Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5497–5501, July 1989
Genetics

Dysfunction of chromosomal loop attachment sites: Illegitimate recombination linked to matrix association regions and topoisomerase II

(nuclear organization/immunoglobulin genes/mutations/nonhomologous recombination/chromatin)

ANN O. SPERRY*, VERONICA C. BLASQUEZ*, AND WILLIAM T. GARRARD†

Department of Biochemistry, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235

Communicated by James Bonner, April 3, 1989

ABSTRACT **A family of A+T-rich sequences termed MARs ("matrix association regions") mediate chromosomal loop attachment. Here we demonstrate that several MARs both specifically bind and contain multiple sites of cleavage by topoisomerase II, a major protein of the mitotic chromosomal scaffold. Interestingly, "hotspots" of enzyme cutting occur within the MAR of the mouse immunoglobulin κ-chain gene at the breakpoint of a previously described chromosomal translocation. Since topoisomerase II can mediate illegitimate recombination in prokaryotes, we explored further the possibility that MARs might be targets for this process in eukaryotes. We found that a MAR had been deleted from one of the two rabbit immunoglobulin κ-chain genes and that MARs reside next to a long interspersed repetitive element within the recombination junction of a human ring chromosome 21. These results, taken together with other accounts of nonhomologous recombination, lead to the proposal that a dysfunction of MARs is illegitimate recombination.**

Just as in prokaryotic chromosomes (1), DNA within eukaryotic interphase nuclei and mitotic chromosomes is organized into topologically constrained looped domains averaging 50–100 kilobases (kb) in length (2, 3). To search for DNA sequences that mediate chromosomal loop attachment, we developed an *in vitro* assay that localizes MARs ("matrix association regions") within cloned genes (4). This approach can be complemented by a nuclear "halo" mapping procedure (5), which uses nuclear fractionation of endogenous sequences to identify "scaffold-attached regions"(SARs) (6). Significantly, both assays identify the same fundamental class of anchorage sequences (4, 7, 8). MARs (or SARs) are at least 200 base pairs (bp) long, are A+T-rich, contain topoisomerase II (Topo II) consensus sequences, sometimes reside near cis-acting regulatory sequences, and are evolutionarily conserved, and their nuclear binding sites are abundant (>10,000 per mammalian nucleus) (4, 6, 7, 9–14).

Particularly intriguing is the presence of Topo II consensus sequences within MARs (4, 11, 15). This protein is a component of the mitotic chromosomal scaffold (16) and certain nuclear matrix preparations (17) and appears to be required both for *in vitro* mitotic chromosome condensation and nuclear assembly (18). Thus, it was initially thought that Topo II may be primarily responsible for chromosomal loop organization through direct physical interaction with MAR sequences, not only in the mitotic chromosome but also in the interphase nucleus (4). More recently, however, it has been shown that significant levels of Topo II are present only in dividing and not in resting somatic cells (19, 20), yet chromosomal loops and MAR sequence binding sites are abundant in nuclei of quiescent cells (4, 8, 10). Furthermore, the *Drosophila* Topo II consensus sequence of Sander and Hsieh (15), with which MARs share homology, may not necessarily predict the actual cutting sites in mammalian DNA (21). These important observations led us to investigate whether MARs do in fact specifically interact with Topo II. We demonstrate for the two MARs studied here that these elements do indeed specifically bind Topo II.

Besides the structural role of Topo II in mitotic chromosomes (16), the enzyme also serves key dynamic functions in both the relaxation of supercoiled DNA domains (22, 23) and the decatenation of intertwined DNA loops (24). However, these catalytic roles may provide a mutational load on the genome because Topo II can introduce double-stranded DNA breaks under adverse conditions (25). Furthermore, the eukaryotic enzyme can catalyze nonhomologous recombination *in vitro* (26), and the prokaryotic counterparts, DNA gyrase, and phage T4 Topo II, probably mediate illegitimate recombination *in vivo* (27–30). Since MARs appear to be the natural sites of action of this enzyme in living cells, we were prompted to explore the possible association of MARs with recombinogenic regions. Significantly, we demonstrate here that the *in vivo* breakpoint of a previously described chromosomal translocation (31) corresponds to a "hotspot" of *in vitro* DNA cleavage by Topo II within the MAR of the mouse immunoglobulin κ-chain gene. Furthermore, we found that a MAR has been deleted during rabbit immunoglobulin κ-chain gene evolution (32) and that MARs reside at the recombination junction of a human ring chromosome 21 adjacent to a long interspersed repetitive element (LINE) (33). These results provide evidence for a dysfunction of MARs in illegitimate recombination, since these sequences are sometimes found at points of DNA insertion, deletion, and translocation.

MATERIALS AND METHODS

DNA Binding Assays. *Drosophila* and HeLa Topo II were purified to homogeneity (34, 35). The binding reaction buffer was 10 mM Tris·HCl, pH 7.9/100 mM NaCl/10 mM $MgCl_2$/50 μg of bovine serum albumin per ml/1.25 mM ATP (36). *Drosophila* enzyme binding reactions contained 150 ng of Topo II, 5 ng of ^{32}P-end-labeled restriction fragments, and 10–50 μg of *Escherichia coli* DNA per ml in a final volume of 20 μl. Reaction mixtures were incubated for 15 min at 30°C, glycerol was added to 10% (vol/vol), and samples were separated by using 4% polyacrylamide gels with 45 mM Tris/45 mM borate/1 mM EDTA buffer. HeLa enzyme-binding reaction mixtures contained 150 ng of Topo II, 15 ng

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MAR, matrix association region; Topo II, topoisomerase II; SAR, scaffold-attached region; LINE, long interspersed repetitive element.

*These authors contributed equally to this investigation.
†To whom reprint requests should be addressed.

of ^{32}P-end-labeled restriction fragments, and 10–100 μg of *E. coli* competitor DNA per ml in 50 μl. Complexes were collected on nitrocellulose filters (37), and the bound DNA was eluted and electrophoretically analyzed (10). Nuclear matrices, isolated from MPC-11 mouse plasmacytoma cells, were incubated with labeled probes in the presence of competitor DNA (10).

Mapping Topo II Cleavage Sites. Reactions mixtures contained 150 ng of enzyme and 10,000 cpm of uniquely end-labeled DNA fragment (about 5 ng) in 20 μl of buffer (10 mM Tris·HCl, pH 7.9/50 mM NaCl/50 mM KCl/0.1 mM EDTA/15 μg of bovine serum albumin per ml) with 10 mM $MgCl_2$, 1.25 mM ATP, and 50 μM VM26 (38). Reactions incubated for 6 min at 30°C were terminated by addition of 2 μl of 10% sodium dodecyl sulfate, 1 μl of 250 mM EDTA, and 2 μl of proteinase K at 1 mg/ml and were incubated for a further 30 min at 37°C. Nuclear extracts of J558L mouse plasmacytoma cells (39) were similarly incubated (21). Purified cleavage products were resolved on sequencing gels (40). Uniquely end-labeled DNA was prepared by digestion of pBSMAR3.1 with *Xba* I (or *Ava* II), followed by 5′-end-labeling with [^{32}P]ATP and polynucleotide kinase. After digestion with *Xho* I (or *Hin*dIII), fragments were gel purified.

Recombinant DNA Clones. Recombinant plasmid pBSMAR-3.1 contains the 343-bp *Hin*dIII–*Dra* I fragment of pG19/45 (4) inserted into the *Hin*dIII and *Hin*cII sites of Bluescript M13. Rabbit κ_1- and κ_2-chain gene segments correspond to *Sst* I fragments of 5.4 and 3.1 kb, respectively, from the joining–constant intron region of the b_4 allotype (32) inserted into a pBR322 derivative. Plasmid pR21BP consists of a 3.8-kb *Eco*RI–*Hin*dIII fragment encompassing the recombination junction of a human ring chromosome 21 inserted into pBR322 (33).

RESULTS

Sequences That Specifically Bind Topo II Include MARs. To determine whether MARs specifically bind Topo II, we selected for study two previously well-characterized genetic loci: the *Drosophila* histone gene repeat, which contains a MAR within the nontranscribed spacer between the *H1* and *H3* genes (4, 5), and the mouse immunoglobulin κ-light-chain gene that contains a MAR within an intron adjacent to an enhancer (4) (Fig. 1*E*). We utilized gel retardation and nitrocellulose membrane filtration assays to study DNA binding to enzymes purified to homogeneity from *Drosophila* embryos and HeLa cells, respectively. Since we found that DNA–Topo II complexes did not enter polyacrylamide gels, the gel retardation assay actually relied on band disappearance. Incubation of ^{32}P-labeled restriction fragments derived from pDhis with Topo II revealed that the 1.76- and 0.66-kb fragments specifically bound the enzyme (see arrows in Fig. 1 *A* and *B*). Importantly, these fragments mapped to the only two regions within the locus that are preferentially cleaved by Topo II *in vitro* (41); the 0.66-kb *Eco*RI–*Hin*fI fragment corresponds to the single MAR of the entire histone gene repeat (Fig. 1*E*).

A 999-bp *Ava* II fragment of the recombinant plasmid pG19/45 specifically bound Topo II (see arrows in Fig. 1 *C* and *D*). Significantly, 335 bp of this fragment corresponded to the single MAR identified within some 20 kb analyzed of the mouse immunoglobulin κ-chain gene locus (4), while the remaining 664 bp contained pBR322 sequences, which exhibited only secondary binding (data not shown). We conclude that these MARs contain evolutionarily conserved Topo II binding sites, but that not all fragments that specifically bind the protein are MARs (eg., the 1.76-kb *Hin*fI pDhis sequence).

"Hot Spots" of Topo II Cleavage Within the Mouse Immunoglobulin κ-Chain Gene MAR Correspond to the Breakpoint of a Chromosomal Translocation. Since MARs specifically bind Topo II, these elements may be targets for rare illegitimate recombination events via aberrant Topo II cleavage (see the Introduction). Although sequence information has been reported for many recombination junctions, the sites of DNA cleavage by Topo II within any MAR have not been previously determined. To investigate these issues, we selected for study the mouse κ-chain gene MAR, since several chromosomal translocations have occurred within this element and the recombination junctions have been characterized (31, 42). Enzymes from *Drosophila*, calf, and mouse each cut within the MAR at many identical positions (Fig. 2*A*), although *Drosophila* Topo II produced additional unique cleavage products (asterisks in Fig. 2*B*). The nucleotide sequences of 15 major cutting sites (sites 1–15 in Fig. 2*B*) bear little homology to each other or to the *Drosophila* consensus cleavage sequence (15) but often occurred near, although not precisely at, the sites predicted by the consensus (Fig. 2*B*). Nevertheless, it is significant that the κ-chain gene MAR contains multiple Topo II cleavage sites that are recognized identically by evolutionarily distant enzymes.

Comparison of the sequences at these Topo II cleavage sites with those located at the breakpoints of previously reported chromosomal translocations revealed a particularly striking relationship. Sites 1–4 occur within the same 14-bp region that was deleted in the plasmacytoma PC7183, in which an imperfect reciprocal chromosomal translocation occurred (31). Furthermore, the 5′-recombination junction

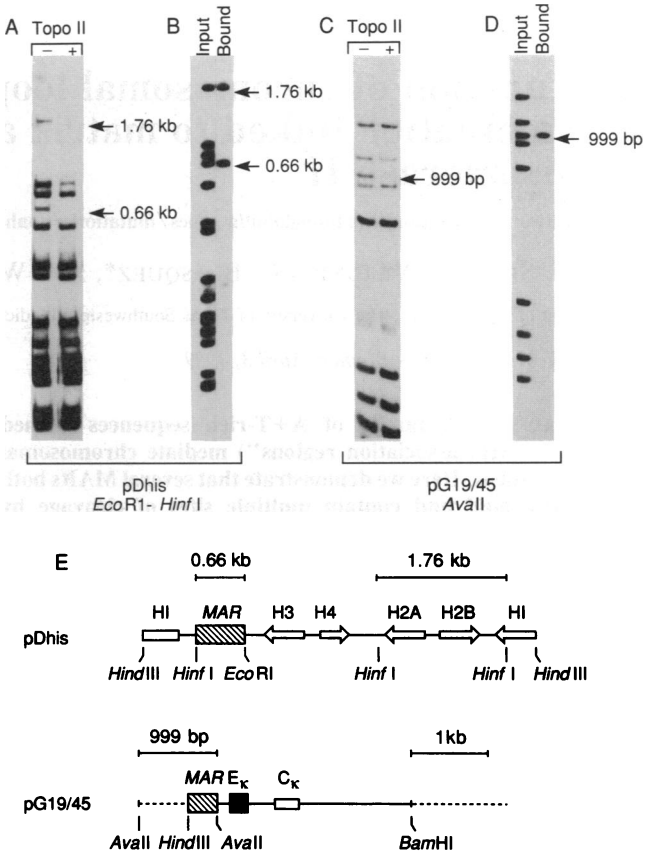

FIG. 1. MAR sequences specifically bind Topo II. (*A* and *C*) ^{32}P-end-labeled restriction fragments incubated with (+) or without (−) *Drosophila* Topo II. Arrows indicate fragments that do not enter gels after binding Topo II. (*B* and *D*) Pure HeLa cell Topo II was incubated with ^{32}P-end-labeled restriction fragments, and protein-bound DNA collected on nitrocellulose filters was separated electrophoretically. Arrows indicate fragments that preferentially bind Topo II. (*E*) Sequences shown include fragments that bind Topo II (⊢), MARs (hatched rectangles), flanking vector sequences attached to fragments that bind (broken lines), transcription units (open arrows), enhancer (E_κ), and constant region exon (C_κ).

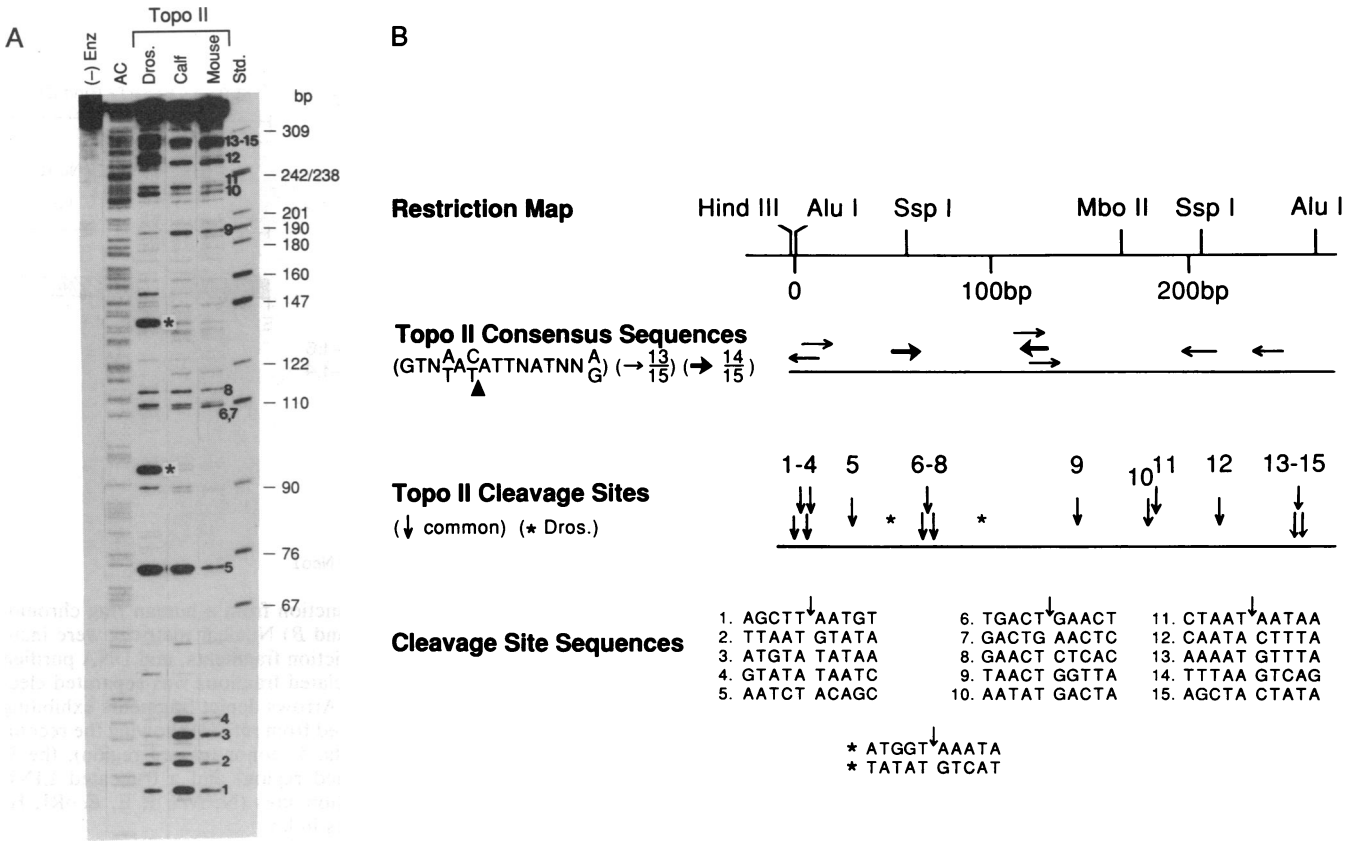

FIG. 2. Positions of Topo II cleavage within the mouse κ-chain gene MAR. (*A*) A uniquely ^{32}P-end-labeled DNA fragment encompassing the MAR was subjected to cleavage at adenine and cytosine (lane AC) or with Topo II from the indicated sources. Samples were separated on a sequencing gel. (*B*) Location of Topo II consensus sequences (15) and the actual sequences at Topo II cleavage sites. The numbers 1–15 refer to selected cleavage sites, while the asterisks depict products specific to the *Drosophila* enzyme. Cleavage sites within the sequence (43) were determined by using fragments labeled at either end. Enz, enzyme; Std., standard.

occurs exactly at the sequence specified by site 2 (31). Interestingly, this "hot spot" also contains two overlapping Topo II consensus sequences (Fig. 2*B*). Therefore, it is possible that illegitimate recombination may occur within MARs via aberrant Topo II cleavage *in vivo*. We explored this possibility further as described below.

Deletion of a MAR Occurred During the Evolution of the Rabbit Immunoglobulin κ-Chain Genes. The rabbit immunoglobulin κ-chain gene locus contains two linked constant regions, termed κ_1 and κ_2, that are believed to have originated by a gene duplication event. The κ_2 gene differs from κ_1 by three deletions (32), two of which overlap a region predicted to contain a MAR (4) (Fig. 3*E*, arrows). To directly test the "MAR deletion hypothesis," we performed nuclear matrix binding assays using restriction fragments of recombinant plasmids carrying these rabbit sequences. In response to increasing unlabeled competitor DNA, a 3.66-kb *Sst* I–*Pvu* II fragment was preferentially retained by matrices relative to other ^{32}P-labeled rabbit or vector sequences (arrow in Fig. 3*A*). This fragment is derived from the joining–constant region of the κ_1 gene (Fig. 3*E*). Significantly, a 3.1-kb fragment encompassing the corresponding region from the κ_2 gene did not specifically bind to matrices and therefore lacks a MAR (Fig. 3*B*). The MAR within the κ_1 gene resides on 1.16-kb *Sst* I–*Xmn* I and 667-bp *Nco* I fragments (arrows in Fig. 3 *C* and *D*, respectively). As summarized in Fig. 3*E*, the common overlapping segments between these two fragments define the boundaries of the MAR. The element is localized just upstream of the enhancer, in the same relative position as in the mouse κ-chain gene (4). Therefore, during the evolutionary divergence of the rabbit κ_1 and κ_2 genes, the MAR was deleted from the primordial κ_2 gene.

The Recombination Junction of a Human Ring Chromosome 21 Contains MARs Separated by a LINE. Wong *et al.* (33) have cloned and sequenced the recombination junction of a human ring chromosome 21 along with the corresponding normal DNA segments. Their analysis revealed that the breakage and reunion sites are surrounded by Topo II consensus sequences and certain A+T-rich sequence motifs that share homology with MAR consensus sequences (4). To directly test whether MARs are located within these regions, we performed nuclear matrix binding assays using the recombinant plasmid pR21BP, which carries the junction fragment of this ring chromosome (Fig. 4*C*). A 3.8-kb *Eco*RI–*Hin*dIII fragment that spans the recombination site exhibited specific binding (arrow in Fig. 4*A*). This fragment contains two MARs (arrows in Fig. 4*B*)—a 1.6-kb *Eco*RI–*Nco* I fragment that spans the junction and a 1.4-kb *Nco* I–*Hin*dIII fragment that resides on the 3′ side of the recombination site (Fig. 4*C*). These MARs are separated by a 750-bp region that contains the 3′ end of a LINE (33), which resides on a 0.8-kb *Nco* I fragment that does not exhibit significant binding (Fig. 4 *B* and *C*). Since LINES can be considered to be markers of DNA damaging events (44), this region may have consisted earlier of a single MAR, which in turn became fragmented. Importantly, in additional studies we have found that the 5′ side of the recombination junction and the corresponding region from normal DNA lack MARs (data not shown). Thus, in the case of this recombination event, only one of the two donor fragments possessed a MAR.

DISCUSSION

Topo II and Chromosomal Loop Attachment at MARs. We demonstrate for the two MARs studied here that these

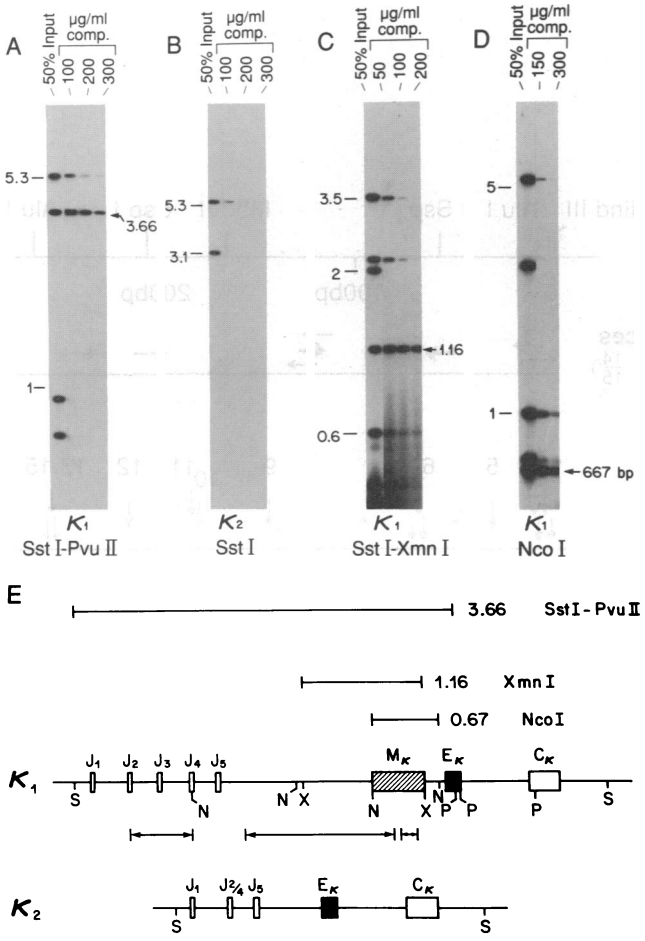

FIG. 3. Rabbit immunoglobulin κ_2-chain gene lacks a MAR. (*A–D*) Nuclear matrices were incubated with ^{32}P-end-labeled restriction fragments, and DNA purified from input and matrix-associated fractions was separated electrophoretically; sizes are shown in kb. The concentrations of *E. coli* competitor (comp.) DNA are indicated at the top of lanes. Arrows depict fragments exhibiting specific binding. (*E*) MAR-containing restriction fragments are depicted by the lines shown above the κ_1-chain gene map; numbers indicate size in kb. Sequences shown include joining region (*J*), MAR (M_κ), enhancer (E_κ), constant region exon (C_κ), and the restriction sites, *Sst* I (S), *Nco* I (N), *Xmn* I (X), and *Pvu* II (P). The major regions absent from the κ_2 gene but present in the κ_1 gene are depicted by the three arrows below the κ_1 map (adapted from ref. 32).

elements specifically bind Topo II. The sequence determinants of Topo II binding and cleavage appear to be evolutionarily conserved, since similar results were obtained by using enzymes from a Dipteran insect and mammals. Furthermore, a SAR-containing intergenic region of a *Drosophila* hsp70 gene prefers to bind this enzyme relative to gene coding sequences (45). These and other MARs with high affinity for Topo II are probably sequestered by this protein at the bases of the DNA loops in metaphase chromosomes, since the enzyme is a major component of the mitotic chromosomal scaffold (16). We also found that sequences that are not MARs can exhibit marked binding specificity (Fig. 1 *A* and *B*) and that not all MARs strongly bind the enzyme [the 3′ MAR of pEH (ref. 10; unpublished results)]. Therefore, although Topo II binding sites may be generally associated with MAR sequences, their occurrence is nevertheless neither a necessary nor sufficient condition to specify a MAR. In addition, since interphase nuclei of resting cells have MAR sequence binding sites (4, 8, 10) but apparently lack Topo II (19, 20), proteins other than this enzyme likely participate in nuclear loop attachment.

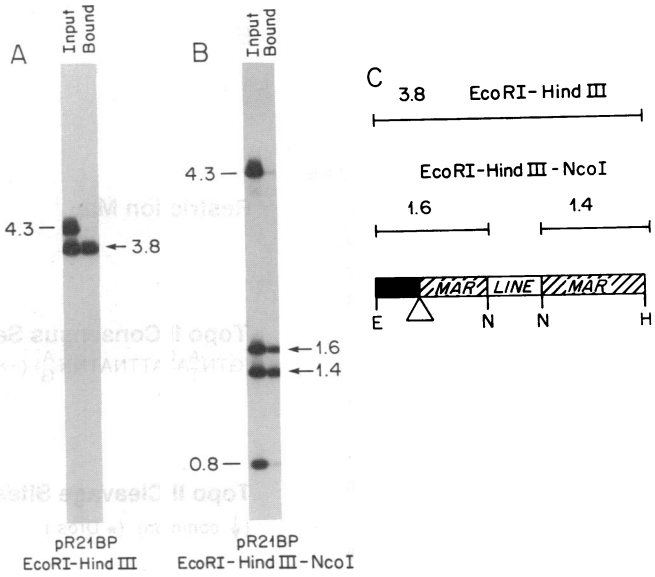

FIG. 4. A recombination junction from a human ring chromosome 21 possesses MARs. (*A* and *B*) Nuclear matrices were incubated with ^{32}P-end-labeled restriction fragments, and DNA purified from the input and matrix-associated fractions was separated electrophoretically; sizes are in kb. Arrows depict fragments exhibiting specific binding. (*C*) Map (adapted from ref. 33) showing the recombination joint (open triangle), the 5′ donor (closed region), the 3′ donor containing MARs (hatched region) and a truncated LINE (open regions), and key restriction sites (N, *Nco* I; E, *Eco*RI; H, *Hin*dIII). Numbers indicate sizes in kb.

Topo II and Illegitimate Recombination at MARs. Illegitimate recombination breakpoints do not necessarily occur at random positions in genomes. Fragmentation sites of at least one of the recombination partners have sometimes been correlated with LINES (44), short interspersed repetitive elements (e.g., *Alu* sequences) (46), topoisomerase I sites (47), and in B or T cells, with switch and variable–diversity–joining region recombination sequences or their corresponding homologs (48). However, the mechanisms of breakage at many sites still remain puzzling. Since those MARs that possess high affinity for Topo II likely interact with the enzyme in living cells, it is logical to predict that these elements may also be targets for illegitimate recombination, particularly in view of the elegant studies performed in prokaryotic systems (see the Introduction).

We have localized MARs at sites of chromosomal DNA insertion, deletion, and translocation. Sequence analysis of the reciprocal products of the chromosomal translocation that occurred in the plasmacytoma PC7183 reveals that only 14 bp was deleted from the κ-chain gene MAR (31), and we have found that this region has four strong Topo II cleavage sites as well as two overlapping consensus sequences. Furthermore, cleavage site 2 corresponds exactly to the sequence at the 5′ junction of the recombination. In the rabbit system, a 160-bp stretch corresponding to the smallest deleted region from the primordial κ_2-chain gene (Fig. 3*E*) is missing from the κ_1-chain gene in certain strains (49). This region within the MAR possesses two Topo II consensus sequences and is cleaved by the enzyme *in vitro* (unpublished results). Therefore, we suspect that Topo II cleavage was instrumental in triggering alterations in both the mouse and rabbit κ-chain immunoglobulin genes.

Chromosome 21 MARs Are Only at One Side of the Recombination Junction. We found MARs at only one of the two parental donor regions that led to the recombination joint in a human ring chromosome 21. The subunit exchange model, based upon the ability of Topo II to mediate illegitimate recombination *in vitro*, predicts that the enzyme would be

responsible for DNA cleavage at each of the two breakpoints to be fused at the recombination junction (26). Since we found that not all sequences that strongly interact with Topo II are MARs, it is possible that the combined actions of Topo II cutting at non-MAR and MAR sequences could lead to recombination via this proposal. Another possibility is that Topo II cleavage may occur at only one of the two recombination breakpoints, since DNA ends are recombinogenic (50). Finally, not all strand breaks at MARs may be linked to Topo II, since another breakpoint within the mouse κ-chain gene MAR does not map at Topo II sites (42). Anchorage sites might also be particularly sensitive to shear forces imposed by the dynamics of the connected DNA loops or to cleavage by other enzyme systems.

Other Examples of MARs at Sites of Recombination. Several previously characterized deletions within the human β-globin gene locus are bordered on one side by the recently identified MAR that resides within the second intron of the β-globin gene (51, 52). Interestingly, in Chinese hamster cells, a MAR occurs at the junction between units of amplification of the dihydrofolate reductase gene (53), and presumptive MARs reside in the junctions of the amplified adenylate deaminase gene (54). Therefore, it seems clear that MARs constitute one class of sequences that are targets for illegitimate recombination, supporting the earlier proposals that chromosomal loop attachment sites might be associated with nonhomologous recombination (55, 56).

We thank Drs. R. Hancock, L. F. Liu, and N. Osheroff for Topo II; M. E. Gaulden for VM26; S. E. Antonarakis, E. E. Max, and C. Wong for recombinant DNA clones; L. F. Liu, R. G. Mage, N. Osheroff, E. F. Vanin, and M. Xu for stimulating discussions; and S.-Y. Huang for technical assistance. These studies were supported by Grants GM22201, GM29935, and GM31689 from the National Institutes of Health, and Grant I-823 from the Robert A. Welch Foundation.

1. Worcel, A. & Burgi, E. (1972) *J. Mol. Biol.* **71,** 127–147.
2. Benyajati, C. & Worcel, A. (1976) *Cell* **9,** 393–407.
3. Paulson, J. R. & Laemmli, U. K. (1977) *Cell* **12,** 817–828.
4. Cockerill, P. N. & Garrard, W. T. (1986) *Cell* **44,** 273–282.
5. Mirkovitch, J., Mirault, M. E. & Laemmli, U. K. (1984) *Cell* **39,** 223–232.
6. Gasser, S. M. & Laemmli, U. K. (1986) *Cell* **46,** 521–530.
7. Izaurralde, E., Mirkovitch, J. & Laemmli, U. K. (1988) *J. Mol. Biol.* **200,** 111–125.
8. Phi-Van, L. & Stratling, W. H. (1988) *EMBO J.* **7,** 655–664.
9. Cockerill, P. N. & Garrard, W. T. (1986) *FEBS Lett.* **204,** 5–7.
10. Cockerill, P. N., Yuen, M.-H. & Garrard, W. T. (1987) *J. Biol. Chem.* **262,** 5394–5397.
11. Gasser, S. M. & Laemmli, U. K. (1986) *EMBO J.* **5,** 511–518.
12. Amati, B. B. & Gasser, S. M. (1988) *Cell* **54,** 967–978.
13. Mirkovitch, J., Spierer, P. & Laemmli, U. K. (1986) *J. Mol. Biol.* **190,** 255–258.
14. Mirkovitch, J., Gasser, S. M. & Laemmli, U. K. (1988) *J. Mol. Biol.* **200,** 101–109.
15. Sander, M. & Hsieh, T. S. (1985) *Nucleic Acids Res.* **13,** 1057–1072.
16. Earnshaw, W. C. & Heck, M. M. S. (1985) *J. Cell Biol.* **100,** 1716–1725.
17. Berrios, M., Osheroff, N. & Fisher, P. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4142–4146.
18. Newport, J. & Spann, T. (1987) *Cell* **48,** 219–230.
19. Heck, M. M. S. & Earnshaw, W. C. (1986) *J. Cell Biol.* **103,** 2569–2581.
20. Fairman, R. & Brutlag, D. L. (1988) *Biochemistry* **27,** 560–565.
21. Darby, B. K., Herrera, R. E., Vosberg, H.-D. & Nordheim, A. (1986) *EMBO J.* **5,** 2257–2265.
22. Brill, S. J. & Sternglanz, R. (1988) *Cell* **54,** 403–411.
23. Giaver, G. N. & Wang, J. C. (1988) *Cell* **55,** 849–856.
24. Holm, C., Goto, T., Wang, J. C. & Botstein, D. (1985) *Cell* **41,** 553–563.
25. Gaulden, M. E. (1987) *Mutagenesis* **2,** 357–365.
26. Bae, Y. S., Kawasaki, I., Ikeda, H. & Liu, L. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2076–2080.
27. Ikeda, H., Kawasaki, I. & Gellert, M. (1984) *Mol. Gen. Genet.* **196,** 546–549.
28. O'Connor, M. B. & Malamy, M. H. (1985) *J. Mol. Biol.* **181,** 545–550.
29. Ikeda, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 922–926.
30. Ripley, L. S., Dubins, J. S., deBoer, J. G., DeMarini, D. M., Bogerd, A. M. & Kreuzer, K. N. (1988) *J. Mol. Biol.* **20,** 665–678.
31. Shapiro, M. A. & Weigert, M. (1987) *Mol. Cell. Biol.* **7,** 4130–4133.
32. Emorine, L. & Max, E. E. (1983) *Nucleic Acids Res.* **11,** 8877–8890.
33. Wong, C., Kazazian, H. H., Jr., Stetten, G., Earnshaw, W. C., VanKeuren, M. L. & Antonarakis, S. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1914–1918.
34. Shelton, E. R., Osheroff, N. & Brutlag, D. L. (1983) *J. Biol. Chem.* **258,** 9530–9535.
35. Miller, K. G., Liu, L. F. & England, P. T. (1981) *J. Biol. Chem.* **256,** 9334–9339.
36. Trask, D. K., DiDonato, J. A. & Muller, M. T. (1984) *EMBO J.* **3,** 671–676.
37. Berent, S. L. & Sevall, J. S. (1984) *Biochemistry* **23,** 2977–2983.
38. Osheroff, N. & Zechiedrich, E. L. (1987) *Biochemistry* **26,** 4303–4309.
39. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
40. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
41. Udvardy, A., Schedl, P., Sander, M. & Hsieh, T.-S. (1985) *Cell* **40,** 933–941.
42. Perlmutter, R. M., Klotz, J. L., Pravtcheva, D., Ruddle, F. & Hood, L. (1984) *Nature (London)* **307,** 473–476.
43. Max, E. E., Maizel, J. V., Jr., & Leder, P. (1981) *J. Biol. Chem.* **256,** 5116–5120.
44. Singer, M. F. & Skowronski, J. (1985) *Trends Biochem. Sci.* **10,** 119–122.
45. Sander, M., Hsieh, T.-S., Udvardy, A. & Schedl, P. (1987) *J. Mol. Biol.* **194,** 219–229.
46. Lehrman, M. A., Schneider, W. J., Sudhof, T. C., Brown, M. S., Goldstein, J. L. & Russel, D. W. (1985) *Science* **227,** 140–146.
47. Bullock, P., Champoux, J. J. & Botchan, M. (1985) *Science* **230,** 954–958.
48. Croce, C. M. (1987) *Cell* **49,** 155–156.
49. Akimenko, M.-A., Mariame, B. & Rougeon, F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5180–5183.
50. Orr-Weaver, T. L. & Szostak, J. W. (1985) *Microbiol. Rev.* **49,** 33–58.
51. Anand, R., Boehm, C. D., Kazazian, H. H., Jr., & Vanin, E. F. (1988) *Blood* **72,** 636–641.
52. Jarman, A. P. & Higgs, D. R. (1988) *EMBO J.* **7,** 3337–3344.
53. Dijkwel, P. A. & Hamlin, J. L. (1988) *Mol. Cell. Biol.* **8,** 5398–5409.
54. Hydrien, O., Debatisse, M., Buttin, G. & de Saint Vincent, B. R. (1987) *EMBO J.* **6,** 2401–2408.
55. Vanin, E. F., Henthorn, P. S., Kioussis, D., Grosveld, F. & Smithies, O. (1983) *Cell* **35,** 701–709.
56. Feinberg, A. P. & Coffey, D. S. (1982) in *The Nuclear Envelope and Nuclear Matrix*, ed. Maul, G. G. (Liss, New York), pp. 293–305.