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High-Frequency Gene Conversion between Repeated Cμ Sequences Integrated at the Chromosomal Immunoglobulin μ Locus in Mouse Hybridoma Cells

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The occurrence of mitotic recombination between repeated immunoglobulin μ gene constant (Cμ) region sequences stably integrated at the haploid chromosomal immunoglobulin μ locus in murine hybridoma cells was investigated. Recombination events are detected as changes in hapten-specific immunoglobulin M production. Recombination occurs with high frequency (0.5 to 0.8%) by a mechanism consistent with gene conversion. A double-strand break repair-like mechanism is suggested by the finding that repair of a 2-bp deletion mutation and a 2-bp insertion mutation occurs with parity in a donor-directed manner. The results also suggest that the gene conversion process is directional in that the 5′ Cμ region sequence is preferentially converted.

During mitotic growth in eukaryotic cells, both reciprocal recombination and gene conversion events can occur between repeated sequences. Reciprocal recombination generates chromosomal rearrangements, such as deletions, inversions, amplifications, and translocations, which although important for the assembly and expression of genes, can also be deleterious to the cell (10). On the other hand, gene conversion transfers genetic information in a nonreciprocal fashion and was first proposed as a mechanism to explain the aberrant segregation of alleles during fungal meiosis (31). In *Saccharomyces cerevisiae*, gene conversion is important in regulating mating-type switching (19, 39). Gene conversion is also thought to be important in maintaining similarity between repeated eukaryotic genomic DNA sequences (7, 32, 33, 41, 49), variation of trypanosome surface glycoproteins (26), somatic diversification of the avian immune system (27), mammalian variable and constant region gene segment evolution (49), and the diversification of the mammalian major histocompatibility complex locus (15a). However, the hallmark of gene conversion in *S. cerevisiae*, that is, the nonreciprocal transfer of genetic information, has been difficult to establish in mammalian cells because of the inability to recover all products of recombination.

Several models have been proposed to account for recombination data. The Holliday model (16) proposes that strands of the same polarity are nicked at homologous sites and then exchanged to produce heteroduplex DNA (hDNA). The Meselson-Radding (Aviemore) model (28), an extension of the Holliday model, also postulates a role for nicking but only on one DNA strand. The nicked strand invades the homologous duplex and serves as a primer for DNA repair synthesis, resulting in the displacement of a single DNA strand. The displaced strand is degraded, and asymmetric hDNA is enlarged by DNA synthesis on the donor strand. In both models, the Holliday junction can be resolved either with or without the exchange of flanking markers. Repair of the hDNA intermediate results in gene conversion. The differential repair of such hDNA has been used to explain disparities in the frequency of converting small deletions and insertions in the fungi *Ascobolus immersus* and *Sordaria brevicollis* (22, 23, 50). On the other hand, the double-strand break repair (DSBR) model (42) has been proposed to explain recombination data for *S. cerevisiae*, in which virtually all mutations are repaired to the wild type with similar efficiencies (31). In the DSBR model, a double-strand cut is made in the recipient duplex and a gap is formed by exonuclease action. Gene conversion results from DNA repair synthesis through information present in the homologous donor duplex. In the DSBR model, two Holliday junctions which can be resolved to produce both crossover and noncrossover configurations are formed.

In this study, we have investigated mitotic recombination in cultured murine hybridoma cells. Our assay detects changes in 2,4,6-trinitrophenyl (TNP)-specific immunoglobulin M (IgM) production as a consequence of recombination between donor and recipient immunoglobulin μ gene constant (Cμ) regions, both integrated at the endogenous TNP-specific haploid chromosomal immunoglobulin μ gene. This system provides a unique opportunity to investigate mitotic recombination at an endogenous, chromosomal mammalian gene. Moreover, it also allows rigorous analysis of the recombination mechanism because hybridoma cells which arise from independent recombination events can be distinguished by the ability to produce TNP-specific IgM, isolated and analyzed at the molecular level. We provide evidence to suggest that recombination between repeated immunoglobulin Cμ regions occurs in a nonreciprocal manner, consistent with a gene conversion mechanism. Of further significance is the fact that gene conversion occurs with high frequency, preferentially in the 5′ Cμ region, and in a donor-directed manner, consistent with a DSBR-like mechanism.

MATERIALS AND METHODS

Hybridoma cell lines and tissue culture. The origins of the Sp6/HL, igm482, and igm10 hybridomas have been described previously (8, 20, 21). The G418-resistant (G418^r) parental hybridomas Im/RCμ482-3 and Im/RCμ-D7 bearing the isogenic immunoglobulin Cμ region duplication integrated at the haploid TNP-specific chromosomal immunoglobulin μ locus were constructed by homologous recombination as previously described (2, 4). Hybridoma cells were grown in Dulbecco's modified Eagle medium containing 13% bovine calf serum (HyClone) and 5.3×10^{-4} M 2-mercaptoethanol. In the case of G418^r hybridomas,

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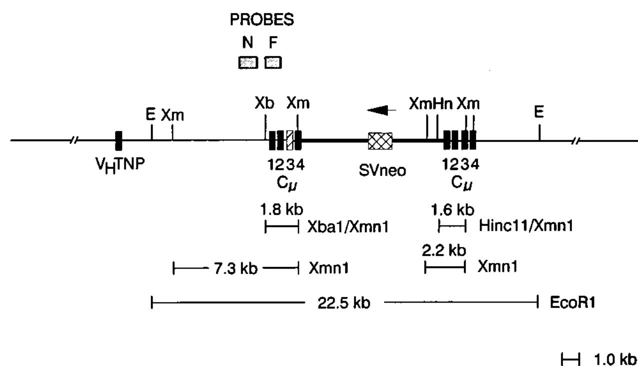

FIG. 1. Structure of the Im/RCμ482-3 haploid chromosomal immunoglobulin μ gene. The sizes (in kilobases) of fragments generated by various restriction enzymes and the positions of DNA probe fragments used in Southern blot hybridization analysis are indicated. Probe N consists of adjacent 475- and 495-bp *Nhe*I fragments, while probe F is an 870-bp *Xba*I-*Bam*HI fragment. The Im/RCμ482-3 chromosomal μ gene was generated by homologous recombination as described previously (4). The homologous recombination event generates a duplication of the chromosomal Cμ region such that the upstream (5′) Cμ region bears the 2-bp Cμ3 deletion (hatched rectangle) of the igm482 hybridoma, whereas the downstream (3′) Cμ region bears the normal Cμ sequence found in the wild-type Sp6/HL hybridoma (8). With the exception of the 2-bp igm482 deletion, which destroys an *Xmn*I restriction enzyme site in Cμ3, the Cμ regions of igm482 and Sp6/HL are isogenic (8, 20, 21). In the wild-type Sp6/HL μ gene, the Cμ3 and Cμ4 *Xmn*I sites are separated by 224 bp (13). Abbreviations: E, *Eco*RI; Hn, *Hinc*II; Xb, *Xba*I; Xm, *Xmn*I; V_HTNP, TNP-specific heavy chain variable region; SVneo (cross-hatched box), neomycin phosphotransferase gene of pSV2neo (37).

Dulbecco's modified Eagle medium was supplemented with G418 at an active concentration of 600 μg/ml.

Assay for recombinant cells. TNP-specific plaque assays (2, 11, 17) were used to assay recombinant plaque-forming cells (PFCs). PFCs were isolated as described previously (3), cloned, and saved for analysis. Recombinants which generated noncytolytic IgM were identified by plating parental hybridoma cells on 96-well microtiter plates. Following colony growth, culture supernatants were assayed for the ability to lyse TNP-coupled sheep erythrocytes in spot tests (1). Noncytolytic hybridoma clones were isolated from plates and saved for analysis.

Growth of hybridoma cells. To measure hybridoma growth, approximately 10^6 exponentially growing hybridoma cells were removed from the culture medium, washed, and resuspended in 10 ml of Dulbecco's modified Eagle medium supplemented with G418 in a tissue culture flask. Flasks were placed at 37°C, and at 24-h intervals, the number of viable cells per milliliter was determined by trypan blue exclusion. The generation time (G) was calculated as follows: $G = t/3.3 \log (N_t/N_0)$, where t is time, N_t is the number of cells per milliliter at time t, and N_0 is the number of cells per milliliter at time zero.

DNA and IgM analysis. High-molecular-weight DNA was isolated from the various hybridoma cell lines by the method of Gross-Bellard et al. (14). Restriction enzymes were purchased from Bethesda Research Laboratories, New England Biolabs, Inc., and Boehringer Mannheim Biochemicals and used in accordance with manufacturer specifications. Agarose gel electrophoresis, Southern blotting onto nitrocellulose, and hybridization were performed as previously described (36, 45). ^{32}P-labelled DNA probe fragments were prepared by using the Multiprime DNA labelling system (Amersham). IgM was biosynthetically labelled with [^{35}S]methionine, purified by binding to goat anti-mouse μ-chain-specific serum coupled to protein A-Sepharose beads, and eluted with 0.5% sodium dodecyl sulfate (SDS). Disulfide bonds were reduced with 2-mercaptoethanol and the μ and κ chains were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (21) and visualized by fluorography.

RESULTS

Recombination between repeated immunoglobulin Cμ regions integrated at the chromosomal immunoglobulin μ locus. The haploid chromosomal immunoglobulin μ gene in the Im/RCμ482-3 hybridoma was constructed by gene targeting (4) (Fig. 1). Briefly, the wild-type Sp6/HL hybridoma, which bears a single copy of the TNP-specific chromosomal μ gene and makes cytolytic, polymeric IgM(κ) specific for hapten TNP, was used as the recipient for gene transfer. The Cμ region of the igm482 hybridoma (an Sp6/HL-derived mutant which bears a 2-bp deletion in the third constant region domain of the μ gene [Cμ3] [8]) was introduced into Sp6/HL cells on the pSV2neo (37)-derived vector pRCμ482 (4). Homologous recombination between vector-borne and chromosomal Cμ regions resulted in the integration of a single copy of pRCμ482 into the Sp6/HL chromosomal μ gene, generating a duplication of the immunoglobulin Cμ region. Thus, in Im/RCμ482-3, the upstream (5′) Cμ region bears the 2-bp igm482 Cμ3 deletion and is present in its normal position to be expressed 3′ of the TNP-specific heavy chain variable region (V_HTNP). The 5′ mutant igm482 Cμ region is separated from the isogenic downstream (3′) wild-type Sp6/HL Cμ region by the integrated pSV2neo vector. The 2-bp igm482 deletion results in the synthesis of a truncated μ chain which lacks the Cμ4 domain (8). Since the Cμ4 domain is required for the assembly of cytolytic, polymeric IgM (6), TNP-specific IgM synthesized by Im/RCμ482-3 (and igm482) is monomeric and fails to lyse TNP-coupled sheep erythrocytes (4).

TABLE 1. Correction of the 2-bp deletion in the 5′ immunoglobulin Cμ region

Cell line	No. of cells screened	No. of PFCs	Frequency[a]
Im/RCμ482-3	6.6×10^5	861	0.0013
Im/RCμ-D7/7[b]	1.5×10^7	0	$<0.7 \times 10^{-7}$
Sp6/HL	300	227	0.76
igm482	1.0×10^7	0	$<1.0 \times 10^{-7}$

[a] Based on three experiments.

[b] The chromosomal μ gene in the parental hybridoma Im/RCμ-D7 was constructed by homologous recombination (2) and bears the wild-type Sp6/HL 5′ Cμ region and the mutant igm482 3′ Cμ region separated by the integrated pSV2neo transfer vector. Im/RCμ-D7/7 is a representative recombinant generated by intrachromosomal gene conversion between the Im/RCμ-D7 Cμ regions. In Im/RCμ-D7/7, both 5′ and 3′ Cμ regions bear the 2-bp igm482 deletion (described in Results).

In principle, the 5′ igm482 Cμ region could act as the recipient for recombination with the 3′ wild-type Cμ region. Such a recombination event is expected to correct the 2-bp igm482 deletion, allowing recombinant cells to be detected as PFCs in a TNP-specific plaque assay (2). Indeed, G418^r TNP-specific PFCs are detected in the Im/RCμ482-3 cell line (frequency, 0.0013) (Table 1). The generation of PFCs is dependent on the presence of the 3′ wild-type Cμ region and thus is likely due to homologous recombination, because PFCs are not detected in the Im/RCμ-D7/7 hybridoma, in which both of the 5′ and 3′ immunoglobulin Cμ regions bear the 2-bp igm482 deletion (frequency, $<0.7 \times 10^{-7}$). Table 1 also presents the efficiencies of detecting TNP-specific PFCs in wild-type Sp6/HL (frequency, 0.76) and mutant igm482 (frequency, $<1 \times 10^{-7}$) hybridomas as controls.

The finding that the 5′ mutant igm482 Cμ region acts as a recombination recipient prompted investigation into whether it can also serve as a recombination donor, allowing insertion of the 2-bp igm482 deletion into the 3′ wild-type Cμ region. A recombinant of this type would be incapable of generating PFCs because both of the 5′ and 3′ immunoglobulin Cμ regions would contain the 2-bp igm482 deletion. To screen for recombinants of this type, Im/RCμ482-3 cells were cloned (0.1 cell per well) and approximately 5×10^4 cells from each of 955 individual colonies were examined for TNP-specific PFCs in Cunningham chambers (11). Colony screening revealed no colonies that did not contain PFCs, indicating that the frequency of inserting the 2-bp igm482 deletion into the 3′ wild-type Cμ region is <1/955 (<0.0010).

Interestingly, 8 of these 955 colonies caused complete lysis of

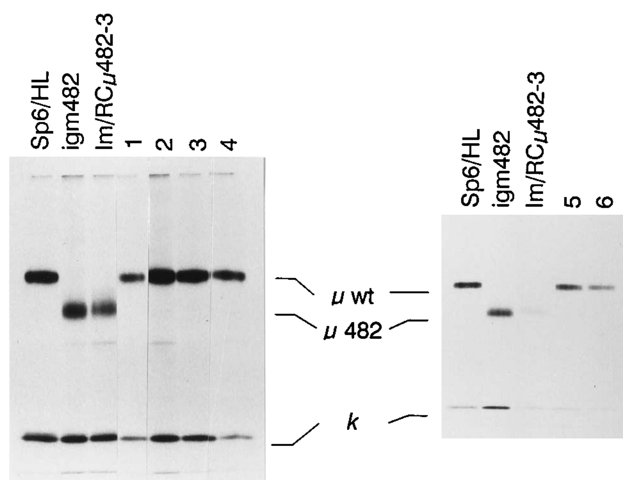

FIG. 2. Production of wild-type μ chains in lytic cell lines. Lytic cell lines (lanes 1 to 6) obtained from Im/RCμ482-3 colony assays along with various control hybridomas were biosynthetically labelled with [^{35}S]methionine. Intracellularly labelled material was immunoprecipitated with goat anti-mouse μ-chain-specific antisera coupled to protein A-Sepharose beads and eluted with 0.5% SDS. Following reduction of disulfide bonds with 2-mercaptoethanol and separation by SDS-PAGE, the μ and κ chains were visualized by fluorography (21). The positions of the TNP-specific, wild-type Sp6/HL and mutant igm482 μ chains (μ wt and μ 482, respectively) and the TNP-specific κ chain (κ) are indicated.

the TNP-coupled sheep erythrocytes in Cunningham chambers, suggesting a high frequency of PFCs in these cultures. Lytic colonies might be mixed, composed of Im/RCμ482-3 cells as well as recombinant PFCs generated early in the growth of each colony. Since PFCs and Im/RCμ482-3 cells grow with the same generation time (17 h), a mixed culture should be revealed by synthesis of both wild-type Sp6/HL and mutant igm482 μ chains. However, examination of intracellular μ chains produced by representative lytic cultures reveals only the full-length Sp6/HL wild-type μ chain, clearly distinguished from the approximately 12-kDa-smaller igm482 μ chain (Fig. 2). Also, control experiments (data not shown) reveal that lytic cultures and wild-type Sp6/HL cells produce TNP-specific PFCs with similar efficiency. Thus, lytic colonies are homogeneous, composed only of PFCs. This suggests that each lytic colony is derived from an individual recombinant cell deposited in the culture well. Accordingly, we conclude that the frequency of correcting the 2-bp deletion in the 5′ Cμ region of Im/RCμ482-3 by homologous recombination is 8/955 (0.008), not 0.0013 as reported in Table 1. The difference in detecting recombinant cells by plaque and colony assays may indicate that cells which have recently undergone recombination do not synthesize and/or secrete enough IgM to be detected by the plaque assay. The disparity in the frequency of recombination between the 5′ and 3′ Cμ regions is addressed below.

PFCs are generated by gene conversion. Ten representative G418^r PFCs were isolated from the Im/RCμ482-3 culture, and their μ gene structure was analyzed by Southern blot hybridization analysis. The restriction enzyme sites and corresponding fragment sizes for the Im/RCμ482-3 haploid chromosomal μ gene along with the positions of various DNA probe fragments are presented in Fig. 1. To indicate the specificities of probe fragments for the chromosomal μ gene, we have included DNA from the cell line igm10, an Sp6-derived mutant that lacks the TNP-specific chromosomal μ gene.

The results discussed above suggest that the 2-bp deletion in the 5′ Cμ region has been repaired by homologous recombination in PFCs. For verification, we examined the results of an *Xba*I-*Xmn*I digest of PFC genomic DNA probed with fragment F (Fig. 3A). The 2-bp deletion in the igm482 hybridoma destroys an *Xmn*I site normally found in the Sp6/HL Cμ3 exon (8, 13). Thus, a 1.6-kb *Xba*I-*Xmn*I wild-type fragment is found in Sp6/HL, whereas a 1.8-kb *Xba*I-*Xmn*I mutant fragment is present in igm482 and Im/RCμ482-3 hybridomas. All PFCs have lost the 1.8-kb *Xba*I-*Xmn*I fragment, indicating that the mutant igm482 Cμ region is absent, and bear, instead, the 1.6-kb *Xba*I-*Xmn*I wild-type Cμ region fragment expected for correct homologous recombination. In addition, PFCs also contain the 3′ Cμ region on an approximately 2.2-kb *Xmn*I fragment (Fig. 1), which suggests that it bears the wild-type sequence (Fig. 3C).

The chromosomal μ gene flanking structures in Im/RCμ482-3 and PFCs were compared by using an *Eco*RI digest analyzed with probe fragment N (Fig. 3B). The Sp6/HL and igm482 chromosomal μ genes are present on a 12.5-kb *Eco*RI fragment (2). The insertion of the 10-kb pRCμ482 targeting vector into the Sp6/HL chromosomal μ gene generates the 22.5-kb *Eco*RI chromosomal μ gene fragment visible in Im/RCμ482-3. All PFCs also bear the 22.5-kb *Eco*RI fragment, indicating that they have retained the overall Im/RCμ482-3 chromosomal μ gene structure.

Either gene conversion or double reciprocal recombination is expected to have generated G418^r PFCs. A *Hin*cII-*Xmn*I digest of PFC DNA analyzed with probe F was used to distinguish between these possibilities. As presented in Fig. 1, this double digest generates a 1.6-kb *Hin*cII-*Xmn*I 3′ wild-type Cμ region fragment in Im/RCμ482-3. In PFCs, the size of the *Hin*cII-*Xmn*I 3′ Cμ region fragment differs, depending on the mechanism of recombination. PFCs generated by gene conversion are expected to retain the 1.6-kb *Hin*cII-*Xmn*I 3′ wild-type Cμ region fragment regardless of whether conversion occurs between Cμ regions on the same chromosome or by intrachromatid or sister chromatid gene conversion after DNA replication. In contrast, intrachromosomal or intrachromatid double reciprocal recombination is expected to place the 2-bp deletion in the 3′ Cμ region, generating a 1.8-kb *Hin*cII-*Xmn*I fragment detected with probe F. As shown in Fig. 3C, all PFCs bear the 1.6-kb *Hin*cII-*Xmn*I 3′ wild-type Cμ region fragment in addition to the 7.1-kb *Xmn*I fragment that bears the 5′ wild-type Cμ region.

While the presence of the 1.6-kb *Hin*cII-*Xmn*I 3′ wild-type Cμ region fragment rules out intrachromosomal (and intrachromatid) double reciprocal recombination, it does not distinguish between gene conversion and unequal sister chromatid double reciprocal recombination. Unequal sister chromatid double reciprocal recombination generates two different cells following cell division; one is a PFC identical to that formed by gene conversion, and the second is a non-PFC that bears the 2-bp igm482 deletion in both 5′ and 3′ Cμ regions. Recombinant cells in which the 5′ and 3′ Cμ regions bear wild-type or mutant igm482 sequences have similar generation times; the Im/RCμ-D7-derived recombinant (D7/7) which bears 5′ and 3′ mutant igm482 Cμ regions (Table 1, footnote *b*) has a generation time of 16.4 h, similar to those of the parental Im/RCμ-D7 cell line (17.8 h) and hybridoma Im/RCμ482-3/7 (PFC isolate no. 7 in Fig. 3), which bears 5′ and 3′ wild-type Sp6/HL Cμ regions (17.4 h). Thus, these two cell types are completely viable in culture and, therefore, are expected at the same frequency following unequal sister chromatid double reciprocal recombination. However, the Im/RCμ482-3 colony screening described above indicates that PFCs which bear 5′

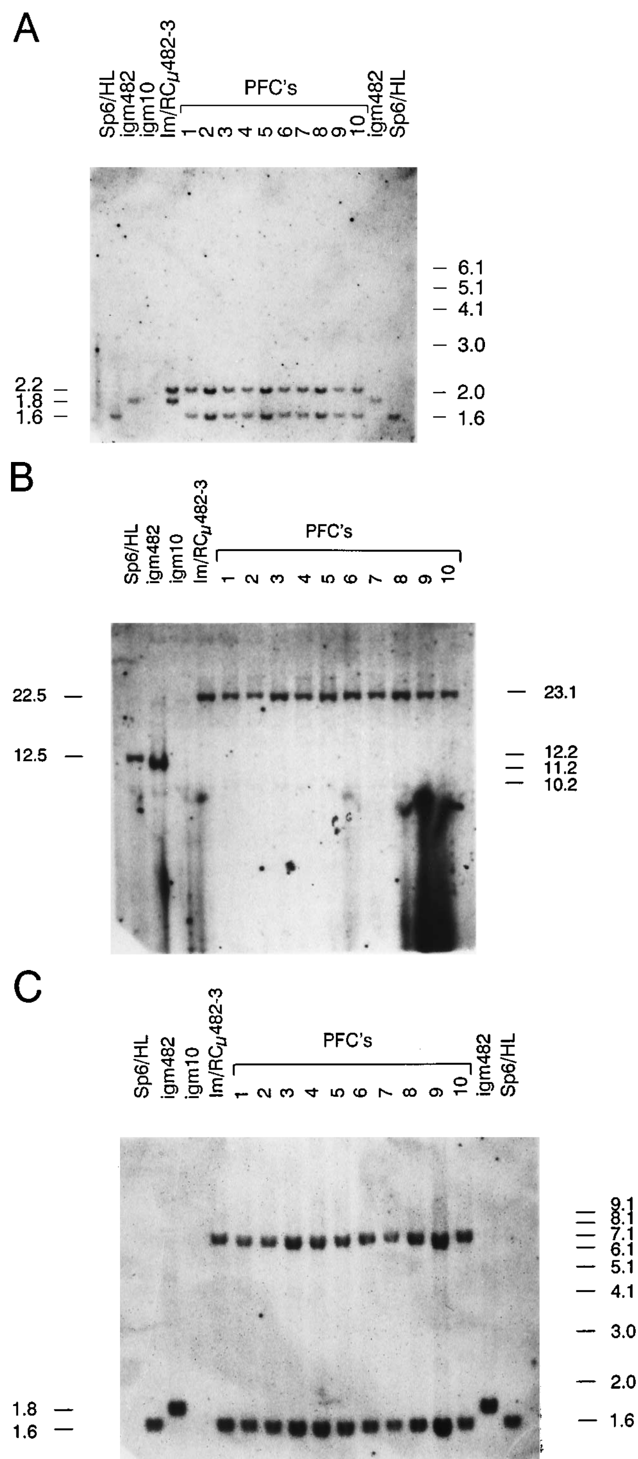

FIG. 3. Analysis of μ gene structure in PFCs. High-molecular-weight DNA from the indicated hybridoma cell lines was digested with *Xba*I-*Xmn*I (A), *Eco*RI (B), and *Hin*cII-*Xmn*I, electrophoresed through agarose gels with consistencies of 1.0% (A and C) and 0.7% (B), and blotted to nitrocellulose. The results presented in panels A and C were obtained following hybridization with probe fragment F, while probe N was used to obtain the results shown in panel B. In panel C, the 1.6- and 1.8-kb μ gene fragments in the Sp6/HL and igm482 control cell lines, respectively, were generated after digestion of the respective genomic DNA with *Xba*I-*Xmn*I. The sizes (in kilobases) of fragments of interest and the positions of DNA marker bands (1-kb ladder [Gibco/BRL] and *Hin*dIII-digested λ DNA) are presented on the left and right, respectively.

and 3′ wild-type Cμ regions are detected at least eightfold more frequently than non-PFCs in which both Cμ regions bear the 2-bp igm482 deletion. This result is inconsistent with a mechanism of unequal sister chromatid double reciprocal recombination, suggesting that PFCs are generated by gene conversion instead.

Repair of a deletion mutation and an insertion mutation by gene conversion. Gene conversion repairs the 2-bp igm482 deletion in the 5′ Im/RCμ482-3 Cμ region by using the 3′ wild-type Cμ region as the conversion donor with a frequency of 0.008. The reverse reaction, in which the 2-bp igm482 deletion is transferred from the 5′ Cμ region into the 3′ wild-type Cμ region, occurs at least eightfold less frequently (frequency, <0.001). These values are significantly different ($P < 0.005$; χ^2 test). This suggests either that gene conversion repairs the 2-bp igm482 deletion more than eight times more efficiently than the corresponding 2-bp insertion (the wild-type sequence) or that gene conversion is influenced by the position of the recipient Cμ region in the μ locus.

To distinguish between these possibilities, gene conversion was examined in the related hybridoma cell line Im/RCμ-D7, in which the isogenic wild-type and mutant igm482 chromosomal Cμ regions are reversed; the 5′ Cμ region bears the wild-type sequence, while the 3′ Cμ region bears the 2-bp igm482 deletion (2). The 3′ mutant igm482 Cμ region is expected to act as the gene conversion donor, transferring the 2-bp deletion into the recipient 5′ wild-type Cμ region. Thus, while Im/RCμ-D7 synthesizes the full-length Sp6/HL wild-type μ chain and secretes cytolytic, polymeric TNP-specific IgM (2), recombinant cells are expected to synthesize the igm482 μ chain, allowing their detection as noncytolytic cells in colony assays. Screening 1,036 individual Im/RCμ-D7 colonies revealed that 5 were noncytolytic (frequency, 0.005). According to Southern blot analysis similar to that presented in Fig. 3 (data not shown), all five bear the 2-bp igm482 deletion in both 5′ and 3′ Cμ regions, suggesting that they are generated by gene conversion. From the recombination frequencies and numbers of cell generations, the mean rate of gene conversion in the 5′ Cμ region of Im/RCμ482-3 and Im/RCμ-D7 is 1.5×10^{-4} and 1.3×10^{-4} recombinants per cell generation, respectively, values which are not significantly different ($P = 0.001$; t test). This suggests that the 2-bp deletion mutation and 2-bp insertion mutation, when present in the 5′ Cμ region, are converted with similar efficiency by information contained in the 3′ donor Cμ region. Therefore, these results suggest that the >eightfold-higher frequency of gene conversion in the 5′ Cμ region of Im/RCμ482-3 is not due to preferential correction of the 2-bp deletion but is due to the 5′ Cμ region being a preferred recipient during gene conversion.

DISCUSSION

Intrachromosomal recombination occurs with high frequency between repeated, isogenic immunoglobulin Cμ regions integrated at the haploid chromosomal immunoglobulin μ locus in mouse hybridoma cell lines. The results suggest that recombinant cells arise from the nonreciprocal transfer of information from donor to recipient Cμ regions, consistent with a mechanism of gene conversion. In this study, it was not possible to determine whether gene conversion occurred within a chromosome or, following DNA replication, within a chromatid or unequally between sister chromatids. However, studies of chromatid interactions during intrachromosomal recombination in mouse cells (9) suggest that recombination may involve the interaction of sister chromatids.

In a previous study (1), a comparison between the occur-

rence of single reciprocal recombination events and that of presumed gene conversion events in hybridoma cell lines which harbored the same immunoglobulin Cμ repeats was made. Approximately 33% of recombination events resulted from single reciprocal recombination, while the majority (67%) were attributed to gene conversion. The results of this study suggest that the majority of recombination events between Cμ repeats are in fact bona fide gene conversions.

A mechanism for the gene conversion process is suggested by the finding that a 2-bp deletion mutation and a 2-bp insertion mutation, when present in the 5′ recipient Cμ region, are repaired with parity in favor of the sequence contained in the 3′ donor Cμ region. For this pair of mutations, gene conversion is apparently donor directed rather than genotype directed. While the genotype-directed pathway is consistent with gene conversion that occurs through the repair of hDNA (28), the donor-directed pathway is most consistent with a DSBR-like model (42). Although our studies do not prove that the 2-bp insertion and deletion mutations are repaired by a DSBR mechanism, the observation that the genotypes of recombinants are biased in favor of the downstream donor Cμ region is consistent with this interpretation.

Intrachromosomal gene conversion between repeated immunoglobulin Cμ regions is approximately 10^3- to 10^5-fold higher than the frequency of recombination between different duplicated segments randomly integrated in the genomes of other mammalian cell lines (15, 25, 34, 40, 46). The orientation of the *neo* gene with respect to the transcriptional direction of the chromosomal μ gene, that is, whether it is reversed as in this study or in tandem as reported previously (1), does not appear to influence recombination frequency. Hybridoma cells also do not appear to be unusually recombinogenic, because the frequency of recombination between transferred and chromosomal immunoglobulin genes in hybridoma cells (approximately 10^{-3}) (2, 4, 5) is similar to the frequency of gene targeting for other chromosomal genes in different mammalian cell lines (12, 35, 44).

The unusually high frequency of gene conversion between Cμ repeats encourages further investigation of the mechanism(s) responsible. The apparent involvement of the 5′ Cμ region as a preferred recipient in the conversion process suggests that μ gene transcription and/or protein binding may be important in promoting high-frequency recombination in this chromosomal region, perhaps by facilitating the introduction of DNA breaks which promote recombination. This suggestion is consistent with two sets of models proposed to explain yeast recombination data (19, 31, 38, 39, 43, 47, 48). One set proposes that the process of transcription itself might be responsible, perhaps by increasing DNA accessibility to recombination machinery or to nucleolytic attack, which facilitates recombination. The second set proposes that *cis*-acting sequences and/or special genomic sites promote recombination, perhaps by altering chromatin accessibility or facilitating the binding of proteins important in recombination and/or transcription.

The unusually high frequency of mitotic intrachromosomal gene conversion reported here also differs markedly from the much-lower frequency of mitotic ectopic gene conversion (approximately 10^{-7}) measured previously (3) with the same hybridoma Cμ region repeats. In contrast, during meiosis in mice, intrachromosomal (29) and ectopic (30) gene conversions occur at frequencies of approximately 2 and 0.3%, respectively. This comparison suggests that during mammalian mitotic growth, ectopic recombination is suppressed relative to intrachromosomal recombination. The suppression of mitotic ectopic recombination might reflect the fact that mammalian chromosomes are organized into distinct subnuclear sites in interphase nuclei (24), thus restricting their opportunity for contact. It is also possible that intrachromosomal recombination and ectopic recombination have different requirements during mitosis and meiosis. Intrachromosomal recombination is important in repairing DNA damage (18) and is expected to be retained because it is beneficial. However, the potential deleterious effects of ectopic recombination, for example, the generation of unwanted chromosomal rearrangements (10), may outweigh any potential benefits. Thus, mammalian cells appear to have evolved a mechanism(s) for preserving intrachromosomal recombination and suppressing ectopic recombination.

In summary, the results of this investigation reveal that intrachromosomal gene conversion occurs with unusually high frequency between repeated Cμ region sequences integrated at the chromosomal immunoglobulin μ locus by a suggested DSBR-like mechanism. The preferential involvement of the 5′ Cμ region as the recipient in gene conversion suggests that transcription and/or protein binding may be important in promoting high-frequency recombination in this region. The high frequency of intrachromosomal gene conversion reported here stands in marked contrast to the much-lower frequency of ectopic gene conversion reported previously (3) and suggests that mitotic mammalian cells have evolved constraints on the ability of dispersed repeated sequences to undergo recombination. Continued investigation of this recombination system is expected to illuminate molecular features important in mediating recombination both within and between mammalian chromosomes.

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