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Organization of immunoglobulin heavy chain genes and allelic deletion model

(complementary DNA hybridization/myeloma/recombination of variable and constant region genes)

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ABSTRACT We have assessed the number of times the gene sequence encoding constant regions of mouse immunoglobulin heavy chains γ1, γ2a, and γ3 are represented in the mouse genome by hybridization kinetic analysis. All three genes are present at one copy each per haploid genome in normal tissues and myelomas producing IgM or IgG3. IgG1-producing myelomas, however, contain 1 copy each of the γ1 and γ2a genes and 0.5 copy of the γ3 gene per haploid genome. IgG2b-producing myelomas contain 1 copy of the γ2a gene and 0.5 copy each of the γ1 and γ3 genes per haploid genome. IgG2a-producing myelomas contain 1 copy of the γ2a gene and 0.5 copy each of the γ1 and γ3 genes per haploid genome. In myelomas producing IgA, all three γ genes are represented 0.5 time per haploid genome. In order to account for the results we propose an allelic deletion model: (*i*) The specific deletion of heavy chain constant region genes accompanies the recombination of a variable region gene to a constant region gene. (*ii*) The portion of the chromosome that resides between two joining sequences is excised out of the chromosome. (*iii*) The recombination occurs on one of the alleles. Based on this model we also propose that heavy chain genes are arranged on one chromosome in the following order; variable region genes, unknown spacer sequence, μ, γ3, γ1, γ2b, γ2a, and α.

The genetic control of immunoglobulin synthesis has been a puzzle to molecular biologists as well as to immunologists. The organization of immunoglobulin genes is a key to those unresolved problems unique to immunology, such as the two gene–one polypeptide theory, allelic exclusion, and the origin of antibody diversity.

There are at least seven classes and subclasses of immunoglobulins in the mouse—IgM, IgD, IgG1, IgG2a, IgG2b, IgG3, and IgA—which differ from each other in the amino acid sequences of the constant regions (C) of the heavy (H) chains. Genetic studies have shown that the H chain C genes of the mouse are clustered to such a narrow segment of one chromosome that there have been no recombinants found among 2069 progenies derived from two crosses (1, 2). These C_H genes share a family of variable region (V) genes (3–5) that resides on the same chromosome and is separated from the C_H genes by unknown spacer sequences (6).

Dreyer and Bennett (7) have proposed that V and C are encoded by two separate genes which eventually join to form a single gene. Extensive kinetic studies using probes derived from purified light (L) chain mRNA have produced general agreement in favor of the V–C recombination hypothesis (8–16).

In contrast, there have been few reports of molecular studies on organization of H chain genes (17, 18). This is mainly because purification of H chain mRNA is much more difficult than that of L chain mRNA. Recently we have succeeded in purifying H chain mRNA from mouse myelomas of several subclasses by immunoprecipitation of polysomes (19). Using these probes we have measured the number of H chain gene sequences and shown deletion of H chain c genes in mouse myelomas that produce immunoglobulins of specific classes. Based on these results we will propose an allelic deletion model for the mechanism of V_H–C_H recombination. The H chain c genes are proposed to be aligned on one chromosome in the order V_H, spacer, μ, γ3, γ1, γ2b, γ2a, and α.

MATERIALS AND METHODS

Myeloma tumor lines used in the present study were kindly supplied by M. Potter (National Institutes of Health), T. Kishimoto (Osaka University), M. Kawakami (Kitazato University), and S. Migita (Kanazawa University). Myeloma tumors were propagated and harvested as described (9).

H chain mRNAs were purified from MOPC 31C (IgG1), J606 (IgG3), MPC 11 (IgG2b), and HOPC 1 (IgG2a) myelomas by M. Ono as described (19). The purities of MOPC 31C, HOPC 1, MPC 11, and J606 mRNAs are 97%, 54%, 92%, and 99%, respectively, as determined by hybridization kinetic analysis (9). More detailed characterization will be described elsewhere. Mouse globin and κ L chain mRNAs were prepared from mouse reticulocytes and MOPC 41 myeloma, respectively, as described (9). [^{3}H]cDNAs complementary to mRNAs encoding globin, κ L chain, and H chain were synthesized and purified as described (9, 20). H chain cDNAs derived from MOPC 31C and J606 mRNA are about 1500 bases long; H chain cDNA from HOPC 1 mRNA is about 1800 bases long. The specific radioactivities of globin, κ L chain, and H chain [^{3}H]cDNAs thus prepared were 10, 10, and 16 cpm/pg, respectively. DNAs from tumors and normal tissues were prepared as described (21). DNA was digested twice with RNase (100 μg/ml, 2 hr at 37°) and treated with alkali (0.2 M, 60 min at 37°) before use. DNA was sheared to 800 base-pairs long by sonication.

Hybridization of radioactive probes with cellular DNA was performed as described (9, 14) except that ^{14}C-labeled DNA derived from MOPC 31C myeloma cells was added to all the hybridization reaction mixtures to 610 cpm/mg. The concentrations of cellular DNA, [^{3}H]cDNA, and Na^+ were 9.4 mg/ml, 350 pg/ml, and 1 M, respectively. The hybrids formed were assayed by S1 nuclease digestion.

RESULTS AND DISCUSSION

Characterization of Probes. H chain mRNAs were purified from MOPC 31C (γ1), HOPC 1 (γ2a), MPC 11 (γ2b), and J606

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Abbreviations: V and C, variable and constant regions; H and L chains, heavy and light chains; cDNA, synthetic DNA complementary to mRNA; C_0t value, product of concentration (mol/liter) of nucleotide sequences of DNA and time (sec) of incubation.

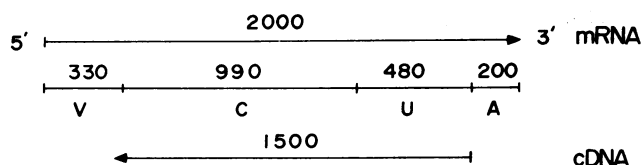

FIG. 1. Diagram of H chain mRNA and its cDNA. V, variable region sequence; C, constant region sequence; U, untranslated sequence; A, poly(A). Numbers are numbers of bases.

(γ3) myelomas by immunoprecipitation of polysomes (19). The mRNA preparations obtained ran as homogeneous bands upon polyacrylamide gel electrophoresis in formamide, with a molecular weight of 700,000 (i.e., 2000 bases).

The cDNA preparations used for the present study were about 1500 bases long as determined by polyacrylamide gel electrophoresis in formamide. Because we did not see any difference in the extent of hybridization of cDNA between homologous and heterologous H chain mRNAs of the identical subclass, the cDNA preparations represent most of the C gene sequences but not of the V gene sequences. A diagram of H chain mRNA and its cDNA is shown in Fig. 1. Nucleotide sequence analyses of MOPC 31C cDNA inserted in plasmids indicated that the untranslated sequence of about 500 nucleotides is present at the 3′ end of mRNA (unpublished data).

Reiteration Frequency of the γ1 Gene. MOPC 31C [^{3}H]-cDNA was hybridized to a vast excess of cellular DNA derived from MOPC 31C myeloma. Reassociation of mouse unique DNA was followed by the addition of a trace amount of ^{14}C-labeled MOPC 31C DNA. All C_0t analyses were carried out in similar double-label experiments under the identical conditions.

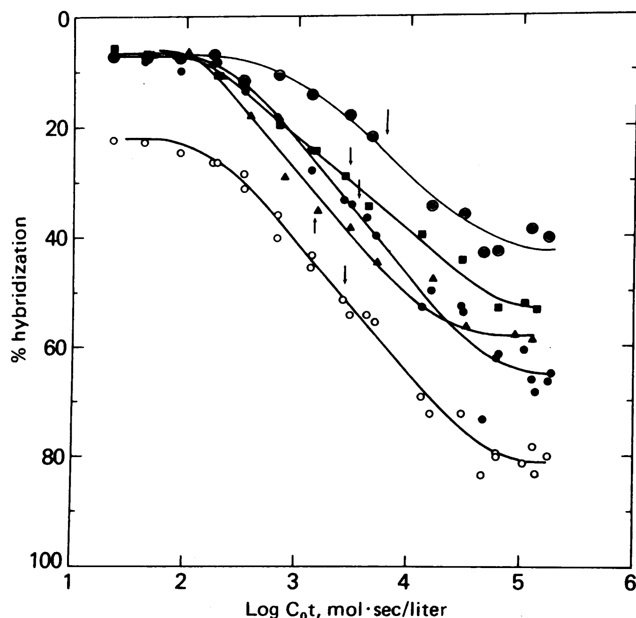

FIG. 2. Hybridization kinetic analyses of MOPC 31C cDNA with total cellular DNA from MOPC 31C (IgG1) and MOPC 511 (IgA) myelomas. MOPC 31C [^{3}H]cDNA was hybridized to MOPC 31C DNA or MOPC 511 DNA. In separate experiments, κ L chain [^{3}H]cDNA and globin [^{3}H]cDNA were hybridized to total cellular DNA from MOPC 31C myeloma; these reactions were carried out as above except that concentrations of globin [^{3}H]cDNA and κ chain [^{3}H]cDNA were 540 and 800 pg/ml, respectively. Hybridization profiles of MOPC 31C [^{14}C]DNA in three experiments were superimposable. Arrows indicate $C_0t_{1/2}$ points. ○, MOPC 31C [^{14}C]DNA; ●, MOPC 31C cDNA with MOPC 31C DNA; ●, MOPC 31C cDNA with MOPC 511 DNA; ■, κ chain cDNA; ▲, globin cDNA.

Table 1. Reiteration frequency of the γ1 gene

Origin of DNA: Myeloma	H chain class produced	Copy/haploid: γ1	Lκ	Globin
MOPC 104E	μ	1.0		
J606	γ3	1.1		
MOPC 31C	γ1	0.79	0.93	1.9
MOPC 70A	γ1	0.82		
MC 101	γ1	1.03		
MPC 11	γ2b	0.42	1.1	
NP 2	γ2b	0.39	1.1	
MOPC 141	γ2b	0.59		
UPC 10	γ2a	0.60		
RPC 5	γ2a	0.56		
MOPC 511	α	0.48		
MOPC 315	α	0.52		
MOPC 41	None	0.53	1.0	1.8
RPC 20	None	0.44		
Liver	—	1.3	1.0	1.9
Spleen	—	1.0		
Kidney	—	0.96		
Thymus	—	1.3		
Newborn mice	—	1.1	1.0	

As shown in Fig. 2, γ1 cDNA hybridized with a $C_0t_{1/2}$ value of 3500, whereas that of mouse unique sequences is 2700. Comparison of these $C_0t_{1/2}$ values yields a reiteration frequency of 0.8 copy per haploid genome for the γ1 gene sequence in MOPC 31C DNA (22). We have tested the congruity of the cDNA·DNA hybrids by thermal stability to S1 nuclease digestion (14). The γ1 cDNA hybrid formed with MOPC 31C DNA showed a sharp melting profile with a t_m value of 90.6°, indicating that congruently matched duplexes were formed. As control experiments, κ L chain cDNA derived from MOPC 41 mRNA and globin cDNA were hybridized to the same batch of MOPC 31C DNA. $C_0t_{1/2}$ values for the κ chain cDNA and globin cDNA were 2900 and 1400, respectively, indicating that the κ chain and globin genes are represented 0.93 and 1.9 times per haploid genome, respectively, in agreement with previous reports (8–14, 21). Similar experiments were carried out with DNA derived from many myelomas and normal tissues. As summarized in Table 1, the γ1 gene sequence is present as 1 copy per haploid genome in IgG1-, IgM-, and IgG3-producing myelomas as well as in normal mouse tissues.

On the other hand, when the γ1 cDNA was hybridized to DNA derived from an IgA-producing myeloma (MOPC 511), the hybridization kinetics were quite different from those obtained for MOPC 31C DNA. As shown in Fig. 2, a $C_0t_{1/2}$ value of 6200 was obtained, compared to 3000 for mouse unique copy DNA (not shown). The results yield a reiteration frequency of 0.48 copy per haploid genome. The extent of hybridization reached approximately 42% which is smaller than the value (65%) obtained with MOPC 31C DNA. The possibility that reduction in the extent of hybridization is due to a partial deletion of γ1 sequence was excluded by the following experiments. First, cDNA preparations of different length (500, 1000, and 1500 bases) gave the identical extent of hybridization. Second, the extent of hybridization reached about 65% when the ratio of DNA to cDNA was doubled.

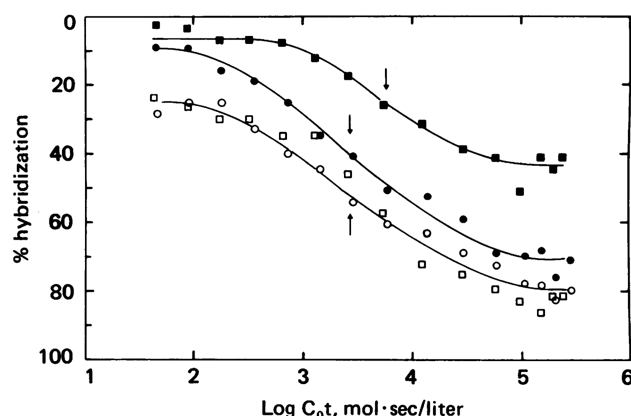

FIG. 3. Hybridization kinetic analyses of J606 cDNA with total cellular DNA from J606 and MOPC 70A myelomas. Arrows indicate $C_0t_{1/2}$ points. ○, J606 [^{14}C]DNA; ●, J606 [^{3}H]cDNA with J606 DNA; □, MOPC 70A [^{14}C]DNA; ■, J606 [^{3}H]cDNA with MOPC 70A DNA.

In nine lines of myeloma producing IgG2a, IgG2b, IgA, or Bence Jones protein, the number of γ1 gene copy ranged from 0.39 to 0.60 (Table 1). The results indicate that the average number of the γ1 gene copy is reduced to 0.5 copy per haploid genome in these myelomas. Our technique is sensitive enough to distinguish 1 copy per haploid from 2 copies per haploid because the number of gene copies encoding globin and κ chain remained constant regardless of the reduction in the γ1 gene copy. Obviously, the results can be interpreted in two ways. One possibility is that chromosomal abnormalities in these myelomas happen to have decreased the apparent reiteration frequency of the γ1 gene (23). The alternative possibility, which we prefer, is that the γ1 gene is specifically deleted from one of the alleles in mouse myelomas that produce immunoglobulins of the defined classes (i.e., IgG2a, IgG2b, and IgA).

Reiteration Frequency of the γ3 Gene. In order to test the possibilities mentioned above, we assessed the number of times the gene sequences corresponding to the γ3 cDNA probe are represented in various myelomas by hybridization kinetic analysis. Typical examples of such experiments are shown in Fig. 3, in which J606 [^{3}H]cDNA was hybridized to a vast excess of cellular DNA derived from J606 (IgG3) and MOPC 70A (IgG1) myelomas, respectively. The results are quite clear. The $C_0t_{1/2}$ values for DNAs from J606 and MOPC 70A are 2700 and 5800, respectively, compared to 2700 for unique copy mouse DNA. These values are equivalent to reiteration frequencies of 1.0 and 0.46 per haploid genome for DNAs from J606 and MOPC 70A, respectively.

Similar experiments were carried out with DNAs derived from 11 other myelomas and 3 tissues (Table 2). IgM- and IgG3-producing myelomas, as well as normal tissues, contain 1 copy of the γ3 gene sequence per haploid genome whereas IgG1-, IgG2a-, IgG2b-, and IgA-producing myelomas contain only 0.5 copy per haploid genome. The maximal extents of hybridization decreased in concordance with reduction in the reiteration frequency. It is worth emphasizing that IgG1-producing myelomas MOPC 31C, MOPC 70A, and MC 101 have lost 0.5 copy of the γ3 gene sequence per haploid genome. Nevertheless, they retain 1 copy of the γ1 gene sequence per haploid. Because both γ1 and γ3 gene sequences have been shown to be located on a small segment of one chromosome, it is unlikely that the reduction in the number of the γ3 gene copy is attributable to general chromosomal abnormalities in these myelomas.

Table 2. Reiteration frequency of the γ3 and γ2a genes

Origin of DNA			
Myeloma	H chain class produced	Copy/haploid γ3	Copy/haploid γ2a
MOPC 104E	μ	0.85	0.94
J606	γ3	1.0	1.20
FlOPC 21	γ3	0.82	0.93
MOPC 31C	γ1	0.67	1.13
MOPC 70A	γ1	0.52	—
MC 101	γ1	0.54	—
MPC 11	γ2b	0.52	1.36
NP 2	γ2b	0.5	—
MOPC 141	γ2b	0.63	—
UPC 10	γ2a	0.52	1.2
RPC 5	γ2a	0.68	—
HOPC 1	γ2a	—	1.2
MOPC 511	α	0.46	0.56
MOPC 315	α	0.37	0.60
Liver	—	1.18	—
Spleen	—	—	1.0
Kidney	—	0.88	1.0
Newborn mice	—	0.9	—

Reiteration Frequency of the γ2a and γ2b Genes. Determination of the copy number of the γ2 gene represented in the myeloma genome provided further evidence for the specific deletion of the γ genes in mouse myelomas (Table 2). Approximately 1 copy of the γ2a gene sequence is present per haploid genome in DNAs derived from normal tissues and myelomas producing IgM, IgG3, IgG1, IgG2b, and IgG2a. Only 0.5 copy of the γ2a gene is present per haploid genome in DNA derived from myelomas producing IgA.

Preliminary results indicate that the γ2b gene is represented once per haploid genome in DNAs derived from spleen and myelomas producing IgM, IgG3, IgG1, and IgG2b whereas it is reduced to approximately 0.5 copy per haploid genome in DNAs from myelomas producing IgA.

Summary of γ Gene Deletions. Taking all these results together, we are inclined to conclude that the reduction in the number of the γ gene copy in mouse myelomas is due to the specific deletion of the particular genes from one of the alleles. A summary of our interpretation is shown in Table 3. Assuming that the H chain genes are arranged on a chromosome in the order V_H genes, spacer sequence, μ gene, γ3 gene, γ1 gene, γ2b gene, γ2a gene, and α gene, a whole set of the results can be easily explained by deletion of a chromosomal segment from one of the alleles. Apparently, the deletion starts next to the C_H gene expressed in each myeloma, suggesting that the deletion

Table 3. Summary of deletion of γ genes

Origin of DNA	Copy/haploid γ3	γ1	γ2b	γ2a
IgM producer	1	1	1	1
IgG3 producer	1	1	1	1
IgG1 producer	0.5	1	1	1
IgG2b producer	0.5	0.5	1	1
IgG2a producer	0.5	0.5	0.5	1
IgA producer	0.5	0.5	0.5	0.5
Nonlymphatic tissues	1	1	1	1

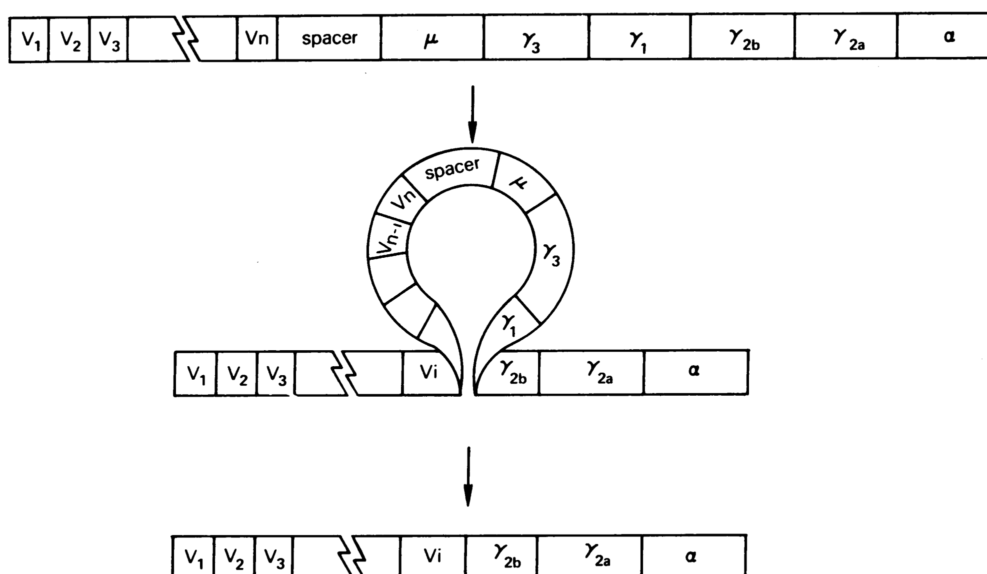

FIG. 4. Allelic deletion model for V_H–V_C recombination. The diagram shows recombination of a V_i sequence with the γ2b gene, resulting in deletion of the μ, γ3, and γ1 genes.

of the C_H genes is related to expression of the remaining C_H gene.

Allelic Deletion Model. In order to put all these results together, we propose a model that we call the "allelic deletion model." We assume that the specific deletion of C_H gene sequences accompanies the V–C gene recombination which takes place only on one of the alleles, as shown in Fig. 4. Upon joining of the V_H and C_H gene sequences the portion of the chromosome that resides between the two sequences is excised out of the chromosome. The deletion of the γ genes in myelomas producing the specific classes of immunoglobulin reflects the relative location of the class-specific C_H genes that have undergone recombination with a V_H gene. Given the deletion profile shown in Table 3, it is possible to assume that C_H genes are arranged in the order (μ, γ3), γ1, γ2b, γ2a, and α genes. It is also reasonable to put the μ gene between the V_H sequences and the γ3 sequence. Otherwise, the γ3 gene should be deleted in the IgM-producing myeloma.

We think that a given V_H gene recombines successively with different C_H genes during immunodifferentiation of a single lymphocyte. Inasmuch as C_H gene deletion accompanies the V–C recombination, differentiation proceeds irreversibly from an IgM-producer to an IgG- or IgA- producer according to a linear arrangement of C_H genes. We have no information concerning the location of the δ gene because there has been no IgD-producing mouse myeloma available. We speculate that the δ gene is located between the μ gene and the γ3 gene because the δ gene is expressed prior to the γ genes but after the μ gene during the course of differentiation (24, 25). But we reserve the possibility that the δ gene is an exception to the model.

The proposed order of C_H genes is compatible with recent genetic studies using rabbit allotypes (26–28), which indicate that the μ gene is closest to the V_H genes and that the α gene is farthest from the V_H genes. Preud'homme *et al.* (29) reported that MPC 11 myeloma cells (IgG2b producer), when treated with mutagens, gave rise to mutant clones that produced γ2a H chains. This report is also in agreement with the proposed order of the γ2b and γ2a genes. Lieberman and Potter (30) proposed the order γ2a, γ2b, γ1, and α genes on the basis of the assumption that an H chain gene allele of the Japanese wild mouse has arisen from recombination between two alleles of inbred strains BALB/c and C57BL/6. However, different conclusions are drawn if different combinations are chosen as parent alleles.

Concordance of the Allelic Deletion Model with Immunological Phenomena. Any model for the immunoglobulin genes has to explain such unique immunological observations as allelic exclusion and switch of the immunoglobulin class synthesized in a single lymphocyte. Genetic studies using allotype markers have shown that only one of the alleles of immunoglobulin genes is expressed in activated B lymphocytes (31–33). Genetic information for the V_H and C_H which are genetically linked on one parental chromosome are coordinately expressed, which is referred to as *cis* expression (34–36). Apparently, the allelic deletion model postulates allelic exclusion and *cis* expression by nature. Tonegawa *et al.* (37) reported that rearrangement of κ chain genes occurs on both alleles. It remains to be seen whether different mechanisms operate on the L and H gene systems.

The switch within a clone from μ chain synthesis to γ chain synthesis is well established as the normal process by which B lymphocytes begin to secrete IgG, and this easily explains the simultaneous presence of both IgM and IgG on the cell surface (38–40). It is not clear, however, whether this switch may include a period in which a single cell simultaneously synthesizes μ and γ chains and, in particular, μ and γ mRNAs. According to the allelic deletion model the successive recombination of a V_H gene sequence, first with a μ gene and then with a γ gene, can account for the shift of the immunoglobulin class synthesized in a lymphocyte. The switch to the reverse direction has not been found and is very difficult to explain by this model. In view of the relatively long half-life of immunoglobulin proteins (96 hr) and mRNAs (14 hr) (41, 42), we do not have to postulate the concurrent transcription of μ and γ gene sequences within a single lymphocyte. A similar explanation may be applicable to the simultaneous presence of IgM and IgD on the cell surface (43).

Although no experimental data are available, all the possible mechanisms thus far proposed to account for the V–C recombination include (*i*) the copy-insertion model (5, 44), (*ii*) the translocation model (45), (*iii*) the inversion model (37), and (*iv*)

the looping-out excision model (46). None of the models except the looping-out excision model can explain our results.

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