Determination of the absolute handedness of knots and catenanes of DNA

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DNA winds about itself in a right-handed or left-handed fashion at several structural levels. The double helix is generally right-handed and is given a (+) sign by convention, whereas supercoiling of the helix axis is always (−) in the cell[1,2]. The winding in higher -order forms such as knots and catenanes is unknown, and this has impeded elucidation of the mechanisms of their formation and resolution by replication, recombination and topoisomerase action[3–6]. We introduce here a procedure for determining the handedness of DNA winding by inspection of electron micrographs of DNA molecules coated with *Escherichia coli* RecA protein. We demonstrate the validity of the method and show that DNA topoisomerase I of *E. coli*[7] generates an equal mixture of (+) and (−) duplex DNA knots, and that one product of recombination by resolvase of transposon Tn*3* (refs 8, 9) is a catenane of uniquely (+) sign.

We have adopted a sign convention that can be applied uniformly to the winding in catenanes, knots, supercoils and double helices. The plane projection of two crossing DNA segments can form a node in two topologically distinct ways (for supercoils, see Fig. 1*a*, *b*). The crossing in a left-handed supercoil is given a (−) sign and that in a right-handed supercoil a (+) sign. The arrows show the relative direction of the two segments along the DNA backbone. To determine the sign of any node, both the relative direction and the overlay of the crossing DNA segments must be distinguished.

It is straightforward to determine the direction of crossing segments in intramolecular nodes in supercoiled or knotted rings, because the path of DNA connecting the segments can be followed. To describe the sign of a knot then, it is only necessary to determine reliably which of the crossing segments overlies the other. This has not been possible by electron microscopic examination of conventionally stained and shadowed DNA because resolution of the two segments at the junction is poor, with rare exceptions[17]. To overcome this problem, we sought a protein coat for the DNA that would enhance visualization of the nodes; *E. coli* RecA protein[10,11] proved ideal. In the presence of ATPγS, it binds cooperatively to duplex DNA to form a stiffened striated complex about 100 Å in diameter[12]. Figure 2 shows RecA-decorated trefoil knots that have been tied in nicked rings by *E. coli* DNA topoisomerase I. The three nodes are (+) in the upper knot (Fig. 2*a*) and (−) in the lower knot (Fig. 2*b*).

To validate our procedure for determining the chirality of knots, we took advantage of the topological requirement that trefoils have either three (−) (Fig. 1*c*) or three (+) (Fig. 1*d*) nodes. Thus, every knot provided two internal checks on the accuracy of each node assignment. A mask was placed over a micrograph so that only a single node of a knot was visible at a time. Three observers independently scored which of the crossing segments was on top. Ninety-two per cent of the nodes in the 47 trefoils analysed were assigned the same sign as the other two nodes of the knot. By considering exclusively knots where the consensus sign for all three nodes was the same, the probability of incorrectly ascribing sign to a knot is negligible.

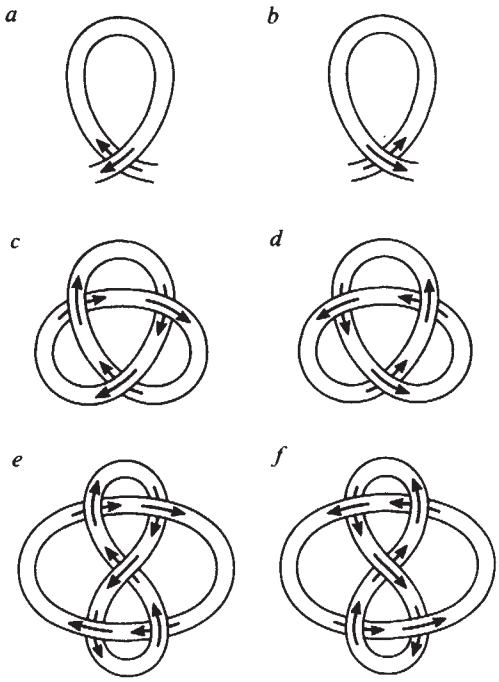

Fig. 1 Topology of nodes, trefoil knots and figure-8 catenanes. Sign of nodes: as illustrated for supercoils, two DNA segments can cross in plane projection to form nodes of two topological types: (−) in *a* and (+) in *b*. The arrows indicate the path of the connecting DNA. Node sign is a local property dictated only by the overlay and relative direction of the crossing segments. The absolute direction of the arrows is arbitrary because traversing the path in the opposite direction maintains the sign. Trefoil knots: these can have either three (−) nodes (*c*) or three (+) nodes (*d*). Figure-8 catenanes: four of the nodes of figure-8 catenanes are intermolecular: two are (+) and two are (−). The central node describes a supercoil entrapped by the intermolecular linkage and thereby defines the two stereoisomers of figure-8 catenanes. The (−) catenane (*e*) contains a left-handed supercoil and the (+) catenane (*f*) a right-handed supercoil.

Of the 40 knots generated by topoisomerase I that met this restriction, 18 were (+) and 22 were (−). The difference of this ratio from 1 is not statistically significant. In knotting DNA rings, DNA topoisomerase I passes a duplex DNA segment through the intact strand remaining at a nick[13], thereby changing the sign of the crossed segments. As topoisomerase I produced (+) and (−) knots equally, (+) and (−) nodes must be formed and inverted equally in its active centre. Previously topoisomerase I had been shown to invert (−) nodes of only one sign, because it relaxed (−), but not (+), supercoils[7]. This substantiates the view of topoisomerases as enzymes that can promote permeation of DNA segments through each other without regard to sign or orientation of the crossing segments.

By contrast with the racemic topoisomerase I products, the site-specific recombination enzyme Tn*3* resolvase generated a unique stereoisomer. This enzyme promotes recombination between directly repeated resolution sites in a supercoiled plasmid to give two linked daughter rings[8,9]. About 99% of the recombinants are singly interlinked catenanes[9]. When the products were nicked to remove supercoils, a minor fraction was revealed that had a higher electrophotoretic mobility. We expected that these would be conventional doubly-interlinked catenanes but found instead that they have the novel topological form of a figure-8 catenane. The two possible stereoisomers of figure-8 catenanes (Fig. 1*e*, *f*), can be distinguished simply by inspection of the path and overlay of the DNA. In contrast to standard catenanes, the relative direction of the two component rings need not be distinguished. In the simplest representation of a figure-8 catenane (Fig. 1*e*, *f*), there are two (+) and two

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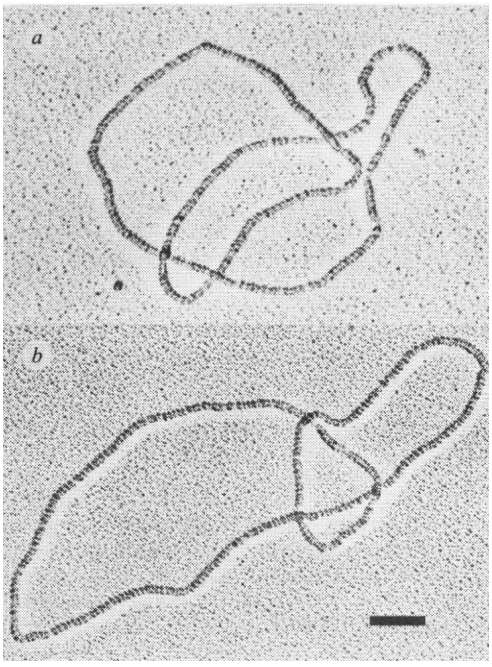

Fig. 2 Electron micrographs of RecA protein-coated trefoil knots generated by *E. coli* DNA topoisomerase I. Forty microgrammes of plasmid pRR51 (ref. 8) were singly nicked by pancreatic DNase I in the presence of ethidium bromide and knotted by treatment with 60 μg of *E. coli* topoisomerase I in a 2.4-ml reaction mixture containing 30% (v/v) glycerol, 20 mM Tris-HCl (*p*H 7.6), 20 mM KCl, 8 mM potassium phosphate, 6 mM $MgCl_2$ and 0.5 mM dithiothreitol. After 30 min at 52 °C, the products were separated by electrophoresis through a 0.8% agarose gel and the trefoil knots (about 10% of the product) isolated by electroelution. Twenty-five nanogrammes of the purified DNA were incubated at 37 °C with 1 μg of RecA protein[15] in a 20-μl reaction mixture containing 25 mM triethanolamine chloride (*p*H 7.6), 2.5 mM magnesium acetate and 2 mM rATP. After 5 min, 0.5 mM ATPγS was added to stabilize the complexes, and 30 min later glutaraldehyde was added to 0.2%. After an additional 15 min incubation, the reaction mixture was applied to a 0.5 ml Sepharose 2B (Pharmacia) column and eluted with 5 mM magnesium acetate. The drops were collected on Parafilm and adsorbed to a freshly glow discharged carbon-coated grid for electron microscopy[16]. The specimens were washed by floating on 5 mM magnesium acetate for 20 min, dehydrated in absolute ethanol and rotary shadowed at an angle of 7° with carbon–platinum. The striations on the DNA correspond to the right-handed helical coating by RecA[12] and provide an internal control for the correct reading of the photographic negatives. Scale bar, 1,000 Å.

(−) intermolecular nodes that entrap a supercoil in one of the component rings. Because the signs of the intermolecular nodes cancel, the sign of the whole molecule is that of the lone intramolecular node. The catenane with the left-handed supercoil (Fig. 1*e*) is (−), and the one with the right-handed supercoil (Fig. 1*f*) is (+). We determined the sign of the resolvase product as above by masking the DNA so that only one of the five nodes was visible at a time and scoring the overlying DNA segment. For the 16 molecules analysed by two observers, 93% of the node signs were consistent. After eliminating three molecules whose structure was ambiguous, the consistency was 98%. All 13 of the remaining figure-8 catenanes were (+) (see Fig. 3).

Three features of the recombination mechanism are implied by this single result, barring the unlikely possibility that (−) catenanes were also produced but selectively lost. First, resolvase pairs the recombination sites and rearranges the broken DNA strands in a well defined fashion to generate a product with unique handedness. Second, if an achiral DNA intermediate were generated in recombination, then resolvase must form an asymmetric complex with the intermediate[14]. Third, the

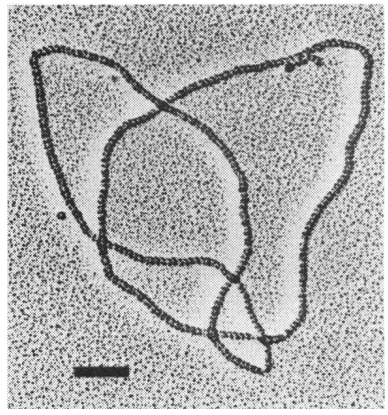

Fig. 3 Electron micrograph of a figure-8 catenane produced by Tn*3* resolvase. 225 μg of plasmid p51A[2], containing directly repeated copies of the recombination site, were treated with resolvase and nicked with DNase I in the presence of ethidium bromide as described earlier[9]. The products were separated by agarose gel electrophoresis and about 1% were figure-8 catenanes which migrated faster than the predominant product of singly interlinked daughter rings. The figure-8 catenanes were isolated, coated with RecA protein and prepared for electron microscopy as described in Fig. 2 legend. Scale bar, 1,000 Å.

catenated product of recombination contains an entrapped (+) supercoil, but the substrate is negatively supercoiled. Thus, catenation here can hardly result from a passive redistribution of supercoil windings as has been suggested for another recombination system[4], but instead must be directed by the enzyme.

All mechanisms for generation of knots and catenanes make implicit predictions of sign, and these can now be tested directly. Conversely, the sign of naturally occurring knots and catenanes will illuminate the history of their formation since we have shown that enzymes leave a characteristic topological fingerprint. Such structural studies are greatly facilitated by the DNA visualization technique used here because it allows unambiguous identification of complex topological forms by distinguishing nodes resulting from true linkage from accidental foldovers.

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Erratum

In the article 'Embryonic lethal mutation in mice induced by retrovirus insertion into the α1(I) collagen gene' by Angelika Schnieke, Klaus Harbers and Rudolf Jaenisch, *Nature* **304,** 315–320 (1983), on page 317, Figure 3*B* and Figure 2*B* were transposed.