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
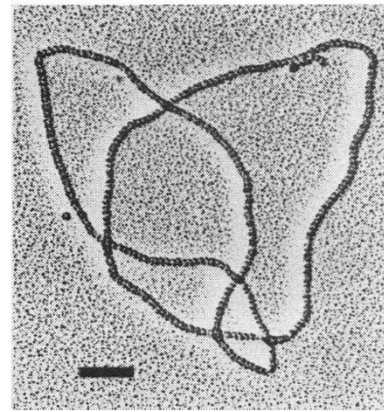

Fig. 3 Electron micrograph of a figure-8 catenane produced by Tn3 resolvase. 225 μg of plasmid p51A[2], containing directly repeated copies of the recombination site, were treated with resolvase and nicked with DNase I in the presence of ethidium bromide as described earlier[9]. The products were separated 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.