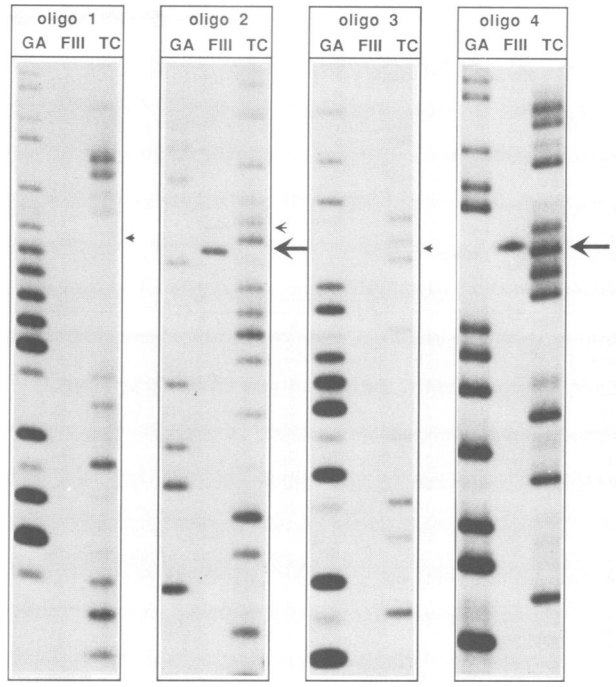

Fig. 4. Mapping of the cleavage sites produced by calf thymus endonuclease. Four uniquely 5′-^{32}P-end labelled junctions (as indicated in lane headings) were incubated with fraction III (0.31 μg) for 45 min as described in Materials and methods. The products of the reactions were analysed by denaturing PAGE. G+A and T+C sequence ladders flank the fraction III cleavage reactions (lane FIII). Only part of each ladder is shown. Major and minor sites (as observed on over-exposed autoradiographs) are indicated by large and small arrows. To assign the cleavage sites, allowances were made to compensate for the nucleoside eliminated in the chemical sequencing reaction (Maxam and Gilbert, 1980).

effect and was routinely included in the standard assay. There was no requirement for ATP (data not shown).

Cleavage of pseudo-cruciform DNA

To determine whether the calf thymus endonuclease cleaves other types of structures that serve as models for Holliday junctions, we prepared a pseudo-cruciform DNA structure. Single-stranded DNA from M13mp9/8 and M13mp8/13, which contain inverted repeats that hybridize to form a pseudo-cruciform junction (Gough and Lilley, 1985), were annealed to form duplex DNA. Following treatment with *Eco*RI and *Hin*fI, the 630 bp fragment containing the junction was purified and ^{32}P-end labelled at both 3′-termini (Figure 6A).

When the fragment was treated with T4 endonuclease VII (Figure 6B, lane b) or the calf thymus nuclease (lane c), the DNA was cleaved to produce two product bands as detected by non-denaturing polyacrylamide gel electrophoresis. Digestion with the single-stranded nuclease S1, which cleaves the single-stranded hairpin loops, produced bands of faster mobility (lane d). These results indicate that the calf thymus endonuclease cleaves pseudo-cruciform DNA in a manner similar to T4 endonuclease VII. Subsequent experiments in which the products were analysed by denaturing gel electrophoresis confirmed that cleavage occurred close to the base of the junction (data not shown).

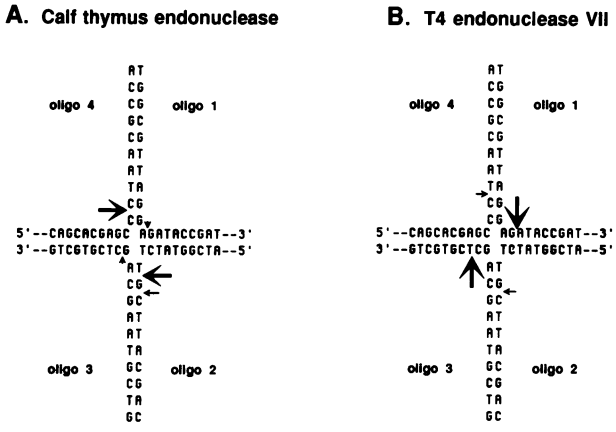

Fig. 5. Central core of the junction indicating the major and minor sites of cleavage. (**A**) Cleavage by calf thymus endonuclease. (**B**) Cleavage by T4 endonuclease VII (data compiled from Parsons *et al.*, 1990). Major and minor cleavage sites are indicated by large and small arrows respectively.

Similarly, we have observed that cruciform structures extruded from supercoiled plasmids are also a substrate for the calf thymus endonuclease (data not shown).

Table I. Reaction requirements.

	Relative activity (%)
Complete reaction mixture (pH 6.7)	100
$-Mg^{2+}$	< 5
−K glutamate	58 (100[a])
+200 mM glutamate	20
+50 mM NaCl	34[a]
+200 mM NaCl	< 1[a]
boil 2 min	< 3[a]
+proteinase K+SDS	< 1[a]
complete reaction mixture (pH 7.5)	8

Reaction mixtures containing 3 pmol ^{32}P-end labeled junction DNA were incubated in standard buffer (50 mM sodium phosphate pH 6.7, 5 mM $MgCl_2$, 50 mM K glutamate, 1 mM DTT, 100 μg/ml BSA) with 0.31 μg fraction III for 45 min, as described in Materials and methods. Reaction products were analysed by denaturing PAGE and visualized by autoradiography. The relative cleavage was determined using an LKB Ultrascan XL Laser densitometer and expressed as a percentage relative to the reaction under standard conditions.

[a] K glutamate was omitted and the percentage cleavage was determined relative to the −K glutamate reaction. Treatment with proteinase K (2 mg/ml) and SDS (0.5%) was for 15 min at 37°C.

Discussion

In this paper, we describe the preliminary characterization of a partially purified enzyme from calf thymus tissue that resolves model Holliday junctions. Based on several lines of evidence, we suggest that this endonuclease is specific for junction DNA. (i) The enzyme cleaved junction DNA into unbranched duplex DNA without any subsequent processing of the duplex product. (ii) Single-stranded DNA fragments containing the DNA sequences present in the junction were not cleaved. (iii) Precise mapping of the cleavage sites showed that cuts were introduced at diametrically opposed positions located one to two residues from the junction point. (iv) Cleavage of the junction occurred in one orientation only (single-strand specific endonucleases that might resolve a 'breathing junction' would cut all four strands). (v) Other types of junctions including pseudo-cruciforms and cruciform DNA structures served as targets for the enzyme.

The junction used in the present experiments has been used in studies with T4 endonuclease VII (Parsons *et al.*, 1990), and the results obtained with T4 endonuclease VII and the calf thymus activity can be compared directly. T4 endonuclease VII cuts this DNA by the introduction of nicks at positions that are symmetrically related across the junction point. Cleavage occurs in one orientation (strands 1 and 3, Figure 5B), at sites located two residues to the 3′-side of the junction. Similarly, the calf thymus endonuclease cleaves the DNA in one orientation, at the 3′-side of the junction, at sites located one residue from the junction point. In comparison with T4 endonuclease VII, the calf thymus activity cleaves the junction in the opposite orientation (by nicking strands 2 and 4, Figure 5A). It is of interest that the same DNA substrate is cleaved by T7 endonuclease I with equal efficiency in both orientations, by cleavage of strands 1 and 3, or by cleavage of strands 2 and 4 (C.A.Parsons and S.C.West, unpublished data). The differences between the three junction-resolving enzymes may be a reflection of the way in which they bind the junction into their catalytic site in preparation for cleavage.

The relative molecular mass of the partially purified calf thymus endonuclease is ~75 kd as determined by gel filtration. However, at this stage of purification, we cannot rule out the possibility that the activity is an active fragment of a larger protein or is part of a complex. In comparison, the T4 and T7 encoded junction-specific nucleases have M_r of ~40 kd, and are composed of two identical peptide subunits (Kemper and Garabett, 1981; Dunn and Studier, 1983; Barth *et al.*, 1988).

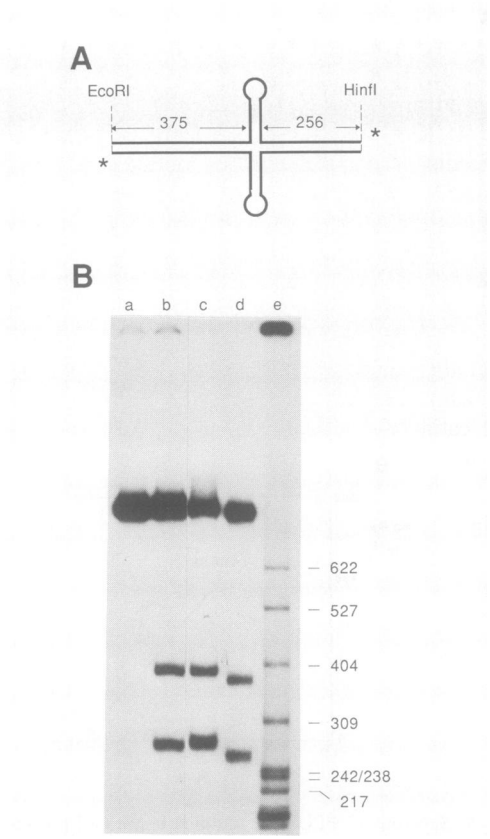

Fig. 6. Cleavage of pseudo-cruciform DNA. **(A)** Structure of the *Eco*RI−*Hin*fI fragment showing the position of the junction. DNA was ^{32}P-end labelled at both 3′-termini as indicated by asterisks. **(B)** Pseudo-cruciform DNA (lane a) was treated with 30 U T4 endonuclease VII (lane b), 0.62 μg calf thymus fraction III (lane c) or 2.5 U S1 nuclease (lane d) under the appropriate buffer conditions. The cleavage products were analysed by electrophoresis through 5% polyacrylamide. Lane e, marker fragments produced by *Msp*I cleavage of pBR322.

As far as we are aware, the calf thymus endonuclease has not been described previously and differs from the mammalian enzymes DNase I (Kunitz, 1950), DNase II (Bernardi, 1968), DNase III (Lindahl *et al.*, 1969b), DNase IV (Lindahl *et al.*, 1969a; Ishimi *et al.*, 1988), the Ca^{2+}, Mg^{2+}-dependent endonuclease (Ishida *et al.*, 1974) and DNase VI (Pedrini *et al.*, 1976). In addition, any relationship between the calf thymus activity and presumed junction-resolving activities from human cell-free extracts (Waldman and Liskay, 1988) or partially purified placental extracts (Jayaseelan and Shanmugam, 1988) is unknown. These preliminary reports suggested the presence of an activity that resolves Holliday junctions *in vitro*, but neither presented evidence showing that resolution occurred by specific nucleolytic cleavage of the junction.

What is the role of this nuclease within the cell? The T4 and T7 junction-resolving proteins play an important role in phage packaging (Kemper and Janz, 1976; Tsujimoto and Ogawa, 1978), and in genetic recombination and repair-related processes (Mizuuchi *et al.*, 1982; Kemper *et al.*, 1984; Kleff and Kemper, 1988). Mutations in the genes encoding the endonucleases result in mutants that have partially recombination-deficient phenotypes (Kerr and Sadowski, 1975; Tsujimoto and Ogawa, 1978; Miyazaki *et al.*, 1983). At this time, any proposed function for the calf thymus endonuclease, such as a role in recombination or repair, is purely speculative. However, it is of interest that proteins similar to the *E.coli* RecA protein have been partially purified from somatic tissue (Hsieh *et al.*, 1986; Cassuto *et al.*, 1987; Ganea *et al.*, 1987; Fishel *et al.*, 1988), indicating that the process of recombinational repair may be an important cellular function in growing cells.

Materials and methods

DNA

Junction DNA was made by annealing oligonucleotides
1 (5′-GACGCTGCCGAATTCTGGCGTTAGGAGATACCGATAAGCT-TCGGCTTAA-3′),
2 (5′-CTTAAGCCGAAGCTTATCGGTATCTTGCTTACGACGCTAG-CAAGTGATC-3′),
3 (5′-TGATCACTTGCTAGCGTCGTAAGCAGCTCGTGCTGTCTAG-AGACATCGA-3′ and
4 (5′-ATCGATGTCTCTAGACAGCACGAGCCCTAACGCCAGAATT-CGGCAGCGT-3′)
(Kallenbach *et al.*, 1983). Oligonucleotides were synthesized by phosphoramidite chemistry on an Applied Biosystems 380B DNA synthesizer. Gel purified oligonucleotides (2.25 nmol) were 5′-end labelled using [γ-^{32}P]ATP and polynucleotide kinase. Following kinase inactivation, each labelled oligonucleotide was annealed with excess (3 nmol) of the three complementary oligonucleotides. Annealing was carried out by incubation for 2 min at 95°C, followed by 10 min at 65°C, 37°C, 25°C and 0°C each. Junction DNA was purified by gel electrophoresis. Linear duplex DNA was made by annealing oligonucleotides 1 and 5 (5′-CTTAAGCCGAAGCTTATCGGTATCTCCTAACGCCAGAATTCGGC-AGCGT-3′). DNA concentrations are expressed in moles of nucleotide residues.

Pseudo-cruciform DNA was prepared by annealing single-stranded DNA of the phages M13mp8/13 and M13mp9/8 (Gough and Lilley, 1985), followed by digestion with *Eco*RI and *Hin*fI. DNA fragments containing the pseudo-cruciform structure were purified by gel electrophoresis and 3′-^{32}P-end labelled with DNA polymerase I (Klenow fragment).

Enzymes

Restriction endonucleases, DNA polymerase I and S1 nuclease were purchased from Bethesda Research Laboratories and T4 polynucleotide kinase was from Pharmacia. Homogeneous T4 endonuclease VII (60 000 units/μl) was a gift of Dr B.Kemper.

Preparation of junction-cutting activity from calf thymus

1 kg fresh calf thymus was stripped of fatty tissue, cut into 2 cm^3 pieces and homogenized in ice-cold extraction buffer (50 mM Tris−HCl pH 7.5, 1 mM EDTA, 100 mM NaCl, 0.5 mM DTT) containing 1 mM phenylmethylsulphonyl fluoride (PMSF), 1.9 μg/ml aprotinin, 0.5 μg/ml leupeptin, pepstatin, chymostatin and *N*α-*p*-tosyl-L-lysine chloromethyl ketone. The tissue was homogenized in a final volume of 5 l, and after 1 h on ice was centrifuged at 13 000 *g* for 30 min. The supernatant (fraction I: 1.3 mg/ml) was diluted to 16 l with dilution buffer (50 mM Tris−HCl pH, 7.5, 1 mM EDTA, 0.5 mM DTT), and 3.5 l of phosphocellulose slurry (equilibrated in dilution buffer containing 20 mM NaCl) was added and mixed. After standing for 1 h, the supernatant was removed and replaced by a further 10 l of dilution buffer. The suspension was mixed and stored overnight at 4°C.

The supernatant was removed and a minimal volume (~1 l) of dilution buffer containing 500 mM NaCl was added to elute protein from the phosphocellulose. Following centrifugation at 8500 *g* for 10 min, the phosphocellulose pellet was re-washed. The two supernatants were pooled and 56 g/l $(NH_4)_2SO_4$ was added. After 30 min, the precipitate was removed by centrifugation. Additional $(NH_4)_2SO_4$ (288 g/l) was added to the supernatant and after 1 h, the precipitate was collected by centrifugation, resuspended in 100 ml R buffer [50 mM Tris−HCl pH 7.5, 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, 0.1 mM PMSF] and dialysed for 5 h (fraction II). In some preparations, the pellets were frozen in dry ice/acetone and stored at −80°C.

50 ml of fraction II (5 mg/ml) was applied to a phosphocellulose column (100 cm^3) equilibrated with R buffer. Protein was eluted with a 1 l gradient (0−500 mM KCl) in R buffer. Fractions were collected and aliquots assayed for junction-cutting activity. The peak of activity eluted at 300−400 mM KCl (fraction III: 0.4 mg/ml).

An aliquot of fraction III (100 μl) was applied to a Superose 12 HR 10/30 gel filtration FPLC column (Pharmacia) equilibrated with R buffer supplemented with 0.5 M KCl. The column was run at 0.2 ml/min and 0.5 ml fractions were collected. Fractions containing the junction-cutting activity (fraction IV) were identified and stored at −80°C. The relative molecular weight of the activity was determined by comparison with protein markers (Biorad). The protein markers were detected by the Bradford assay.

Endonuclease assays

Cleavage of synthetic four-way junctions. The standard reaction (10 μl) contained 50 mM sodium phosphate pH 6.7, 5 mM $MgCl_2$, 50 mM potassium glutamate, 1 mM dithiothreitol and 100 μg/ml bovine serum albumin. ^{32}P-end labelled junction DNA (0.3 μM) was incubated for 30−60 min at 37°C with varying amounts of protein. For neutral PAGE, reactions were deproteinized by addition of one tenth volume of stop buffer [20 mg/ml proteinase K, 5% (w/v) SDS, 100 mM Tris−HCl pH 7.5, 50 mM EDTA] and incubated at 37°C for 15 min. DNA products were electrophoresed through 10% polyacrylamide gels using a Tris−borate buffer system. For denaturing PAGE, reactions were stopped by the addition of an equal volume of loading buffer [80% (v/v) formamide, 50 mM Tris−HCl pH 8.0, 1 mM EDTA, 0.1% (w/v) xylene cyanol, 0.1% (w/v) bromophenol blue] and heated at 95°C for 3 min. Samples were loaded onto 12% polyacrylamide gels containing 7 M urea. Gels were dried and radiolabelled DNA detected by autoradiography.

Cleavage of pseudo-cruciform DNA. Reactions (30 μl) containing 3′-^{32}P-end labelled pseudo-cruciform DNA (0.05 μM) were digested with T4 endonuclease VII or calf thymus endonuclease for 45 min at 37°C, or with S1 nuclease for 15 min at 25°C. The reaction buffer for T4 endonuclease VII was 50 mM Tris−HCl pH 8.0, 10 mM $MgCl_2$ and 1 mM dithiothreitol. S1 nuclease buffer was 30 mM sodium acetate pH 4.6, 50 mM NaCl, 1 mM $ZnCl_2$ and 5% (v/v) glycerol.

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References

Barth,K.A., Powell,D., Trupin,M. and Mosig,G. (1988) *Genetics*, **120**, 329−343.
Bernardi,G. (1968) *Adv. Enzymol.*, **31**, 1−49.
Bianchi,M.E. (1988) *EMBO J.*, **7**, 843−850.
Bianchi,M.E., Beltrame,M. and Paonessa,G. (1989) *Science*, **243**, 1056−1059.
Cassuto,E., Lightfoot,L.A. and Howard-Flanders,P. (1987) *Mol. Gen. Genet.*, **208**, 10−14.
Churchill,M.E.A., Tullius,T.D., Kallenbach,N.R. and Seeman,N.C. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4653−4656.
Cooper,J.P. and Hagerman,P.J. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 7336−7340.
de Massy,B., Studier,F.W., Dorgai,L., Appelbaum,E. and Weisberg,R.A. (1984) *Cold Spring Harbor Symp. Quant. Biol.*, **49**, 715−726.
de Massy,B., Weisberg,R.A. and Studier,F.W. (1987) *J. Mol. Biol.*, **193**, 359−376.
Duckett,D.R., Murchie,A.I.H., Diekmann,S., Von Kitzing,E., Kemper,B. and Lilley,D.M.J. (1988) *Cell*, **55**, 79−89.
Dunn,J.J. and Studier,F.W. (1983) *J. Mol. Biol.*, **166**, 477−535.
Elborough,K.M. and West,S.C. (1988) *Nucleic Acids Res.*, **16**, 3603−3614.
Fishel,R.A., Detmar,K. and Rich,A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 36−40.
Ganea,D., Moore,P., Chekuri,L. and Kutcherlapati,R. (1987) *Mol. Cell. Biol.*, **7**, 3124−3130.

Gough,G.W. and Lilley,D.M.J. (1985) *Nature*, **313**, 154–156.
Hsieh,P., Meyn,M.S. and Camerini-Otero,R.D. (1986) *Cell*, **44**, 885–894.
Ishida,R., Alkiyoshi,H. and Takahashi,T. (1974) *Biochem. Biophys. Res. Commun.*, **56**, 703–710.
Ishimi,Y., Claude,A., Bullock,P. and Hurwitz,J. (1988) *J. Biol. Chem.*, **263**, 19723–19733.
Jayaseelan,R. and Shanmugam,G. (1988) *Biochem. Biophys. Res. Commun.*, **156**, 1054–1059.
Jensch,F., Kosak,H., Seeman,N.C. and Kemper,B. (1989) *EMBO J.*, **8**, 4325–4334.
Kallenbach,N.R., Ma,R.I. and Seeman,N.C. (1983) *Nature*, **305**, 829–831.
Kemper,B. and Garabett,M. (1981) *Eur. J. Biochem.*, **115**, 123–132.
Kemper,B. and Janz,E. (1976) *J. Virol.*, **18**, 992–999.
Kemper,B., Jensch,F., Depka-Prondzynski,M., Fritz,H.J., Borgmeyer,U. and Mizuuchi,K. (1984) *Cold Spring Harbor Symp. Quant. Biol.*, **49**, 815–825.
Kerr,C. and Sadowski,P.D. (1975) *Virology*, **65**, 281–285.
Kleff,S. and Kemper,B. (1988) *EMBO J.*, **7**, 1527–1535.
Kunitz,M. (1950) *J. Gen. Physiol.*, **33**, 349–362.
Lilley,D.M.J. and Kemper,B. (1984) *Cell*, **36**, 413–422.
Lindahl,T., Gally,J.A. and Edelman,G.M. (1969a) *Proc. Natl. Acad. Sci. USA*, **62**, 597–603.
Lindahl,T., Gally,J.A. and Edelman,G.M. (1969b) *J. Biol. Chem.*, **244**, 5014–5019.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–560.
Miyazaki,J., Ryo,Y. and Minagawa,T. (1983) *Genetics*, **104**, 1–9.
Mizuuchi,K., Kemper,B., Hays,J. and Weisberg,R.A. (1982) *Cell*, **29**, 357–365.
Mueller,J.E., Kemper,B., Cunningham,R.P., Kallenbach,N.R. and Seeman,N.C. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 9441–9445.
Müller,B.M., Jones,C., Kemper,B. and West,S.C. (1990) *Cell*, **60**, 329–336.
Parsons,C.A. and West,S.C. (1988) *Cell*, **52**, 621–629.
Parsons,C.A., Kemper,B. and West,S.C. (1990) *J. Biol. Chem.*, **265**, 9285–9289.
Pedrini,A.M., Ranzani,G., Pedrali Noy,G.C.F., Spadari,S. and Falaschi,A. (1976) *Eur. J. Biochem.*, **70**, 275–283.
Symington,L.S. and Kolodner,R. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7247–7251.
Tsujimoto,Y. and Ogawa,H. (1978) *J. Mol. Biol.*, **125**, 255–273.
Waldman,A.S. and Liskay,R.M. (1988) *Nucleic Acids Res.*, **16**, 10249–10266.
West,S.C. (1989) In Eckstein,F. and Lilley,D.M.J. (eds), *Nucleic Acids and Molecular Biology*. Springer-Verlag, Berlin, Vol. 3, pp. 151–154.
West,S.C. (1990) *BioEssays*, **12**, 151–154.
West,S.C. and Korner,A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 6445–6449.

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