MOLECULAR AND CELLULAR BIOLOGY, Oct. 1992, p. 4422–4432
0270-7306/92/104422-11$02.00/0

Vol. 12, No. 10

Ectopic Recombination within Homologous Immunoglobulin μ Gene Constant Regions in a Mouse Hybridoma Cell Line

MARK D. BAKER[1]* AND LEAH R. READ[2]

Departments of Molecular Biology and Genetics[1] and of Veterinary Microbiology and Immunology,[2] University of Guelph, Guelph, Ontario, Canada N1G 2W1

Received 12 September 1991/Returned for modification 13 December 1991/Accepted 8 July 1992

We have transferred a pSV2neo vector containing the wild-type constant region of the immunoglobulin μ gene (Cμ) into the mutant hybridoma igm482, which bears a 2-bp deletion in the third constant-region exon of its haploid chromosomal μ gene (Cμ3). Independent igm482 transformants contain the wild-type immunoglobulin Cμ region stably integrated in ectopic chromosomal positions. We report here that the wild-type immunoglobulin Cμ region can function as the donor sequence in a gene conversion event which corrects the 2-bp deletion in the mutant igm482 chromosomal Cμ3 exon. The homologous recombination event restores normal immunoglobulin M production in the mutant cell.

Recombination is the process by which genetic information is exchanged between DNA duplexes. In mammalian cells, the nature and frequency of recombination events within DNA sequences have important consequences for genome stability and evolution. Recombination can occur by either gene replacement (gene conversion or double reciprocal crossover) or single reciprocal recombination. Gene conversion has the property of transferring information in a nonreciprocal fashion and is therefore an important mechanism in maintaining the sequence homogeneity within repeated sequences and members of multigene families and in generating genetic diversity (5, 16, 64, 66). Conversely, reciprocal crossovers can generate chromosomal rearrangements such as translocations, deletions, amplifications, and inversions, which are important for the assembly and expression of genes but can also be deleterious to the cell (11). There is a need to learn more about the nature of recombinations occurring within mammalian cells in view of their importance in shaping the mammalian genome.

We have reported previously that homologous recombination occurs with high frequency within a single pair of immunoglobulin μ gene constant regions (Cμ) located 10 kb apart 3′ of the endogenous rearranged heavy-chain variable region in a mouse hybridoma cell line (3). The homologous recombination events consist of both gene conversion and single reciprocal crossover. In the present study, we have investigated whether homologous recombination also occurs within the same immunoglobulin Cμ duplicates when they are not closely linked in the hybridoma genome. To study this, we have used the mutant hybridoma igm482, which bears a 2-bp deletion in the third constant-region exon of its haploid chromosomal μ gene (Cμ3) (6). We have generated independent transformants of igm482 in which the wild-type Cμ region is stably integrated in an ectopic chromosomal position not closely linked to the mutant igm482 chromosomal μ gene. Our results show that the integrated wild-type Cμ region can act as the donor sequence in a gene conversion event which corrects the igm482 mutant Cμ region, restoring normal immunoglobulin M (IgM) production in the mutant cells.

* Corresponding author.

MATERIALS AND METHODS

Cell lines and plasmids. The origin of the hybridoma cell lines Sp6/HL, igm482, and igm10 and the methods used for cell culture have been described previously (4, 6, 30, 31). The construction of the 10-kb plasmid pTCμ (Fig. 1A) has also been reported (4). In brief, the 4.3-kb *Xba*I fragment containing the wild-type Cμ region from the hybridoma Sp6/HL was converted to a *Sal*I fragment and cloned into the *Sal*I site of a modified pSV2neo vector. The *Sal*I site also contains the recognition sequence for the enzyme *Hin*cII. There are no *Xba*I sites in the pTCμ vector. The plasmid vector pRCμ482 bears the same Cμ region as pTCμ except for the introduction of the 2-bp igm482 mutation in the Cμ3 exon by oligonucleotide-directed mutagenesis (4, 68).

Isolation of igm482 transformants. The pTCμ vector (50 μg) was linearized by digestion with *Hpa*I (Fig. 1A), extracted with phenol and chloroform, precipitated with ethanol, resuspended in phosphate-buffered saline, and transferred into 2×10^7 igm482 cells by electroporation with successive 700-V, 25-μF pulses at approximately 0°C with a Bio-Rad Laboratories Gene Pulser. The cells were resuspended in complete Dulbecco's modified Eagle's medium (DMEM) containing 13% bovine calf serum and 0.0035% 2-mercaptoethanol in a tissue culture flask at a density of 10^5 cells per ml. The flask was placed at 37°C for 12 h, and cell survival was determined by trypan blue dye exclusion. After growth for an additional 2 days, one portion of cells was distributed into microtiter plates at a limiting dilution in complete DMEM containing 600 μg of G418 per ml to measure the frequency of G418-resistant ($G418^r$) transformants. The frequency of $G418^r$ transformants was calculated from the Poisson distribution. The remaining culture was resuspended in complete DMEM containing G418 for 10 to 14 days to select stable $G418^r$ igm482 transformants. Independent $G418^r$ igm482 transformants were isolated by cloning in microtiter plates.

Isolation of recombinant PFCs. Plaque-forming cells (PFCs) were detected in cultures of the $G418^r$ igm482 transformants by a trinitrophenyl (TNP)-specific plaque assay (4, 27). To facilitate the recovery of the PFCs, the plaque assay was modified so that cells were plated in a top layer of 0.4% low-melting-point agarose (Sea-Plaque; FMC BioProducts). In each plaque assay, we routinely plated 2×10^7

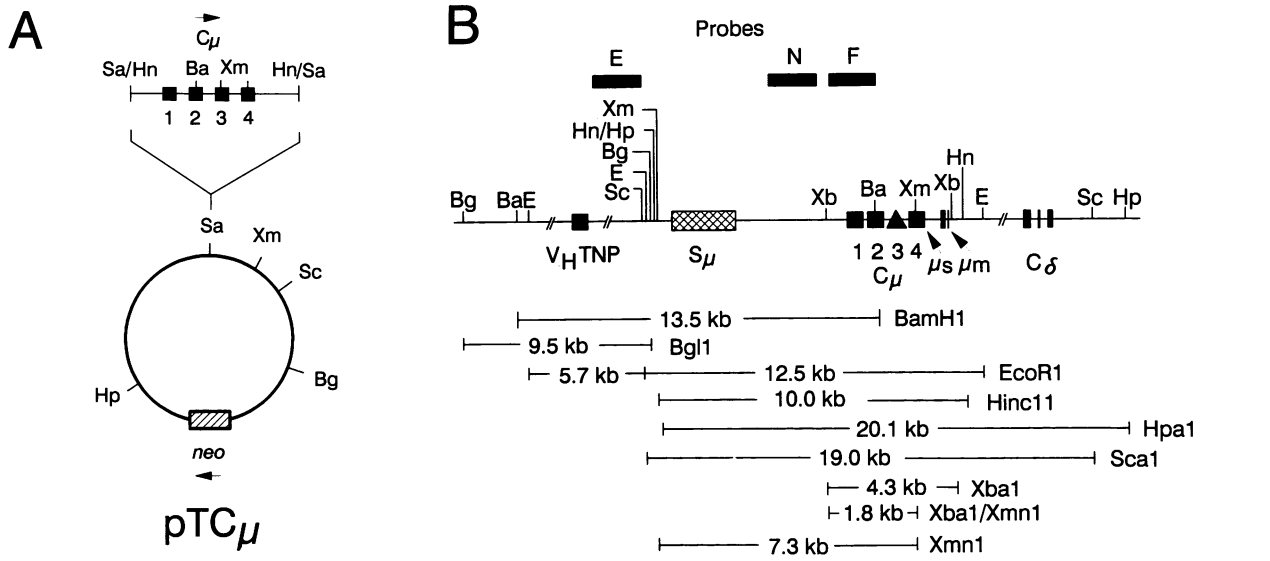

FIG. 1. Structure of the pTCμ transfer vector and the igm482 chromosomal immunoglobulin μ gene. (A) The 10-kb pTCμ transfer vector was constructed as described previously (4). Briefly, the 4.3-kb *Xba*I fragment containing the four exons (solid boxes) for the four domains of the Cμ region was converted to a *Sal*I fragment and then cloned into the *Sal*I site of a derivative of pSV2neo (circle) which lacks the *Bam*HI site and which has the *Eco*RI site converted to a *Sal*I site. The *Sal*I site also contains the recognition sequence for the enzyme *Hin*cII. The arrows designate the tandem orientation of the Cμ region and the *neo* gene (hatched box) of pSV2neo (52). The 10-kb vector pRCμ482 (4) bears the same Cμ region as pTCμ except for the introduction of the 2-bp igm482 mutation in the Cμ3 exon by oligonucleotide-directed mutagenesis (68). In pRCμ482, the orientation of the Cμ region is opposite that of *neo*. (B) The sizes of fragments that the indicated restriction enzymes should generate are shown for the mutant igm482 chromosomal immunoglobulin μ gene. The mutant igm482 Cμ3 exon is indicated (▲). With the exception of the 2-bp deletion, which destroys the *Xmn*I site in the igm482 Cμ3 exon, the chromosomal μ genes of the mutant igm482 and the wild-type Sp6/HL hybridomas are expected to be identical (4, 6, 30). In Sp6/HL, the Cμ3 and Cμ4 *Xmn*I sites are separated by 224 bp (23). Therefore, in the wild-type Sp6/HL, the *Xba*I-*Xmn*I and *Xmn*I fragments detected with probe F are 1.6 and 7.1 kb, respectively (4; our unpublished results). Probe E is a 1,983-bp *Bam*HI-*Eco*RI fragment derived from the *Eco*RI site in the major Cμ intron and the *Bam*HI site 5′ of the unrearranged immunoglobulin JH3 segment: probe F is an 870-bp *Xba*I-*Bam*HI fragment; probe N consists of adjacent 475-bp and 495-bp *Nhe*I fragments. Abbreviations: Ba, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; Hn, *Hin*cII; Hp, *Hpa*I; Sa, *Sal*I; Sc, *Sca*I; Xb, *Xba*I; Xm, *Xmn*I; Cμ, μ gene constant region; *neo*, neomycin phosphotransferase gene of pSV2neo (52); Sμ, the μ gene switch region (cross-hatched box); V_HTNP, TNP-specific heavy-chain variable region.

igm482 transformant cells. The PFCs within each igm482 transformant culture were recovered by picking the hybridoma cells within each plaque with a sterile microcapillary pipette. The recovered cells were transferred to complete DMEM containing G418 in microtiter wells. Following cell growth, the PFCs were subjected to an additional round of purification by this method and then isolated by cloning at a limiting dilution.

DNA and IgM analysis. High-molecular-weight DNA was prepared from the various cell lines by the method of Gross-Bellard et al. (24). Restriction enzymes were purchased from New England Biolabs, Inc., Boehringer Mannheim Biochemicals, and Bethesda Research Laboratories, Inc., and were used in accordance with the specifications of the manufacturers. DNA electrophoresis was conducted at 2 V/cm through agarose gels of the consistency indicated in the figure legends. DNA blotting onto nitrocellulose was performed by the method of Southern (51). ^{32}P-labeled DNA probe fragments were prepared with the Multiprime DNA-labeling system (Amersham). Hybridizations were conducted as described previously (60). IgM was biosynthetically labeled with [^{35}S]methionine and purified by binding to dinitrophenyl-Sepharose beads. The μ and κ chains were visualized after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and autoradiography as described before (31, 48).

RESULTS

Detection of homologous recombination. We have previously described the system for detecting homologous recombination within immunoglobulin Cμ regions (3, 4). In brief, it is based on the wild-type hybridoma Sp6, which bears a single copy of the μ gene and makes IgM(κ) specific for the hapten TNP (30, 31). The mutant hybridoma igm482 was derived from Sp6 (subclone Sp6/HL [6]) and bears a 2-bp deletion in the exon encoding the third domain of Cμ (Cμ3), resulting in the production of a truncated μ chain lacking the Cμ4 domain (6). The IgM bearing this mutant μ chain is monomeric and does not activate complement (noncytolytic). Homologous recombination is assayed by the ability of the wild-type Sp6/HL Cμ region to restore the wild-type μ gene in the mutant igm482 (4). Wild-type cells are conveniently detected as PFCs because they synthesize IgM containing a full-length μ chain that is able to bind TNP-coupled sheep erythrocytes, activate the complement cascade to lyse the erythrocytes, and form a plaque (4, 27). Wild-type cells make plaques on TNP-coupled sheep erythrocytes with an efficiency of approximately 0.5 to 0.8, whereas for igm482 hybridoma cells, the efficiency is $<10^{-7}$ (4).

Structure of igm482 transformants. The restriction enzyme sites and corresponding fragment sizes for the haploid chromosomal igm482 μ gene as well as the origins of the probe

fragments are presented in Fig. 1B. Probe fragments E and N contain sequences present in the chromosomal μ gene but not in the pTCμ vector. Probe F contains sequences present in both chromosomal and vector-borne Cμ regions. The probe fragment G (not shown) is the 762-bp fragment from the *neo* gene of pSV2neo (52).

To generate igm482 transformants bearing the integrated wild-type Cμ region, the vector pTCμ (Fig. 1A) was linearized with *Hpa*I and transferred to igm482 cells by electroporation. The igm482 cells surviving the electroporation were selected for resistance to the drug G418 (52). Following drug selection, the G418-resistant (G418^r) population was cloned at a limiting dilution, and 12 representative G418^r igm482 transformants were isolated. The G418^r igm482 transformants are designated by the prefix R/TCμH. From the number of igm482 cells surviving the electroporation and the frequency of G418^r transformants, we calculate that the 12 transformants are derived from a G418^r population consisting of approximately 2,900 independent G418^r cells.

The enzyme *Sca*I cuts once within the pTCμ vector (Fig. 1A). We analyzed an *Sca*I digest of the R/TCμH transformant DNAs with the *neo* probe fragment G (data not shown) and found that each G418^r transformant contains a different pTCμ vector integration pattern, indicating that each is an independent cell line. The IgM made by the mutant igm482 bears a truncated μ chain lacking the Cμ4 domain (6) and can be distinguished from the normal IgM of the Sp6 wild-type hybridoma by a TNP-specific hemagglutination assay (31). We used this test to verify that each of the 12 independent G418^r igm482 transformants continues to make the mutant, monomeric IgM characteristic of igm482 (4, 6). This suggests that in each transformant, the transferred pTCμ vector has integrated in an ectopic chromosomal position(s) not closely linked to the igm482 chromosomal μ gene. Moreover, since the igm482 hybridoma contains on average 68 chromosomes (49), it might be expected that in most, if not all, of the independent R/TCμH transformants, the wild-type Cμ region is integrated in a chromosome different from murine chromosome 12, the location of the immunoglobulin μ heavy-chain gene.

As shown in Fig. 1A, digestion of the pTCμ transfer vector with *Hin*cII will release the 4.3-kb wild-type Cμ region. In addition, a 10-kb chromosomal μ gene fragment would also be generated following *Hin*cII digestion of igm482 DNA analyzed with probe fragment F (Fig. 1B). Thus, for a random integration of the pTCμ transfer vector, analysis of a *Hin*cII digest of the R/TCμH transformant DNAs with probe fragment F should reveal the presence of both fragments, that is, the 10-kb igm482 chromosomal μ gene fragment and the 4.3-kb fragment(s) encompassing the pTCμ vector-borne wild-type Cμ region. The results of this experiment (Fig. 2) show that all transformants contain one or more copies of the full-length 4.3-kb *Hin*cII wild-type vector-borne Cμ region clearly resolved from the 10-kb *Hin*cII haploid igm482 chromosomal μ gene fragment.

We used densitometry to compare the intensity of the vector-borne 4.3-kb wild-type Cμ region with that of the single-copy 10-kb igm482 μ gene fragment to estimate the number of copies per cell of the intact integrated wild-type Cμ region. This analysis reveals that the R/TCμH transformants contain variable numbers of the integrated wild-type Cμ region, ranging from 1 to approximately 40 copies per cell (Table 1).

As shown in Fig. 2, the igm482 chromosomal *Hin*cII μ gene fragment in the R/TCμH-4 transformant is reduced to approximately 9.5 kb. One interpretation for this result is

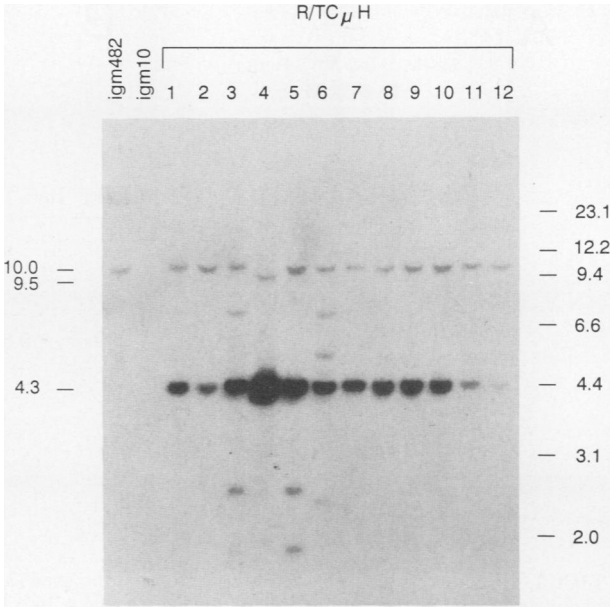

FIG. 2. Copy number of the integrated pTCμ transfer vector in the R/TCμH transformants. DNA from the indicated cell lines was digested with *Hin*cII, electrophoresed through a 0.7% agarose gel, blotted to nitrocellulose, and hybridized with radioactive probe fragment F. The sizes (in kilobases) of each band of interest are shown to the left of the blot. The sizes (in kilobases) of relevant marker bands are indicated to the right of the blot and consist of both *Hin*dIII-digested λ DNA and a 1-kb ladder (GIBCO BRL).

that the pTCμ vector has integrated into the igm482 chromosomal μ gene *Hin*cII fragment (Fig. 1B). However, this is unlikely, because the R/TCμH-4 transformant continues to synthesize the TNP-specific mutant igm482 μ chain. Also, if a copy of the 10-kb pTCμ vector (which does not contain an *Eco*RI recognition site) has integrated into the igm482 chromosomal μ gene, it should convert the *Eco*RI fragment (which contains the 10-kb chromosomal *Hin*cII fragment) from its normal size of 12.5 kb (Fig. 1B) to a fragment of

TABLE 1. Ectopic homologous recombination within immunoglobulin Cμ regions

R/TCμH transformant	Cμ copy no./cell[a]	Frequency (10^{-7}) range of PFCs[b]
1	5	1–5
2	3	2–13
3	12	14–25
4	40	4–21
5	11	23–28
6	11	1–4
7	13	1–5
8	11	6–15
9	10	<0.5
10	6	1–2
11	2	<0.5
12	1	<0.5

[a] Derived from the ratio of the intensities of the 4.3-kb *Hin*cII vector-borne wild-type immunoglobulin Cμ region and the single-copy 10.0-kb *Hin*cII igm482 chromosomal immunoglobulin μ gene fragment shown in Fig. 2.

[b] PFCs were detected by a TNP-specific plaque assay as described in Materials and Methods. We have presented the range of PFC frequencies for each of the R/TCμH transformants. These data are based on at least two independent platings for each transformant cell line. <0.5, no PFCs detected.

≥22.5 kb when *Eco*RI-digested R/TCμH-4 DNA is analyzed with probe fragment N. The result of this experiment again reveals the deletion in the R/TCμH-4 chromosomal μ gene; that is, the *Eco*RI μ gene fragment was approximately 0.5 kb smaller than the normal 12.5-kb fragment (data not shown). Thus, the deletion does not result from the integration of the pTCμ transfer vector into the chromosomal μ gene of R/TCμH-4.

Homologous recombination within immunoglobulin Cμ regions. We have previously shown that homologous recombination within the Sp6 wild-type Cμ region and the mutant igm482 chromosomal μ gene corrects the 2-bp igm482 mutation, generating recombinant cells that produce cytolytic IgM that are detected as PFCs in the TNP-specific plaque assay (4). We have therefore tested for homologous recombination of this type in the R/TCμH transformants bearing the stably integrated wild-type Cμ region. As shown in Table 1, G418^r PFCs are generated within cultures of 9 of the 12 independent R/TCμH transformants. PFCs were not reproducibly detected in three of the R/TCμH transformants (frequency, $<0.5 \times 10^{-7}$). Among the plaque-forming R/TCμH transformants, the frequency of PFCs is not markedly different and does not appear to correlate with the number of copies of the intact 4.3-kb wild-type Cμ region per cell. For these cell lines, we estimate the rate of recombination to be approximately 10^{-7} per cell generation (14). In control experiments, we did not detect PFCs among six independent subclones of the igm482 hybridoma (frequency, $<0.5 \times 10^{-7}$), indicating that reversion of the igm482 mutation does not account for the PFC frequencies shown in Table 1. We have also generated independent G418^r transformants of igm482 derived from the transfer of the *Hpa*I-linearized vector pRCμ482 (Fig. 1A), which bears the same 2-bp deletion found in the Cμ3 exon of the igm482 chromosomal μ gene (4) (Fig. 1B). The frequency of G418^r PFCs among 16 independent pRCμ482 transformants tested was $<0.5 \times 10^{-7}$. These data suggest that PFCs are generated by homologous recombination with the wild-type but not the mutant igm482 Cμ region.

Analysis of the igm482 chromosomal μ gene locus and pTCμ vector integration sites in the R/TCμH transformants. To study homologous recombination within the integrated wild-type Cμ region and the mutant Cμ region of the igm482 chromosomal μ gene, we first characterized the transformants with respect to the structure of the igm482 haploid chromosomal μ gene locus and the number of pTCμ vector integration sites. As discussed above, the R/TCμH transformants vary in pTCμ vector integration sites and copy number. To facilitate the interpretation of Southern blotting experiments and also to minimize the likelihood that the integrated wild-type Cμ region would be closely linked to the igm482 mutant chromosomal μ gene, we have concentrated our studies on the G418^r igm482 transformants R/TCμH-1, -2, and -8. In these (plaque-forming) transformants, the integrated Sp6 wild-type Cμ region is present in low copy number and in an arrangement which appears to be relatively less complicated than that of the others (Table 1 and Fig. 2).

We first characterized the structure of the igm482 chromosomal Cμ region in the R/TCμH transformants. As shown in Fig. 1B, using probe fragment N to analyze igm482 DNA digested separately with the enzymes *Hpa*I and *Sca*I should generate chromosomal μ gene fragments of 20.1 and 19.0 kb, respectively. The pTCμ transfer vector (Fig. 1A) contains a single *Sca*I site and was linearized prior to electroporation with *Hpa*I; thus, its integration within this region of the igm482 μ gene in the R/TCμH transformants would be expected to yield novel *Hpa*I and *Sca*I fragments of unpredictable sizes. However, our analysis of this chromosomal μ gene region in the R/TCμH transformants clearly reveals the presence of the 20.1-kb *Hpa*I (Fig. 3A) and 19.0-kb *Sca*I (Fig. 3B) igm482 μ gene fragments. The 10-kb pTCμ transfer vector does not contain the recognition site for *Eco*RI. Therefore, its integration into the 12.5-kb *Eco*RI fragment of the igm482 chromosomal μ gene (Fig. 1B) is expected to increase the size of this fragment to ≥22.5 kb in the R/TCμH transformants when analyzed with probe fragment N. However, the results of this experiment reveal that the R/TCμH transformants contain the 12.5-kb *Eco*RI μ gene fragment expected for igm482 (data not shown).

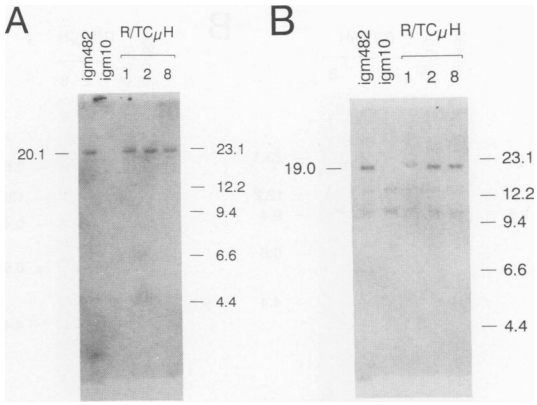

FIG. 3. Analysis of the chromosomal immunoglobulin Cμ region in the R/TCμH transformants. DNA from the indicated cell lines was digested with (A) *Hpa*I or (B) *Sca*I, electrophoresed through 0.7% agarose gels, blotted to nitrocellulose, and hybridized with probe fragment N. The sizes (in kilobases) of each band of interest are shown to the left of each blot. The sizes (in kilobases) of relevant marker bands are indicated to right of each blot and consist of both *Hin*dIII-digested λ DNA and a 1-kb ladder (GIBCO BRL).

We have also examined the region surrounding the igm482 TNP-specific heavy-chain variable region (V_HTNP). As shown in Fig. 1B, using probe E on igm482 DNA digested separately with *Bgl*I and *Eco*RI is expected to yield V_HTNP fragments of 9.5 and 5.7 kb, respectively. The integration of the pTCμ vector into this region in the R/TCμH transformants would be expected to change the sizes of these fragments. Since the pTCμ vector contains one *Bgl*I site (Fig. 1A), its integration into the 9.5-kb *Bgl*I V_HTNP fragment would generate a novel *Bgl*I fragment of an unpredictable size. On the other hand, integration of the 10-kb pTCμ vector (which lacks an *Eco*RI site) into the 5.7-kb *Eco*RI V_HTNP fragment would increase its size to ≥15.7 kb. As shown in Fig. 4A and B, all R/TCμH transformants contain the 9.5-kb *Bgl*I and 5.7-kb *Eco*RI igm482 V_HTNP fragments, respectively.

Irrelevant joining-region and cross-hybridizing fragments are detected with probe N (Fig. 3A and B) and with probe E (Fig. 4A and B). To indicate these irrelevant fragments, we have included the cell line igm10, an Sp6-derived mutant which lacks the TNP-specific μ gene (6, 31). In summary, we have analyzed an approximately 30-kb region in the R/TCμH transformants which includes the expressed TNP-specific igm482 chromosomal μ gene. Our results clearly show that the structure of the R/TCμH chromosomal DNA in this region is identical to that found in igm482 (Fig. 1B), thus confirming and extending our previous conclusion that the vector-borne wild-type immunoglobulin Cμ region in the

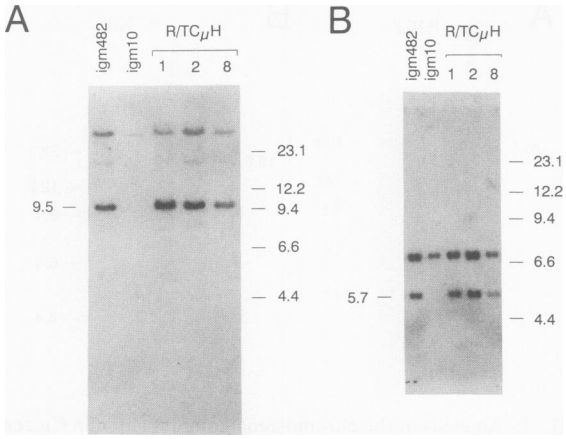

FIG. 4. Analysis of the chromosomal immunoglobulin V_HTNP region in the R/TCμH transformants. DNA from the indicated cell lines was digested with (A) *Bgl*I or (B) *Eco*RI, electrophoresed through 0.7% agarose gels, blotted to nitrocellulose, and hybridized with probe fragment E. The sizes (in kilobases) of each band of interest are shown to the left of each blot. The sizes (in kilobases) of relevant marker bands are indicated to the right of each blot and consist of both *Hin*dIII-digested λ DNA and a 1-kb ladder (GIBCO BRL).

R/TCμH transformants is in an ectopic chromosomal position not closely linked to the igm482 chromosomal μ gene.

We next characterized the R/TCμH transformants with respect to the structure and number of pTCμ vector integration sites. The analysis of the transformant DNAs digested separately with *Sca*I (Fig. 5A and B) and *Bam*HI (Fig. 5C and D), enzymes which cut once within the 10-kb pTCμ vector (Fig. 1A), with probe fragments F (Fig. 5A and C) and G (Fig. 5B and D) reveals the presence of a predominant repeated 10-kb pTCμ vector band, suggesting that the copies of the wild-type immunoglobulin Cμ region are integrated in a tandem (head-to-tail) configuration in the R/TCμH-1, -2, and -8 transformants. With the exception of the 19.0-kb *Sca*I and 13.5-kb *Bam*HI chromosomal μ gene fragments (Fig. 1B), visible in Fig. 5A and C, respectively, the additional bands in Fig. 5A through D likely represent the junction fragments generated by vector integration. An analysis of the junction fragments suggests, as depicted in Fig. 5E, that each transformant bears a tandem wild-type immunoglobulin Cμ region cluster integrated in a single chromosomal position. In the case of R/TCμH-1, integration of the five copies of the wild-type Cμ region in a single chromosomal position is suggested by the 5.3-kb fragment visible in the *Bam*HI digests probed with fragment F (Fig. 5C) and fragment G (Fig. 5D). As shown in Fig. 5E, a deletion has likely removed a portion of the pTCμ vector sequence from the left end of the tandem cluster in this transformant, so that chromosomal DNA is now joined to an internal site within the plasmid. In the case of R/TCμH-2, the presence of the 7.0-kb *Bam*HI fragment visible with probe F (Fig. 5C) and probe G (Fig. 5D) suggests that the three copies of the wild-type Cμ region are also integrated at a single chromosomal site.

An explanation which accounts for the remaining R/TCμH-2 fragments visible in the blots shown in Fig. 5A, B, and D is that a deletion of the entire wild-type Cμ region has occurred in a pTCμ vector which is joined in the opposite orientation to the tandem cluster, as depicted in Fig. 5E. In the case of the transformant R/TCμH-8, only a 10-kb repeated pTCμ vector band is visible in Fig. 5A through D. These data are consistent with the 11 copies of the wild-type Cμ region being integrated at a single chromosomal position but with the deletion of vector sequences and the joining of chromosomal DNA to an internal plasmid site at both ends of the tandem vector cluster (Fig. 5E), so that no junction fragments are formed.

Analysis of μ-chain protein and DNA in PFCs. We have isolated PFCs from the transformants R/TCμH-1, -2, and -8 in order to determine the mechanism of homologous recombination within the integrated wild-type Cμ region and the mutant Cμ region of the igm482 chromosomal μ gene. The PFCs 1/1 and 1/2 were derived from R/TCμH-1; PFCs 2/1, 2/2, and 2/3 were isolated from R/TCμH-2; and PFCs 8/1, 8/2, and 8/3 were derived from R/TCμH-8. We analyzed the μ chains produced by the R/TCμH PFCs by SDS-PAGE. The μ chains of the wild-type and igm482 cell lines differ in molecular mass by approximately 15 kDa and are readily distinguished by their mobility in SDS-PAGE (4). As shown in Fig. 6, all R/TCμH PFCs produce full-length μ chains indistinguishable from the Sp6 wild-type μ and have apparently ceased making the igm482 mutant μ chain found in the original R/TCμH transformants.

The 2-bp deletion in the igm482 Cμ3 exon destroys an *Xmn*I restriction enzyme site which would be expected to be restored in the PFCs as a result of homologous recombination with the integrated wild-type Cμ region. In the wild-type hybridoma Sp6/HL, the Cμ3 and Cμ4 *Xmn*I sites are separated by 224 bp (23). Therefore, using probe F on DNA digested with the enzyme combination *Xba*I and *Xmn*I yields a 1.6-kb fragment for the wild-type μ gene, compared with a 1.8-kb fragment for the mutant (Fig. 1B) (4). As shown in Fig. 7A, and with the exception of PFC 2/3 (discussed below), the PFCs contain the 1.6-kb wild-type μ gene fragment clearly resolved from the 1.8-kb mutant igm482 μ gene fragment of the corresponding R/TCμH transformant, indicating that they were generated by homologous recombination with an integrated donor wild-type Cμ region. The pTCμ vector lacks a recognition site for *Xba*I. Therefore, probe F also detects the repeated 7.0-kb *Xmn*I fragment from the head-to-tail integration of the pTCμ vector (Fig. 7B).

We have compared, by densitometry, the intensities of the single-copy 1.8-kb mutant and 1.6-kb wild-type *Xba*I-*Xmn*I chromosomal μ gene fragments with the 7.0-kb *Xmn*I repeated pTCμ vector-borne fragments in each R/TCμH transformant and its corresponding PFCs and found them to be comparable. In addition to these fragments, probe F also detects an approximately 9.5-kb fragment in the R/TCμH-2 transformant and PFCs which likely represents a junction fragment of the vector integration event. A junction fragment should also be visible in the R/TCμH-1 transformant and PFCs. Perhaps this fragment comigrates with the 7.0-kb pTCμ vector band. As shown in Fig. 7A, PFC 2/3, isolated from the R/TCμH-2 transformant, is unusual in that it retains the 1.8-kb *Xba*I-*Xmn*I mutant μ gene fragment yet produces a normal-length μ chain (Fig. 6). The presence of the 1.8-kb igm482 mutant μ gene fragment suggests that in this PFC, the 2-bp deletion in the igm482 Cμ3 exon has not been corrected by homologous recombination with an integrated wild-type Cμ region. The 2/3 cell line could therefore represent a rare case of spontaneous reversion. Alternatively, the reversion to the plaque-forming phenotype could possibly be induced by some part(s) of the process of DNA transfer. Such a process may also account for the approximately 0.5-kb deletion in the transformant R/TCμH-4, discussed above.

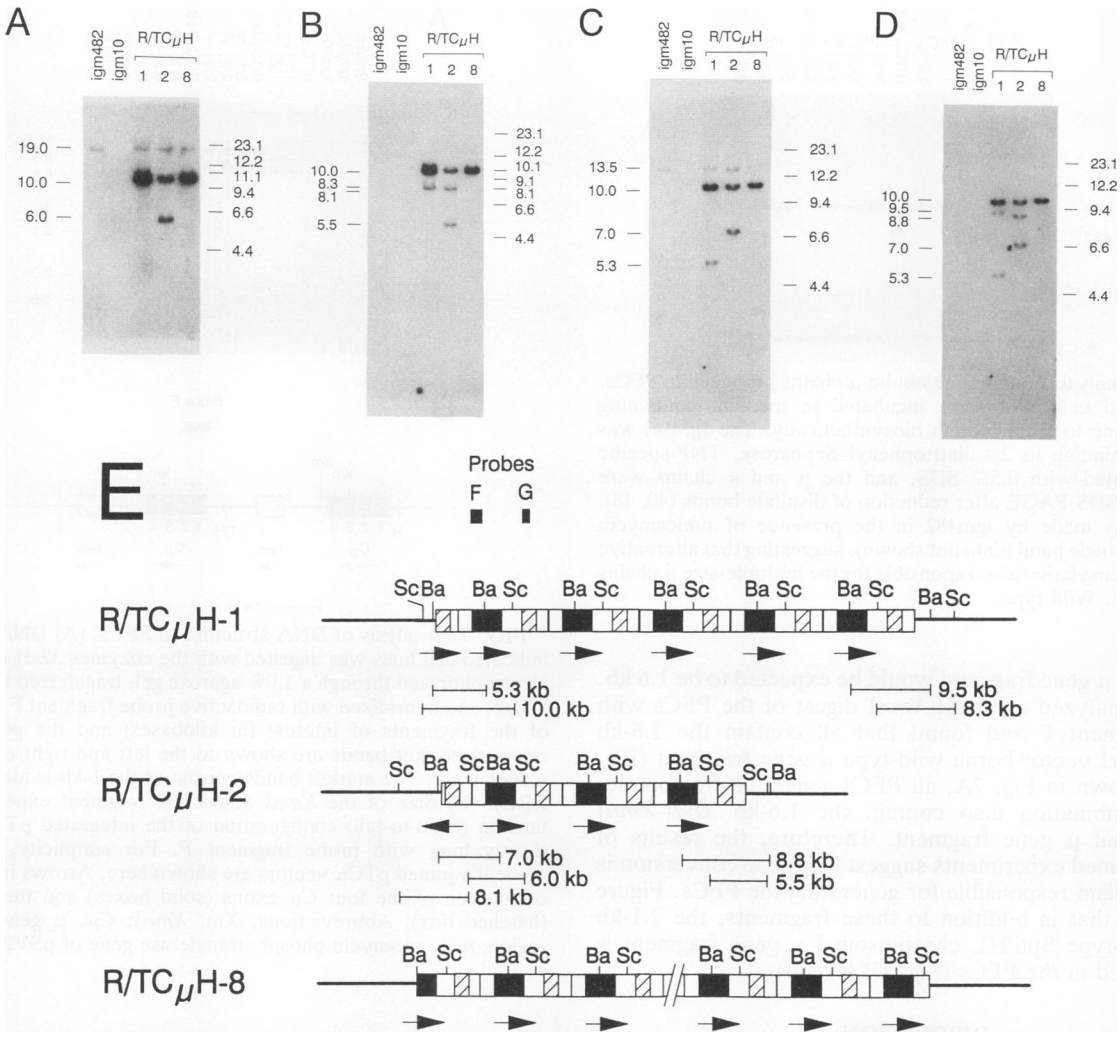

FIG. 5. Structure of integrated pTCμ transfer vector in R/TCμH transformants. DNA from the indicated cell lines was subjected to Southern blot analysis with the following restriction enzymes and probe fragments: (A) *Sca*I, probe F; (B) *Sca*I, probe G; (C) *Bam*HI, probe F; (D) *Bam*HI, probe G. The sizes (in kilobases) of the fragments of interest and the relevant marker bands are indicated to the left and right of each blot, respectively. Marker bands consist of both *Hin*dIII-digested λ DNA and a 1-kb ladder (GIBCO BRL). (E) Tandem integration of the pTCμ transfer vectors at a single chromosomal position in the R/TCμH transformants. The positions of the *Sca*I (Sc) and *Bam*HI (Ba) restriction enzyme sites and the sizes of the junction fragments detected with probe fragments F and G in panels A through D are indicated. In each integrated pTCμ vector, the four wild-type exons of the immunoglobulin Cμ region are represented by the single black box, while the *neo* gene is indicated by the hatched box. As presented in Table 1, the R/TCμH-1, -2, and -8 transformants contain 5, 3, and 11 copies, respectively, of the integrated wild-type immunoglobulin Cμ region. The distance between the *Bam*HI and *Sca*I sites in the pTCμ vector repeats is 10 kb (data not shown). The orientation of the pTCμ vectors within each R/TCμH transformant cluster is indicated by the arrows.

Several mechanisms could account for the generation of PFCs by homologous recombination. A gene replacement event (gene conversion or double reciprocal recombination) occurring within an integrated wild-type Cμ region and the mutant igm482 chromosomal Cμ region would be expected to correct the 2-bp igm482 mutation without changing the flanking μ gene structure. A single reciprocal recombination event would also be expected to correct the igm482 mutation but, in so doing, would generate a rearrangement in the DNA structure 3′ to the Cμ region. The enzyme *Eco*RI can be used to distinguish between these mechanisms. That is, using probe N on DNA digested with *Eco*RI should generate a fragment of 12.5 kb for PFCs generated by gene replacement (Fig. 1B). On the other hand, since the pTCμ vector does not contain an *Eco*RI site, a single reciprocal recombination event occurring within chromosomal and vector-borne Cμ regions is expected to generate an *Eco*RI fragment of an unpredictable size. As shown in Fig. 8A, all PFCs contain the 12.5-kb *Eco*RI fragment, suggesting that they were formed by gene replacement.

We next determined the mechanism of gene replacement in the PFCs, that is, whether it occurred by gene conversion or double reciprocal recombination. As shown in Fig. 8B, a double reciprocal crossover occurring within the donor vector-borne wild-type Cμ region and the recipient mutant igm482 chromosomal Cμ region is expected to generate a 1.8-kb *Hin*cII-*Xmn*I vector-borne Cμ region fragment and a 1.6-kb *Xba*I-*Xmn*I chromosomal μ gene fragment. In the case of gene conversion, both the donor vector-borne wild-type *Hin*cII-*Xmn*I fragment and the recipient chromosomal

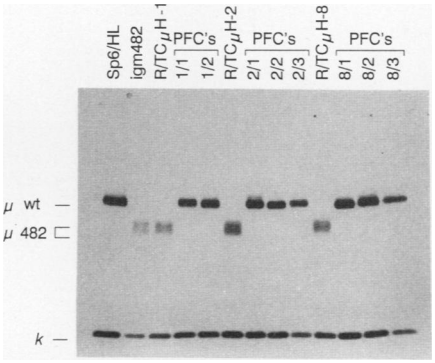

FIG. 6. Analysis of immunoglobulin μ chains produced in PFCs. The indicated cell lines were incubated in medium containing [^{35}S]methionine to label protein biosynthetically. The IgM(κ) was purified by binding to 2,4-dinitrophenyl–Sepharose. TNP-specific IgM was eluted with 0.5% SDS, and the μ and κ chains were analyzed by SDS-PAGE after reduction of disulfide bonds (40, 48). The μ chains made by igm482 in the presence of tunicamycin migrate as a single band (data not shown), suggesting that alternative modes of glycosylation are responsible for the multiple-size μ chains seen here. wt, Wild type.

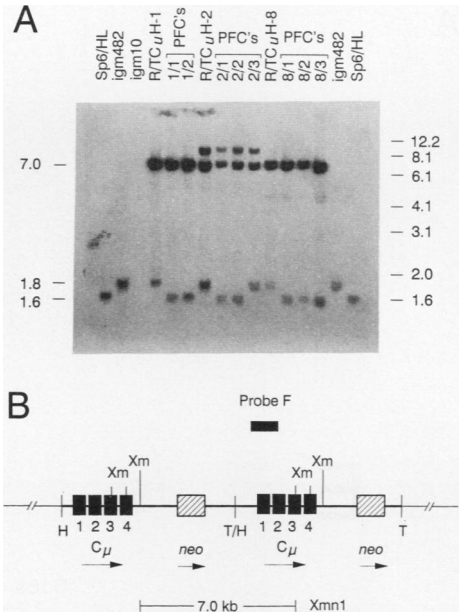

FIG. 7. Analysis of DNA structure in PFCs. (A) DNA from the indicated cell lines was digested with the enzymes *Xba*I and *Xmn*I, electrophoresed through a 1.0% agarose gel, transferred to nitrocellulose, and hybridized with radioactive probe fragment F. The sizes of the fragments of interest (in kilobases) and the positions of relevant marker bands are shown to the left and right of the blot, respectively. The marker bands consist of the 1-kb ladder (GIBCO BRL). (B) Size of the *Xmn*I restriction fragment expected for a tandem (head-to-tail) configuration of the integrated pTCμ vector (heavy line) with probe fragment F. For simplicity, only two tandemly joined pTCμ vectors are shown here. Arrows indicate the orientation of the four Cμ exons (solid boxes) and the *neo* gene (hatched box). Abbreviations: Xm, *Xmn*I; Cμ, μ gene constant region; *neo*, neomycin phosphotransferase gene of pSV2neo (52).

*Xba*I-*Xmn*I μ gene fragment would be expected to be 1.6 kb. We have analyzed a *Hin*cII-*Xmn*I digest of the PFCs with probe fragment F and found that all contain the 1.6-kb *Hin*cII-*Xmn*I vector-borne wild-type μ gene fragment (Fig. 8C). As shown in Fig. 7A, all PFCs generated by homologous recombination also contain the 1.6-kb *Xba*I-*Xmn*I chromosomal μ gene fragment. Therefore, the results of these combined experiments suggest that gene conversion is the mechanism responsible for generating the PFCs. Figure 8C reveals that in addition to these fragments, the 7.1-kb *Xmn*I wild-type Sp6/HL chromosomal μ gene fragment is also detected in the PFCs (Fig. 1B legend).

DISCUSSION

In the present study, we used the igm482 hybridoma, which bears a 2-bp deletion in the Cμ3 exon of its haploid chromosomal immunoglobulin μ gene (6), as the recipient for the transfer of the wild-type immunoglobulin Cμ region contained on a pSV2neo transfer vector (52). In independent igm482 transformants (designated by the prefix R/TCμH), copies of the transferred wild-type immunoglobulin Cμ region are integrated in an ectopic chromosomal position(s) in the hybridoma genome. We report here that the wild-type immunoglobulin Cμ region can act as the donor sequence in an ectopic homologous recombination event with the recipient mutant Cμ region of the igm482 chromosomal μ gene. The homologous recombination event restores normal, cytolytic IgM production in the mutant cells, allowing them to be detected as PFCs. The frequency of homologous recombination among independent R/TCμH transformants is approximately 10^{-7}.

We have analyzed in detail the mechanism of homologous recombination in the R/TCμH-1, -2, and -8 transformants, in which 5, 3, and 11 copies, respectively, of the wild-type immunoglobulin Cμ region are integrated in tandem at a single ectopic chromosomal position not closely linked to the mutant igm482 haploid chromosomal immunoglobulin μ heavy-chain gene. Given the fact that the igm482 hybridoma contains on average 68 chromosomes (49), it seems reasonable to suppose that in each R/TCμH transformant, the tandem vector cluster is integrated in a chromosome different from murine chromosome 12, the location of the igm482 chromosomal μ gene. Our results reveal that in PFCs derived by homologous recombination, the normal μ gene structure has been restored by an apparent gene conversion. We have determined that the vector copy number within each R/TCμH transformant and its corresponding PFCs is comparable, indicating that the tandem vector cluster is stably integrated in these cell lines. The absence of a detectable deletion in the integrated vector sequences in the PFCs argues against the possibility that an extrachromosomal wild-type Cμ region(s) excised by intrachromosomal homologous recombination (3) from the tandem vector cluster participated in the gene conversion event. This suggests that the main gene conversion donor is a stably integrated wild-type immunoglobulin Cμ region. However, we would not expect to detect the deletion of the integrated vector sequences in PFCs in the possible scenario in which an extrachromosomal wild-type Cμ region(s) is generated by vector excision from a sister chromatid if the sister chromatid harboring the deletion did not accompany the gene conversion product following cell division. While it is possible that this scheme may explain the generation of some PFCs, it seems unlikely to be a major mechanism because the gene conversion product and the sister chromatid deleted for the integrated vector sequences would not be expected to be consistently absent from all PFCs.

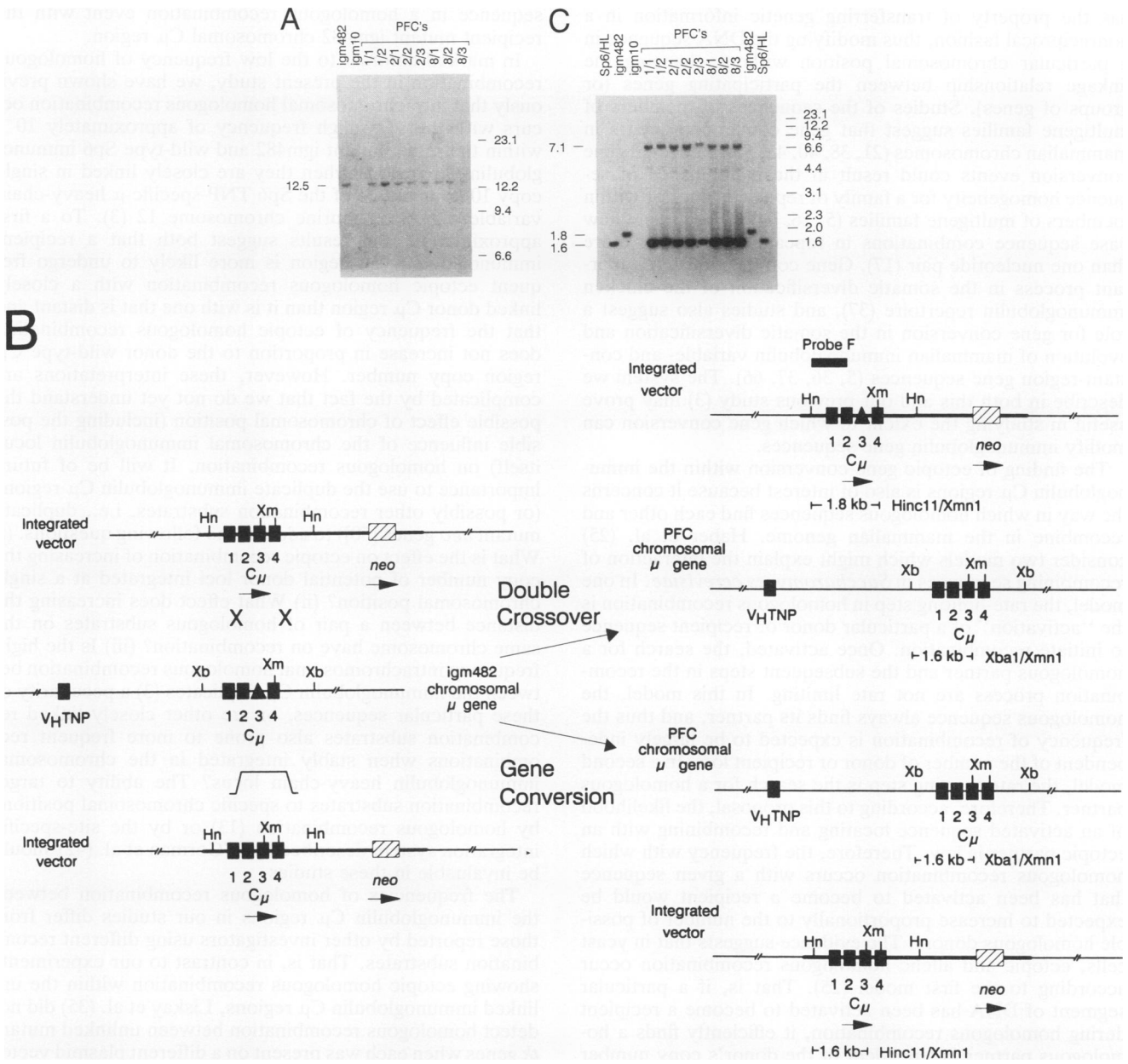

FIG. 8. Analysis of the mechanism of homologous recombination in PFCs. (A) DNA from the indicated cell lines was digested with *Eco*RI, electrophoresed through a 0.7% agarose gel, transferred to nitrocellulose, and hybridized with radioactive probe fragment N. (B) Restriction enzyme sites and DNA fragment sizes expected with probe fragment F for recombinations occurring within donor and recipient immunoglobulin Cμ regions by either gene conversion or double reciprocal crossover. The integrated pTCμ vector containing the four Cμ exons (solid boxes) and the *neo* gene (hatched box) is indicated by the heavy line. The mutant igm482 Cμ3 exon is indicated (▲). (C) DNA from the indicated cell lines was digested with the enzymes *Hin*cII and *Xmn*I, electrophoresed through a 1.0% agarose gel, blotted to nitrocellulose, and hybridized with probe fragment F. The sizes (in kilobases) of each band of interest are shown to the left of the blot. The 1.6-kb and 1.8-kb Sp6/HL and igm482 chromosomal μ gene fragments, respectively, were generated by digestion with the enzyme combination *Xba*I and *Xmn*I. Although not presented in the blot in panel C, probe F also detects the 1.6-kb *Hin*cII-*Xmn*I fragment from the integrated wild-type immunoglobulin Cμ region in the transformants R/TCμH-1, -2, and -8. The positions of the relevant marker bands are indicated to the right of the blots. The marker bands consist of *Hin*dIII-digested λ DNA and a 1-kb ladder (GIBCO BRL). Abbreviations: Hn, *Hin*cII; Xb, *Xba*I; Xm, *Xmn*I; Cμ, the μ gene constant region; V_HTNP, TNP-specific heavy-chain variable region; *neo*, neomycin phosphotransferase gene of pSV2neo (52).

The finding that gene conversion appears to predominate over single (and double) reciprocal recombination is in accordance with our previous experiments (3) and those of others (10, 35, 45). The predominance of gene conversion is not surprising because single reciprocal recombination, especially between ectopic homologous DNA sequences, would generate genomic rearrangements which might be deleterious to the cell. On the other hand, gene conversion

has the property of transferring genetic information in a nonreciprocal fashion, thus modifying the DNA sequence in a particular chromosomal position without changing the linkage relationship between the participating genes (or groups of genes). Studies of the sequences of members of multigene families suggest that gene conversion occurs in mammalian chromosomes (21, 38, 40, 43, 56). Repeated gene conversion events could result in the maintenance of sequence homogeneity for a family of repeated genes or within members of multigene families (5, 16, 64) and generate new base sequence combinations in repeats differing by more than one nucleotide pair (17). Gene conversion is an important process in the somatic diversification of the chicken immunoglobulin repertoire (37), and studies also suggest a role for gene conversion in the somatic diversification and evolution of mammalian immunoglobulin variable- and constant-region gene sequences (5, 36, 37, 66). The system we describe in both this and our previous study (3) may prove useful in studying the extent to which gene conversion can modify immunoglobulin gene sequences.

The finding of ectopic gene conversion within the immunoglobulin Cμ regions is also of interest because it concerns the way in which homologous sequences find each other and recombine in the mammalian genome. Haber et al. (25) consider two models which might explain the interaction of recombining sequences in *Saccharomyces cerevisiae*. In one model, the rate-limiting step in homologous recombination is the "activation" of a particular donor or recipient sequence to initiate recombination. Once activated, the search for a homologous partner and the subsequent steps in the recombination process are not rate limiting. In this model, the homologous sequence always finds its partner, and thus the frequency of recombination is expected to be largely independent of the number of donor or recipient loci. In a second model, the rate-limiting step is the search for a homologous partner. Therefore, according to this proposal, the likelihood of an activated sequence locating and recombining with an ectopic partner is low. Therefore, the frequency with which homologous recombination occurs with a given sequence that has been activated to become a recipient would be expected to increase proportionally to the number of possible homologous donors. The evidence suggests that in yeast cells, ectopic and allelic homologous recombination occur according to the first model (25). That is, if a particular segment of DNA has been activated to become a recipient during homologous recombination, it efficiently finds a homologous partner independent of the donor's copy number or chromosomal position.

In the present study, each independent R/TCμH transformant bears the normal-length (4.3 kb) Sp6 wild-type Cμ region integrated in a different copy number and ectopic chromosomal position. Thus, according to the models above, in order for homologous recombination to occur, an integrated donor immunoglobulin wild-type Cμ region (or the chromosomal igm482 mutant Cμ region) must first become activated and then search the genome for a homologous Cμ region partner with which to recombine. The failure to detect PFCs in 3 of the 12 R/TCμH transformants may indicate that in these cell lines, the 4.3-kb wild-type immunoglobulin Cμ region is integrated in a chromosomal position which is not conducive to homologous recombination. Notwithstanding the result with these three cell lines, the generation of PFCs in the remaining 9 of the 12 independent R/TCμH transformants suggests that there may be many additional ectopic sites in the mammalian genome where the wild-type immunoglobulin Cμ region may act as the donor sequence in a homologous recombination event with the recipient mutant igm482 chromosomal Cμ region.

In marked contrast to the low frequency of homologous recombination in the present study, we have shown previously that intrachromosomal homologous recombination occurs with the very high frequency of approximately 10^{-2} within the same mutant igm482 and wild-type Sp6 immunoglobulin Cμ regions when they are closely linked in single copy 10 kb apart, 3′ of the Sp6 TNP-specific μ heavy-chain variable region on murine chromosome 12 (3). To a first approximation, the results suggest both that a recipient immunoglobulin Cμ region is more likely to undergo frequent ectopic homologous recombination with a closely linked donor Cμ region than it is with one that is distant and that the frequency of ectopic homologous recombination does not increase in proportion to the donor wild-type Cμ region copy number. However, these interpretations are complicated by the fact that we do not yet understand the possible effect of chromosomal position (including the possible influence of the chromosomal immunoglobulin locus itself) on homologous recombination. It will be of future importance to use the duplicate immunoglobulin Cμ regions (or possibly other recombination substrates, i.e., duplicate mutant *neo* genes [50]) to address the following questions. (i) What is the effect on ectopic recombination of increasing the copy number of potential donor loci integrated at a single chromosomal position? (ii) What effect does increasing the distance between a pair of homologous substrates on the same chromosome have on recombination? (iii) Is the high-frequency intrachromosomal homologous recombination between the immunoglobulin Cμ duplicates (3) a peculiarity of these particular sequences, or are other closely linked recombination substrates also prone to more frequent recombinations when stably integrated in the chromosomal immunoglobulin heavy-chain locus? The ability to target recombination substrates to specific chromosomal positions by homologous recombination (13) or by the site-specific integration system described by O'Gorman et al. (42) should be invaluable in these studies.

The frequencies of homologous recombination between the immunoglobulin Cμ regions in our studies differ from those reported by other investigators using different recombination substrates. That is, in contrast to our experiments showing ectopic homologous recombination within the unlinked immunoglobulin Cμ regions, Liskay et al. (35) did not detect homologous recombination between unlinked mutant *tk* genes when each was present on a different plasmid vector stably integrated in the genome of Ltk^- cells (frequency, $<10^{-7}$). Also, the frequency of intrachromosomal homologous recombination between the duplicate immunoglobulin Cμ regions present 10 kb apart at the chromosomal immunoglobulin μ gene locus that we found is approximately 1,000-fold higher than the frequency of intrachromosomal homologous recombination found between duplicate, mutant *neo* (50, 55) or *tk* (35) gene substrates closely linked on plasmid vector sequences stably integrated in the mammalian genome. Differences in the length of homology and the intrachromosomal distance between the recombining regions influence homologous recombination (33–35, 55) and thus may account for some of the differences in recombination frequencies between the *neo* and *tk* genes and the immunoglobulin Cμ regions. However, immunoglobulin genes undergo various recombinations during B-cell ontogeny, and thus, as mentioned above, another factor may be the recombinogenic nature of the immunoglobulin Cμ region or the immunoglobulin locus itself.

Several features of the immunoglobulin locus may render it particularly prone to recombination reactions. The V(D)J recombinations occurring within the chromosomal immunoglobulin genes appear to be correlated with immunoglobulin gene transcription (1, 8, 32, 46). A role for transcription in promoting recombination has also been implicated in other systems (28, 29, 53, 57, 62). It is possible that transcription promotes recombination by making the chromatin more accessible to the recombination machinery. Alternatively, chromatin accessibility may be controlled by other factors (such as histones [19]), and when chromatin is in an "open" configuration, it may facilitate transcription and/or recombination.

Particular DNA sequences within the immunoglobulin locus may also stimulate recombination either directly or indirectly, through effects on transcription and/or chromatin environment. The immunoglobulin switch region contains repeated sequences and is also transcriptionally active (18), features which may be important in rendering it accessible to the switch recombination machinery. Moreover, lymphomas and leukemias are often characterized by translocations between proto-oncogenes and immunoglobulin (and T-cell receptor) genes (7, 26), which may be mediated by the enzymatic machinery that normally rearranges these genes (20, 22, 26, 61). Alternating purine-pyrimidine tracts, which have the potential to form left-handed Z-DNA (9), are present within the immunoglobulin locus (15, 41, 44). Sequences capable of adopting a left-handed Z-DNA conformation have been shown to promote homologous recombination in mammalian cells (9, 12, 54, 63). Wittig et al. (65) have shown that transcription is also associated with Z-DNA formation in mammalian nuclei. One sequence in particular, a $(CA)_{32}$ tract, is located between the Cμ secreted (μs) and Cμ membrane (μm) exons, in the immunoglobulin Cμ recombination substrates (44). $(CA)_n$ tracts have the potential to form Z-DNA (9) and have been reported to induce a transient shift in the pairing of bases in the major groove of B-DNA (58). The involvement of $(CA)_n$ tracts in gene conversion, insertion events, and the enhancement of recombination has been reported elsewhere (59). Thus, these data suggest possible relationships between transcriptional activity, DNA sequence or accessibility, and immunoglobulin gene recombinations. It is also interesting that in both human and murine myeloma tumors as well as mouse myelomas and hybridomas in tissue culture, the rate of mutation in the chromosomal immunoglobulin heavy-chain locus is at least 100- to 1,000-fold higher than the rate of mutations in unlinked chromosomal loci (31, 39, 67). It is possible that both recombination and the high frequency of mutation reflect a common feature of the immunoglobulin genes.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council and the Natural Sciences and Engineering Research Council to M.D.B.

REFERENCES

1. **Alt, F. W., T. K. Blackwell, R. A. Depinho, M. G. Reth, and G. D. Yancopoulos.** 1986. Regulation of genome rearrangement events during lymphocyte differentiation. Immunol. Rev. **89**:5–30.
2. **Alt, F. W., T. K. Blackwell, and G. D. Yancopoulos.** 1987. Development of the primary antibody repertoire. Science **238**: 1079–1087.
3. **Baker, M. D.** 1989. High-frequency homologous recombination between duplicate chromosomal immunoglobulin μ heavy-chain constant regions. Mol. Cell. Biol. **9**:5500–5507.
4. **Baker, M. D., N. Pennell, L. Bosnoyan, and M. J. Shulman.** 1988. Homologous recombination can restore normal immunoglobulin production in a mutant hybridoma cell line. Proc. Natl. Acad. Sci. USA **85**:6432–6436.
5. **Baltimore, D.** 1981. Gene conversion: some implications for immunoglobulin genes. Cell **24**:592–594.
6. **Baumann, B., M. J. Potash, and G. Kohler.** 1985. Consequences of frameshift mutations at the immunoglobulin heavy chain locus of the mouse. EMBO J. **4**:351–359.
7. **Bishop, J. M.** 1989. Oncogenes and clinical cancer, p. 327–358. *In* R. A. Weinberg (ed.), Oncogenes and the molecular origins of cancer. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Blackwell, T. K., M. W. Moore, G. D. Yancopoulos, H. Suh, S. Lutzker, E. Selsing, and F. W. Alt.** 1986. Recombination between immunoglobulin variable region gene segments is enhanced by transcription. Nature (London) **324**:585–589.
9. **Blaho, J. A., and R. D. Wells.** 1989. Left-handed Z-DNA and genetic recombination. Prog. Nucleic Acid Res. Mol. Biol. **37**:107–126.
10. **Bollag, R. J., and R. M. Liskay.** 1988. Conservative intrachromosomal recombination between inverted repeats in mouse cells: association between reciprocal exchange and gene conversion. Genetics **119**:161–169.
11. **Borst, P., and D. R. Greaves.** 1987. Programmed gene rearrangements altering gene expression. Science **235**:658–667.
12. **Bullock, P., J. Miller, and M. Botchan.** 1986. Effects of poly[d(pGpT) · d(pApC)] and poly[d(pCpG) · d(pCpG)] repeats on homologous recombination in somatic cells. Mol. Cell. Biol. **6**:3948–3953.
13. **Capecchi, M. R.** 1989. Altering the genome by homologous recombination. Science **244**:1288–1292.
14. **Capizzi, R. L., and J. W. Jameson.** 1973. A table for the estimation of the spontaneous mutation rate of cells in culture. Mutat. Res. **17**:147–148.
15. **Cohen, J. B., K. Effron, G. Rechavi, Y. Ben-Neriah, R. Zakut, and D. Girol.** 1982. Simple DNA sequences in homologous flanking regions near immunoglobulin V_H genes: a role in gene interaction. Nucleic Acids Res. **10**:3353–3370.
16. **Egel, R.** 1981. Intergenic conversion and reiterated genes. Nature (London) **290**:191–192.
17. **Ernst, J. F., J. W. Stewart, and F. Sherman.** 1981. The *cyc1-11* mutation in yeast reverts by recombination with a nonallelic gene: composite genes determining the iso-cytochromes *c*. Proc. Natl. Acad. Sci. USA **78**:6334–6338.
18. **Esser, C., and A. Radbruch.** 1990. Immunoglobulin class switching: molecular and cellular analysis. Annu. Rev. Immunol. **8**:717–735.
19. **Felsenfeld, G.** 1992. Chromatin as an essential part of the transcription mechanism. Nature (London) **355**:219–224.
20. **Finger, L. R., R. C. Harvey, R. C. A. Moore, L. C. Showe, and C. M. Croce.** 1986. A common mechanism of chromosomal translocation in T- and B-cell neoplasia. Science **234**:982–985.
21. **Flavell, R. A., H. Allen, L. C. Burkly, D. H. Sherman, G. L. Waneck, and G. Widera.** 1986. Molecular biology of the H-2 histocompatibility complex. Science **233**:437–443.
22. **Gerstein, R. M., W. N. Frankel, C. Hsieh, J. M. Durdik, S. Rath, J. M. Coffin, A. Nisonoff, and E. Selsing.** 1990. Isotype switching of an immunoglobulin heavy chain transgene occurs by DNA recombination between different chromosomes. Cell **63**:537–548.
23. **Goldberg, G. I., E. F. Vanin, A. M. Zrolka, and F. R. Blattner.** 1981. Sequence of the gene for the constant region of the μ chain of Balb/c mouse immunoglobulin. Gene **15**:33–42.
24. **Gross-Bellard, M., P. Qudet, and P. Chambon.** 1973. Isolation of high-molecular weight DNA from mammalian cells. Eur. J. Biochem. **36**:32–38.
25. **Haber, J. E., W. Leung, R. H. Borts, and M. Lichten.** 1991. The frequency of meiotic recombination in yeast is independent of the number and position of homologous donor sequences: implications for chromosome pairing. Proc. Natl. Acad. Sci.

USA **88**:1120–1124.
26. **Haluska, F. G., Y. Tsujimoto, and C. M. Croce.** 1987. Oncogene activation by chromosome translocation in human malignancy. Annu. Rev. Genet. **21**:321–338.
27. **Jerne, N. K., C. Henry, A. A. Nordin, H. Fuji, A. M. C. Koros, and I. Lefkovits.** 1974. Plaque-forming cells: methodology and theory. Transplant. Rev. **18**:130–191.
28. **Keil, R. L., and G. S. Roeder.** 1984. Cis-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell **39**:377–386.
29. **Klar, A. J. S., J. N. Strathern, and J. B. Hicks.** 1981. A position-effect control for gene transposition: state of expression of yeast mating-type genes affects their ability to switch. Cell **25**:517–524.
30. **Kohler, G., M. J. Potash, H. Lehrach, and M. J. Shulman.** 1982. Deletions in immunoglobulin mu chains. EMBO J. **1**:555–563.
31. **Kohler, G., and M. J. Shulman.** 1980. Immunoglobulin M mutants. Eur. J. Immunol. **10**:467–476.
32. **Lennon, G. G., and R. P. Perry.** 1990. The temporal order of appearance of transcripts from unrearranged and rearranged Ig genes in murine fetal liver. J. Immunol. **144**:1983–1987.
33. **Liskay, R. M., A. Letsou, and J. L. Stachelek.** 1987. Homology requirement for efficient gene conversion between duplicated chromosomal sequences in mammalian cells. Genetics **115**:161–167.
34. **Liskay, R. M., and J. L. Stachelek.** 1986. Information transfer between duplicated chromosomal sequences in mammalian cells involves contiguous regions of DNA. Proc. Natl. Acad. Sci. USA **83**:1802–1806.
35. **Liskay, R. M., J. L. Stachelak, and A. Letsou.** 1984. Homologous recombination between repeated chromosomal sequences in mouse cells. Cold Spring Harbor Symp. Quant. Biol. **49**:183–189.
36. **Maizels, N.** 1989. Might gene conversion be the mechanism of somatic hypermutation of mammalian immunoglobulin genes? Trends Genet. **5**:4–8.
37. **McCormack, W. T., L. W. Tjoelker, and C. B. Thompson.** 1991. Avian B-cell development: generation of an immunoglobulin repertoire by gene conversion. Annu. Rev. Immunol. **9**:219–241.
38. **Mellor, A. L., E. H. Weiss, K. Ramachandran, and R. A. Flavell.** 1983. A potential donor gene for the *bm1* gene conversion event in the C57BL mouse. Nature (London) **306**:792–795.
39. **Morrison, S. L., and M. D. Scharff.** 1981. Mutational events in mouse myeloma cells. Crit. Rev. Immunol. **3**:1–22.
40. **Nicholls, R. D., N. Fishel-Ghodsian, and D. R. Higgs.** 1987. Recombination at the human α-globin gene cluster: sequence features and topological constraints. Cell **49**:369–378.
41. **Nishioka, Y., and P. Leder.** 1980. Organization and complete sequence of identical embryonic and plasmacytoma κ V-region genes. J. Biol. Chem. **255**:3691–3694.
42. **O'Gorman, S., D. T. Fox, and G. Wahl.** 1991. Recombinase-mediated gene activation and site-specific integration in mammalian cells. Science **251**:1351–1355.
43. **Powers, P. A., and O. Smithies.** 1986. Short gene conversions in the human fetal globin gene region: a by-product of chromosome pairing in meiosis? Genetics **112**:343–358.
44. **Richards, J. E., A. C. Gilliam, A. Shen, P. W. Tucker, and F. R. Blattner.** 1983. Unusual sequences in the murine immunoglobulin μ-δ heavy-chain region. Nature (London) **306**:483–487.
45. **Rubnitz, J., and S. Subramani.** 1986. Extrachromosomal and chromosomal gene conversion in mammalian cells. Mol. Cell. Biol. **6**:1608–1614.
46. **Schlissel, M., and D. Baltimore.** 1989. Activation of immunoglobulin kappa gene rearrangement correlates with induction of germline kappa gene transcription. Cell **58**:1001–1007.
47. **Shimizu, A., and T. Honjo.** 1984. Immunoglobulin class switching. Cell **36**:801–803.
48. **Shulman, M. J., C. Heusser, C. Filkin, and G. Kohler.** 1982. Mutations affecting the structure and function of immunoglobulin M. Mol. Cell. Biol. **26**:1033–1043.
49. **Shulman, M. J., L. Nissen, and C. Collins.** 1990. Homologous recombination in hybridoma cells: dependence on time and fragment length. Mol. Cell. Biol. **10**:4466–4472.
50. **Smith, A. J. H., and P. Berg.** 1984. Homologous recombination between defective *neo* genes in mouse 3T6 cells. Cold Spring Harbor Symp. Quant. Biol. **49**:171–181.
51. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
52. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
53. **Strathern, J. N., A. Klar, J. Hicks, J. Abraham, J. Ivy, K. Nasmyth, and C. McGill.** 1982. Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the MAT locus. Cell **31**:183–192.
54. **Stringer, J. R.** 1985. Recombination between poly[d(GT) · d(CA)] sequence in simian virus 40-infected cultured cells. Mol. Cell. Biol. **5**:1247–1259.
55. **Subramani, S., and J. Rubnitz.** 1985. Recombination events after transient infection and stable integration of DNA into mouse cells. Mol. Cell. Biol. **5**:659–666.
56. **Taub, R. A., G. F. Hollis, P. A. Hieter, S. Korsmeyer, T. A. Waldmann, and P. Leder.** 1983. Variable amplification of immunoglobulin λ light-chain genes in human populations. Nature (London) **304**:172–174.
57. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56**:619–630.
58. **Timsit, Y., E. Vilbois, and D. Moras.** 1991. Base-pairing shift in the major groove of $(CA)_n$ tracts by B-DNA crystal structures. Nature (London) **354**:167–170.
59. **Treco, D., and N. Arnheim.** 1986. The evolutionarily conserved repetitive sequence $d(TG \cdot AC)_n$ promotes reciprocal exchange and generates unusual recombinant tetrads during yeast meiosis. Mol. Cell. Biol. **6**:3934–3947.
60. **Trimble, W. S., M. D. Baker, G. L. Boulianne, H. Murialdo, N. Hozumi, and M. J. Shulman.** 1986. Analysis of hybridoma mutants defective in synthesis of immunoglobulin M. Somatic Cell Mol. Genet. **12**:467–477.
61. **Tsujimoto, Y., J. Gorham, J. Cossman, E. Jaffe, and C. M. Croce.** 1985. The t(14:18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science **229**:1390–1393.
62. **Voelkel-Meiman, K., R. L. Keil, and G. S. Roeder.** 1987. Recombination-stimulating sequences in yeast ribosomal RNA correspond to sequences regulating transcription by RNA polymerase I. Cell **48**:1071–1079.
63. **Wahls, W. P., L. J. Wallace, and P. D. Moore.** 1990. The Z-DNA motif $d(TG)_{30}$ promotes reception of information during gene conversion events while stimulating homologous recombination in human cells in culture. Mol. Cell. Biol. **10**:785–793.
64. **Walsh, J. B.** 1987. Sequence-dependent gene conversion: can duplicated genes diverge fast enough to escape conversion? Genetics **117**:543–557.
65. **Wittig, B., T. Dorbic, and A. Rich.** 1991. Transcription is associated with Z-DNA formation in metabolically active permeabilized mammalian cell nuclei. Proc. Natl. Acad. Sci. USA **88**:2259–2263.
66. **Wysocki, L. J., and M. L. Geftner.** 1989. Gene conversion and the generation of antibody diversity. Annu. Rev. Biochem. **58**:509–531.
67. **Yu, H., and L. A. Eckhardt.** 1986. DNA rearrangement causes a high rate of spontaneous mutation at the immunoglobulin heavy-chain locus of a mouse myeloma cell line. Mol. Cell. Biol. **6**:4228–4235.
68. **Zoller, M. J., and M. Smith.** 1982. Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA. Nucleic Acids Res. **10**:6487–6500.