In the present study, we investigated intrachromosomal homologous recombination in a murine hybridoma in which the recipient for recombination, the haploid, endogenous chromosomal immunoglobulin \( \mu \)-gene bearing a mutation in the constant (C\( \mu \)) region, was separated from the integrated single copy wild-type donor C\( \mu \) region by \( \sim 1 \) Mb along the hybridoma chromosome. Homologous recombination between the donor and recipient C\( \mu \) region occurred with high frequency, correcting the mutant chromosomal \( \mu \)-gene in the hybridoma. This enabled recombinant hybridomas to synthesize normal IgM and to be detected as plaque-forming cells (PFC). Characterization of the recombinants revealed that they could be placed into three distinct classes. The generation of the class I recombinants was consistent with a simple unequal sister chromatid exchange (USCE) between the donor and recipient C\( \mu \) region, as they contained the three C\( \mu \)-bearing fragments expected from this recombination, the original donor C\( \mu \) region along with both products of the single reciprocal crossover. However, a simple mechanism of homologous recombination was not sufficient in explaining the more complex C\( \mu \) region structures characterizing the class II and class III recombinants. To explain these recombinants, a model is proposed in which unequal pairing between the donor and recipient C\( \mu \) regions located on sister chromatids resulted in two crossover events. One crossover resulted in the deletion of sequences from one chromatid forming a DNA circle, which then integrated into the sister chromatid by a second reciprocal crossover.

Much of our knowledge of intrachromosomal recombination in eukaryotes is derived from the study of recombination between closely linked, homologous repeats in yeast (reviewed in Petes et al. 1991) and mammalian cells (reviewed in Bollag et al. 1989). In these systems, homologous recombination can occur either within the chromosome or, following DNA replication, between sister chromatids and can yield simple gene conversion and/or crossover products. However, investigation of intrachromosomal recombination between a single pair of closely linked homologous sequences contrasts sharply with the normal circumstance in eukaryotic cells where repetitive DNA sequences are dispersed throughout the genome (Rubin et al. 1980; Deininger et al. 1981; Hutchison et al. 1989), providing opportunities for combinatorial alignment and recombination to occur. Synapsis between such ectopic sequences might generate chromosomal structures that would not be predicted on the basis of the simple alignment that precedes recombination between a single pair of closely linked substrates. Consequently, these chromosomal structures might behave differently during recombination, generating products that include those not normally observed for recombination between closely linked repeats.

In a previous study (Baker et al. 1996), we reported a mammalian cell line in which a single homologous recombination donor and recipient sequence were separated by a genetic interval of at least 1 Mb along the chromosome. In the present study, we used this system to examine the effect of this large distance on the products of intrachromosomal homologous recombination. In contrast to the simple crossover and conversion products predicted from previous studies of recombination between closely linked sequences, recombination between the well-separated homologous sequences in this study generated recombinants characterized predominantly by three discrete DNA structural patterns. The generation of class I recombinants was consistent with homologous recombination involving a simple crossover between unequally paired sister chromatids, a mechanism predicted from studies of homologous recombination between closely linked repeats. However, a simple mechanism was not satisfactory in explaining the more complex chromosomal rearrangements detected in the class II and class III recombinants. The DNA structures characterizing recombinant classes II and III can be explained by a model in which homologous recombination between unequally paired sister chromatids was always accompanied by an additional crossover event.
MATERIALS AND METHODS

Hybridomas: The origin of the hybridomas Sp6, igm482, igm10, and E9 along with the methods used for hybridoma culture have been described previously (Köhler et al. 1982; Baumann et al. 1985; Baker et al. 1996).

Recombination analysis: The system used to detect homologous recombination in hybridomas has been described previously (Baker et al. 1998). It is based on the wild-type Sp6 mouse hybridoma that bears a single copy of the well-characterized trinitrophenyl (TNP)-specific chromosomal μ-gene and secretes cytokitic, polymeric TNP-specific IgM(κ) (Köhler and Shulman 1980; Köhler et al. 1982). The Sp6-derived mutant hybridoma, igm482, contains a 2-bp deletion in the third exon of the μ-gene constant region (Cμ3). With the exception of the 2-bp deletion, the wild-type Sp6 and mutant igm482 Cμ regions are isogenic. The 2-bp mutant igm482 Cμ3 deletion results in the synthesis of a truncated μ-chain lacking the Cμ4 domain (Baumann et al. 1985). The IgM bearing this mutant μ-chain is monomeric and cannot activate complement-dependent cytolysis of TNP-coupled sheep erythrocytes (Baumann et al. 1985). Homologous recombination is assayed by the ability of the transferred, wild-type Sp6 Cμ region donor sequence to correct the 2-bp deletion in the mutant igm482 chromosomal Cμ region, which acts as the recipient sequence for homologous recombination. This restores production of the normal, TNP-specific (Sp6) μ-chain in the recombinant cells, allowing them to be detected as individual plaque-forming cells (PFC) in a TNP-specific plaque assay (Baker et al. 1998). A recombinant cell is detected as a PFC in the plaque assay with about the same efficiency as a wild-type Sp6 cell (~0.8 PFC/cell), whereas for the mutant igm482 hybridoma, the frequency is <10^-7 PFC/cell (Baker et al. 1998).

DNA and IgM analysis: High-molecular-weight DNA was prepared as described (Gross-Bellard et al. 1973). Restriction enzymes were purchased from New England Biolabs (Beverly, MA) and Bethesda Research Laboratories (Gaithersburg, MD). DNA electrophoresis was conducted through agarose gels of the consistency indicated in the figure legends. DNA blotting onto nitrocellulose was performed as described (Southern 1975). The 32P-labeled DNA probes were prepared using the Multiprime DNA-labeling system (Amersham, Arlington Heights, IL) with hybridizations being conducted according to previously reported methods (Trimbble et al. 1986). Cloning of Cμ-containing EcoRI fragments from hybridoma genomic DNA was accomplished using Lambda DASH II and Lambda ZAP II cloning kits from Stratagene (La Jolla, CA), according to the specified procedures.

The methods for IgM analysis have been described previously (Köhler and Shulman 1980; Köhler et al. 1982; Shulman et al. 1982). In brief, secreted, TNP-specific IgM biosynthetically labeled with 35S methionine was purified by binding to dinitrophenyl-Sepharose beads. Synthesis of intracellular μ-heavy chain in the hybridomas was examined by immunoprecipitation of 35S-labeled material with goat anti-mouse μ-heavy chain specific serum coupled to Protein A-Sepharose beads. The radioactively labeled material, including μ-heavy chains together with any bound κ-light chains, was eluted from the Sepharose beads with 0.5% SDS and visualized by fluorography after SDS-PAGE. The radioactivity present in the intracellular μ- and κ-chains was quantified by phosphorimager and the μ:κ ratio was determined.

Fluorescence in situ hybridization of hybridoma chromosomes: The 10-kb pTCμ vector (Baker et al. 1998) was used as a probe in fluorescence in situ hybridization (FISH). As indicated previously (Baker et al. 1996), this probe identifies the site of the integrated wild-type Sp6 donor Cμ region as well as the endogenous, recipient chromosomal Cμ region in the hybridoma. The pTCμ probe was labeled by nick translation with biotin and detected with fluorescein isothiocyanate (FITC)-avidin followed by biotinylated anti-avidin antibody and avidin-FITC (Baker et al. 1996). Metaphase chromosomes from the E9 hybridoma as well as representative examples of G418® E9 PFC classes I–III were examined by FISH. The images were captured and processed as described previously (Baker et al. 1996).

RESULTS

Structure of recombination recipient and donor Cμ sequence in the E9 hybridoma: The E9 hybridoma was generated as described previously (Baker et al. 1996) by integration of the plasmid pTCμ (a pSV2neo-derived vector bearing the wild-type Cμ region from the Sp6 hybridoma) into the mutant igm482 hybridoma (Köhler and Shulman 1980; Köhler et al. 1982). Southern blot and FISH analysis (Baker et al. 1996) revealed that, like igm482, E9 also contained a single copy of the endogenous, TNP-specific chromosomal μ-gene located near the telomeric end of murine chromosome 12 (Köfler et al. 1992; Figure 1A). In the present study, measurement of the ratio of intracellular μ-heavy chain to κ-light chain in E9 revealed that it was equivalent to that produced by igm482, providing further evidence in support of a single endogenous chromosomal μ-gene (data not shown). In E9, the haploid, chromosomal μ-gene retains the 2-bp mutant igm482 Cμ3 deletion, a mutation that results in production of a truncated μ-chain that, when incorporated into IgM, is unable to effect complement-dependent lysis of TNP-coupled sheep red cells (Baker et al. 1988). With regard to the integrated wild-type Cμ region in E9, previous Southern blot and FISH studies revealed that it had integrated in single copy ~1 Mb from the endogenous, TNP-specific μ-gene (Baker et al. 1996). Southern blot analysis conducted in this study (data not shown) revealed the wild-type Cμ region structure shown in Figure 1B. The known structure of the E9 donor wild-type and recipient mutant igm482 chromosomal Cμ region provided a unique opportunity to investigate the mechanism of intrachromosomal recombination between these well-separated homologous sequences. As described in materials and methods, such homologous recombination can correct the 2-bp mutant igm482 Cμ3 deletion allowing recombinants making normal IgM to be detected as TNP-specific PFC in a sensitive plaque assay (Baker et al. 1998).

Recombinant isolation: To isolate TNP-specific PFC, six separate E9 hybridoma cultures (started from ~50 cells) were expanded in Dulbecco’s modified Eagle’s medium (DME) in the absence of G418 selection to a density of ~10^5 cells/ml. The hybridomas were retested in DME containing 600 μg/ml of active G418 to confirm the stability of the culture. For verification of μ-gene structure, genomic DNA was isolated from the indepen-
Intrachromosomal Homologous Recombination

Figure 1.—Structure of the haploid, mutant igm482 chromosomal immunoglobulin \( \mu \)-gene and the wild-type Sp6 donor C\( \mu \)-region in the E9 hybridoma. (A) The mutant igm482 recipient chromosomal immunoglobulin \( \mu \)-gene. The chromosomal \( \mu \)-locus is transcribed toward the centromere as indicated by the arrow above V\( \text{HTNP} \) (Koer et al. 1992). (B) The wild-type Sp6 donor C\( \mu \)-region in the E9 hybridoma. As described previously (Baker et al. 1996), a small deletion had removed DNA immediately 5' of C\( \mu \)1 in the integrated donor wild-type C\( \mu \)-region. In this study, more detailed Southern blot analysis of the wild-type donor structure revealed that most, if not all, of the C\( \mu \)1 exon was intact as illustrated. This indicated that the single donor wild-type C\( \mu \)-region shared z\( 4 \) kb of homology (indicated by the thickened line) with the haploid, recipient mutant igm482 chromosomal C\( \mu \)-region. In both A and B, only the sizes of relevant restriction enzyme fragments are shown. The mutant igm482 C\( \mu \)3 exon is indicated by the hatched rectangle in A while the pSV2neo backbone portion of the integrated pTC\( \mu \)vector is indicated by the dashed line in B. DNA probe fragment F is an 870-bp XbaI/BamHI fragment while probe G (not shown) is the 762-bp PvuII fragment from the neo gene of the vector pSV2neo (Southern and Berg 1982). E, EcoRI; Xb, XbaI; Xm, XmnI; C\( \mu \), \( \mu \)-gene constant region exons 1-4; S\( \mu \), \( \mu \)-gene switch region; neo, neomycin phosphotransferase gene; V\( \text{HTNP} \), TNP-specific immunoglobulin \( \mu \)-gene heavy chain variable region.

Table 1

<table>
<thead>
<tr>
<th>Independent E9 culture</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Unusual</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>12</td>
<td>14</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>31</td>
<td>54</td>
<td>5</td>
<td>99</td>
</tr>
</tbody>
</table>

Possible intrachromosomal homologous recombination mechanisms capable of generating G418\(^8\) E9 PFC: Intrachromosomal recombination between the homologous donor and recipient C\( \mu \)-region in the E9 hybridoma might occur by gene conversion and/or single reciprocal crossover. Gene conversion is a nonreciprocal mode of homologous recombination. Consequently, conversion of the recipient mutant igm482 chromosomal C\( \mu \)-region by the integrated donor wild-type C\( \mu \)-region will not alter the size or copy number of the participating sequences. Thus, G418\(^8\) E9 PFC generated by gene conversion are expected to bear a wild-type copy of the chromosomal C\( \mu \)-region contained on the 12.5-kb EcoRI \( \mu \)-gene fragment (Figure 1A) along with the 16.8-kb EcoRI fragment containing the donor wild-type C\( \mu \)-region (Figure 1B) and to synthesize the wild-type, TNP-specific \( \mu \)-heavy chain.

The G418\(^8\) E9 PFC might also be generated by intrachromosomal single reciprocal recombination between the homologous donor and recipient C\( \mu \)-regions. Unlike gene conversion, single reciprocal recombination will alter the size and perhaps the number of \( \mu \)-fragments in the PFC in a predictable way. That is, one product of single reciprocal crossover is expected to be a 21.5-kb EcoRI fragment bearing the recombinant, TNP-specific chromosomal \( \mu \)-gene (encoding the wild-type,
Figure 2.—Influence of donor position and orientation on reciprocal crossover. This figure presents the \( \text{C}_\mu \) region products of single reciprocal crossover in PFC and non-PFC resulting from homologous recombination between the single copy, wild-type donor \( \text{C}_\mu \) region in the following positions relative to the haploid, recipient chromosomal \( \mu \)-gene in the hybridoma: (A) 5' direct, (B) 3' direct, (C) 5' inverted, and (D) 3' inverted. Details are presented in results. EcoRI; \( \text{C}_\mu \), \( \mu \)-gene constant region exons 1-4; neo, neomycin phosphotransferase gene; \( \text{V} \_\text{HTNP} \), TNP-specific \( \mu \)-heavy chain gene variable region. The diagrams are not drawn to scale.

TNP-specific \( \mu \)-heavy chain), while the expected reciprocal product is a nonfunctional 7.3-kb EcoRI \( \text{C}_\mu \) region fragment. In addition, as shown next, the products of single reciprocal crossover can provide information about the position and orientation of the integrated donor wild-type \( \text{C}_\mu \) region relative to the recipient mutant igm482 chromosomal \( \text{C}_\mu \) region in the E9 hybridoma.

Figure 2, A–D, presents the four possible wild-type donor \( \text{C}_\mu \) region configurations in E9 along with the predicted single reciprocal crossover products of homologous recombination in PFC and non-PFC following cell division. The wild-type donor \( \text{C}_\mu \) region might be located 5' of the mutant igm482 \( \text{C}_\mu \) region in direct orientation (Figure 2A). In this configuration, the only single crossover mechanism that could generate G418\(^{\text{R}} \)
TNP-specific PFC would be unequal sister chromatid exchange (USCE). In this case, PFC would contain the indicated triplication of \( \mu \)-containing EcoRI fragments, namely, the 16.8-kb donor wild-type \( \text{C}_\mu \) region fragment, the 12.5-kb mutant igm482 chromosomal \( \text{C}_\mu \) region, and the novel 21.5-kb EcoRI fragment bearing the functional recombinant TNP-specific \( \mu \)-heavy chain gene. The reciprocal product of USCE would be the nonfunctional 7.3-kb \( \text{C}_\mu \) region EcoRI fragment. Following mitosis, G418\(^{\text{R}} \)
PFC would be expected to bear the chromosome containing the \( \mu \)-gene triplication while non-PFC would bear the chromosome with the 7.3-kb \( \text{C}_\mu \) region fragment. Thus, PFC would be expected to synthesize both the wild-type Sp6 and mutant igm482
Intrachromosomal Homologous Recombination

Figure 2B presents the expected crossover products if the donor wild-type C\(\mu\) region was 3' of the recipient mutant igm482 C\(\mu\) region in direct orientation. In this donor configuration, G418\(^8\) PFC might be generated by USCE as shown or by intrachromatid (or intrachromosome) single crossover (not shown). In either case, following mitosis, G418\(^8\) PFC are expected to bear the chromosome with the 21.5-kb EcoRI fragment containing the recombinant TNP-specific \(\mu\)-heavy chain gene. Conversely, the chromosome containing the 12.5-kb, 7.3-kb, and 16.8-kb EcoRI \(\mu\)-gene fragment triplication would be present in the non-PFC. Homologous recombination might also occur via crossover with an inverted donor wild-type C\(\mu\) region located either 5' (Figure 2C) or 3' (Figure 2D) of the mutant igm482 recipient chromosomal C\(\mu\) region. For both donor positions, following crossover and cell division, the chromosome containing the 21.5-kb EcoRI fragment bearing the recombinant, TNP-specific \(\mu\)-gene and the reciprocal product, the 7.3-kb EcoRI C\(\mu\) region fragment, would be present in the G418\(^8\) PFC while the chromosome bearing the 16.8-kb wild-type donor C\(\mu\) region and the 12.5-kb mutant igm482 C\(\mu\)-region EcoRI fragments would be present in the non-PFC. The PFC in Figure 2, B-D, are expected to synthesize the wild-type Sp6 but not the mutant igm482 \(\mu\)-heavy chain.

Analysis of \(\mu\)-gene structure and \(\mu\)-chain protein in E9 PFC: To determine the mechanism of homologous recombination, genomic DNA from the E9 PFC was digested separately with EcoRI and XbaI, enzymes that do not cut within the C\(\mu\) region, and probed with the C\(\mu\) specific fragment F. The results revealed two or more C\(\mu\)-hybridizing fragments in each of the 99 G418\(^8\) E9 PFC, and the conclusions drawn from the use of both noncutters were the same. Therefore, only the EcoRI analysis of \(\mu\)-gene structure in representative E9 PFC is presented (Figure 3A). On the basis of the size of the C\(\mu\) fragments, 94/99 G418\(^8\) PFC could be placed into one of three distinct classes (designated classes I-III). In 5/99 PFC, one of the C\(\mu\) fragments was of a slightly different size, making these PFC marginally different from each other and those E9 PFC characterizing classes I-III (data not shown). However, the C\(\mu\) fragment sizes suggest that these latter PFC might be variations of those defining classes I-III. Table 1 indicates the number of PFC from each of the six independent E9 cultures that was assigned to each class. For convenience, Table 2 compares the C\(\mu\) region fragment sizes expected for single reciprocal crossover between the recipient mutant igm482 C\(\mu\) region and the donor wild-type C\(\mu\) region in each of the four possible configurations shown in Figure 2, A-D, with the \(\mu\)-gene fragment sizes observed in PFC recombinant classes I-III (Figure 3A).

Class I PFC: Class I PFC represented 9 of the 99 TNP-specific PFC and were typified by the C\(\mu\) region triplication in recombinants 8, 16, 18, and 26 (Figure 3A).

A

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
</tr>
</thead>
<tbody>
<tr>
<td>class 1</td>
<td>class 2</td>
<td>class 3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>19</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

B

Figure 3.—Analysis of \(\mu\)-gene structure and \(\mu\)-chain protein in E9 PFC. (A) DNA from the indicated cell lines was digested with EcoRI, electrophoresed through a 0.7% agarose gel, blotted to nitrocellulose and hybridized with probe fragment F. The sizes (in kilobases) of the bands of difference are indicated on the left of the blot. (B) For the analysis of \(\mu\)-chains in E9 PFC, IgM was biosynthetically labeled with \(^{35}\text{S}\)methionine and purified by binding to DNP-Sepharose beads. After SDS elution and reduction with 2-mercaptoethanol, the \(\mu\)- and \(k\)-chains were visualized by fluorography following SDS-PAGE as described in materials and methods.

They contained the 12.5-kb mutant igm482 chromosomal EcoRI \(\mu\)-gene fragment (Figure 1A), the 16.8-kb EcoRI fragment bearing the donor wild-type C\(\mu\) region fragment (Figure 1B), and the novel, recombinant 21.5-kb EcoRI \(\mu\)-gene fragment. Reprobing of the cell lines
TABLE 2

<table>
<thead>
<tr>
<th>Predicted crossover products</th>
<th>Observed products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation Size (kb) PFC class Size (kb)</td>
<td></td>
</tr>
<tr>
<td>5' direct 12.5, 16.8, 21.5</td>
<td>Class I 12.5, 16.8, 21.5</td>
</tr>
<tr>
<td>3' direct 21.5</td>
<td>Class II 7.3, 12.5, 21.5</td>
</tr>
<tr>
<td>5' inverted 7.3, 21.5</td>
<td>Class III 12.5, 16.8</td>
</tr>
<tr>
<td>3' inverted 7.3, 21.5</td>
<td></td>
</tr>
</tbody>
</table>

with the probe G (described in Figure 1 legend) revealed hybridization with only the 16.8-kb and 21.5-kb EcoRI C\textsubscript{\mu} region fragments (data not shown). The presence of the 21.5-kb EcoRI C\textsubscript{\mu} region fragment bearing the recombinant, TNP-specific C\textsubscript{\mu} gene and the 12.5-kb EcoRI fragment bearing the mutant igm482 chromosomal C\textsubscript{\mu} gene in the class I PFC was consistent with the synthesis of both the wild-type and mutant C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.

As described next, neither gene conversion nor the single reciprocal crossover mechanisms presented in Figure 2, A–D, could explain the C\textsubscript{\mu} gene structure or C\textsubscript{\mu} region triplication in the class I PFC as explained above under the description of Figure 2A. The C\textsubscript{\mu} region triplication in the class I PFC was exactly the product expected in a PFC generated by USCE between the mutant igm482 recipient chromosomal C\textsubscript{\mu} region and a 5' directly oriented donor wild-type C\textsubscript{\mu} region (Figure 2A; Table 2) and was not consistent with the products expected for wild-type donor configurations in Figure 2, B–D.
Intrachromosomal Homologous Recombination

Figure 4.—Structure of the participants and products of homologous recombination. (A) The recipient mutant igm482 chromosomal $\mu$-gene; (B) the cloned, 16.8-kb EcoRI fragment bearing the E9 wild-type donor $\mu$ region along with the cloned products of single reciprocal crossover between the donor and recipient $\mu$ regions; namely, (C) the 18.6-kb EcoRI fragment bearing the recombinant TNP-specific $\mu$-gene and (D) the 7.3-kb EcoRI fragment bearing the nonfunctional recombinant $\mu$ region. The TNP-specific $\mu$-heavy chain variable region was not part of the cloned EcoRI segment and so its normal position is indicated by a dotted line in A and C. The alignment of the $\mu$ regions together with the solid and open stars indicate how the recipient (A) and donor (B) $\mu$ regions are linked by single reciprocal crossover in the recombinant products (C and D). $\mu$-gene constant region exons 1–4; $\mu$-gene switch region; neo, neomycin phosphotransferase gene; $V_{H}$ TNP, TNP-specific $\mu$-heavy chain variable region. The diagrams are not drawn to scale.

Figure 5 also presents the FISH analysis of representative metaphases of G418® E9 PFC 16, 3, and 2, members of PFC classes I, II, and III, respectively. The results indicated that all $\mu$-hybridizing sequences were confined to a single chromosome in the PFC. Multiple $\mu$-hybridizing signals were not always detected. In some PFC, only one or two $\mu$ signals were visible but they were considerably larger than in E9, suggesting that they resulted from more than one $\mu$-hybridizing sequence. The failure to consistently resolve multiple $\mu$-hybridizing sequences as discrete FISH signals was not surprising especially considering that the interval separating the sequences was small relative to the length of murine chromosome 12 ($\sim$128 Mb; Mouse Genome Database, The Jackson Laboratory, Bar Harbor, ME). For example, in our earlier study (Baker et al. 1996), the $\sim$1-Mb separation between the donor and recipient $\mu$ sequences in E9 did not consistently lead to their resolution as discrete signals on both sister chromatids.

The $\mu$-gene switch ($S_\mu$) sequence as indicated by the presence of the 3.3-kb XbaI fragment in the cloned recombinant $\mu$-gene (in boldface in Figure 4C) as opposed to the 6.2-kb XbaI $S_\mu$ fragment that is present in the corresponding position of the chromosomal $\mu$-gene (in boldface in Figure 4A; data not shown). The $S_\mu$ deletion in the recombinant TNP-specific $\mu$-gene was expected because as shown previously the $S_\mu$ sequence is unstable during cloning in Escherichia coli; that is, a similar-sized $S_\mu$ deletion (2.8 kb) also occurred during cloning of the chromosomal $\mu$-gene from the wild-type Sp6 hybridoma (Ochi et al. 1983). However, the cloned $\mu$-gene is otherwise wild-type as evidenced by its ability to restore normal IgM production following transfer to the appropriate recipient mutant hybridoma (Ochi et al. 1983). When the 2.9-kb $S_\mu$ deletion in the recombinant TNP-specific $\mu$-gene was taken into account, the restriction enzyme fragment sizes observed in the recombinant products were precisely those predicted for linkage between the wild-type donor and recipient mutant igm482 $\mu$-gene through a single reciprocal crossover event between the homologous $\mu$ regions. Thus, structural analysis of the cloned $\mu$ regions genomic DNA fragments verified that the 21.5-kb and the 7.3-kb EcoRI $\mu$ region fragments observed in the G418® E9 PFC were indeed the products of single reciprocal crossover between the homologous wild-type donor and mutant igm482 recipient $\mu$ regions.

**Fluorescence in situ hybridization:** To determine the genomic location of the various EcoRI $\mu$ region fragments in the class I–III PFC, FISH analysis was performed on hybridoma metaphase chromosomes (Figure 5). For FISH, biotinylated pTC$\mu$ vector DNA was used as a probe; hybridizing signals are revealed in yellow. The vector pTC$\mu$ contains the wild-type donor Sp6 $\mu$ region inserted into pSV2neo as described (Baker et al. 1988) and the $\mu$ region detects all $\mu$-bearing sequences in the hybridoma genome (Baker et al. 1996). In E9 hybridoma, the single copies of both the donor wild-type Sp6 $\mu$ region and recipient mutant igm482 $\mu$ region are revealed near the telomere of murine chromosome 12 as reported previously (Baker et al. 1996). The visualization of separate FISH signals suggests that the donor and recipient $\mu$ sequence are separated by a distance of $\sim$1 Mb along the chromosome (Baker et al. 1996).
between the donor and recipient Cμ regions generated TNP-specific PFC that were detected by a sensitive plaque assay (Baker et al. 1988). Recovery and analysis of G418R TNP-specific PFC revealed the following: (i) G418R PFC could be placed into three distinct classes (class I–III PFC) on the basis of the size of Cμ-hybridizing fragments; (ii) FISH analysis of metaphase chromosomes from representative G418R class I–III PFC indicated that all Cμ-hybridizing sequences were clustered at the telomeric end of murine chromosome 12 near the haploid, recipient mutant igm482 Cμ-locus; (iii) structural analysis of cloned genomic DNA confirmed that the recombinant 21.5-kb and 7.3-kb EcoRI Cμ-region fragments were the reciprocal products of a single crossover event between the donor wild-type Cμ region contained on the 16.8-kb EcoRI fragment and the mutant igm482 recipient chromosomal Cμ region present on the 12.5-kb EcoRI fragment; and (iv) all PFC classes synthesized both the wild-type and mutant Cμ-heavy chains.

The Cμ region structures in the class I PFC were precisely those predicted for USCE between the haploid recipient mutant igm482 Cμ region and a single copy, directly oriented, 5' donor wild-type Cμ region as illustrated in Figure 2A. This mechanism is redrawn in a slightly different form in Figure 6A to allow comparison with recombination mechanisms in Figure 6, B and C, that are proposed to explain PFC classes II and III, respectively. As shown in Figure 6A, USCE exchange at position 1 followed by cell division would generate a PFC bearing the chromosome containing the indicated triplication of Cμ-region-containing fragments as well as a non-PFC bearing the chromosome containing the reciprocal crossover product, the 7.3-kb EcoRI Cμ region fragment. In the class I PFC, the presence of the 21.5-kb EcoRI recombinant Cμ-gene fragment and the 12.5-kb EcoRI mutant igm482 Cμ-gene explain the synthesis of both the wild-type and mutant igm482 Cμ-heavy chains.

The Cμ region structures in the class I PFC were precisely those predicted for USCE between the haploid recipient mutant igm482 Cμ region and a single copy, directly oriented, 5' donor wild-type Cμ region as illustrated in Figure 2A. This mechanism is redrawn in a slightly different form in Figure 6A to allow comparison with recombination mechanisms in Figure 6, B and C, that are proposed to explain PFC classes II and III, respectively. As shown in Figure 6A, USCE exchange at position 1 followed by cell division would generate a PFC bearing the chromosome containing the indicated triplication of Cμ-region-containing fragments as well as a non-PFC bearing the chromosome containing the reciprocal crossover product, the 7.3-kb EcoRI Cμ region fragment. In the class I PFC, the presence of the 21.5-kb EcoRI recombinant Cμ-gene fragment and the 12.5-kb EcoRI mutant igm482 Cμ-gene explain the synthesis of both the wild-type and mutant igm482 Cμ-heavy chains, respectively. As described next, the generation of PFC classes II and III was also consistent with a directly oriented, 5' donor wild-type Cμ region given a slight modification of the proposed class I mechanism.

The proposed class II mechanism (Figure 6B) involves the same chromosome alignment and USCE event between donor and recipient Cμ regions at position 1. However, in conjunction with crossover at position 1, a second crossover at position 2 is proposed. In the event the second crossover occurred first, crossover at position 1 would incorporate the DNA circle and both reciprocal Cμ region products into the recipient sister chromatid. At mitosis, the daughter cell receiving this chromosome would be a class II PFC bearing the 7.3-kb EcoRI Cμ region fragment, the 21.5-kb EcoRI fragment bearing the functional, recombinant TNP-specific Cμ-gene, and the 12.5-kb EcoRI fragment bearing the mutant igm482 chromosomal Cμ region (note that the 7.3- and 21.5-kb EcoRI Cμ-gene fragments in the class II

**DISCUSSION**

The mechanism of homologous recombination was examined in the E9 hybridoma in which the recipient mutant igm482 chromosomal Cμ region and the donor wild-type Cμ region were each present in a single copy and separated by ~1 Mb along the hybridoma chromosome (Baker et al. 1996). Homologous recombination

**Figure 5.**—Fluorescence in situ hybridization of hybridoma chromosomes. FISH was performed on hybridoma metaphase chromosomes as described in materials and methods. The E9 hybridoma along with representative metaphases of G418R TNP-specific PFC from class I (16), class II (2), and class III (2) is presented. The FITC-labeled 10-kb vector pTCμ (yellow signal) was used as a probe (Baker et al. 1988). As shown previously (Baker et al. 1996), the Cμ portion of this probe identifies both the mutant igm482 chromosomal Cμ region and the wild-type donor Cμ region in the hybridoma. For all hybridomas, the pTCμ probe localized to murine chromosome 12 in at least 80% of 20 well-spread metaphases.
PFC are the same reciprocal crossover products produced by the class I USCE mechanism in Figure 6A. The other product of mitosis, the daughter cell receiving the chromosome harboring the 16.8-kb wild-type donor Cμ region, would be a non-PFC. This mechanism explains the Cμ region fragments in the class II PFC (Figure 3A) as well as synthesis of both wild-type and mutant igm482 μ-chains. However, in generating the class II PFC, the possibility cannot be ruled out that USCE at position 1 occurred first, generating an intermediate with the class I structure (Figure 6A). However, before cell division, this intermediate underwent a second crossover that linked the 7.3-, 21.5-, and 12.5-kb EcoRI Cμ region fragments.

The class III PFC contained EcoRI fragments bearing the 16.8-kb wild-type donor Cμ region and the 12.5-kb mutant igm482 chromosomal μ-gene (Figure 3A). The absence of any novel fragments suggested that these PFC might be explained by simple intrachromosomal gene conversion. However, a more complicated mechanism was suggested by the finding that class III PFC synthesized both the wild-type and mutant igm482 μ-heavy chains. These results can be explained by the mechanism in Figure 6C. Gene conversion occurred between unequally paired donor wild-type and recipient mutant igm482 Cμ regions at position 1, correcting the 2-bp mutant igm482 Cμ3 deletion and generating the wild-type Sp6 chromosomal μ-gene. The template for gene conversion might have been the donor wild-type Cμ region on the same chromatid or on the sister chromatid (as indicated by the dashed arrows at position 1 in Figure 6A). In conjunction with gene conversion, a crossover event occurred at position 3, generating a DNA circle; both the gene conversion and crossover event might have been associated. Integration of the DNA circle (containing the conversion product, the wild-type μ-gene) into the recipient sister chromatid by a crossover event within the indicated region of perfect
sequence identity would generate the structure shown. The duplication generated by the crossover event explains the production of both wild-type and mutant \( \mu \)-chains, the equivalent intensities of the EcoRI fragments bearing the 16.8-kb wild-type donor \( \mu \)-region and the 12.5-kb chromosomal \( \mu \)-gene (Figure 3A), and the FISH results indicating localization of the \( \mu \)-fragments on a single chromosome in the class III PFC. However, a variation of this model involving only two crossovers might also explain the class III PFC (not shown). In this alternative, the gene conversion event at position 1 is accompanied by a single illegitimate unequal crossover between the sister chromatids that link the \( \mu \)-region fragments together on the same chromosome. In both the class II and class III PFC, the position of the proposed second crossover and, therefore, the chromosomal distance separating the \( \mu \)-segments in the PFC is unknown.

Previous studies examining intrachromosomal homologous recombination between closely linked sequences in mammalian cells revealed that the recombination products were simple gene conversion and/or crossover (excision) events (Lin and Sternberg 1984; Liskay et al. 1984; Smith and Berg 1984; Rubnitz and Subramani 1986; Baker 1989; Baker and Read 1995). However, in this study, the recombination products characterizing PFC classes II and III were more complex than predicted on the basis of these simple recombination mechanisms. The models presented in Figure 6, A–C, to explain recombination between the well-separated homologous \( \mu \)-regions in this study have not been proposed previously to explain recombination between homologous repeats in mammalian cells.

As depicted in Figure 6A, the least frequent PFC class I (9/99 PFC) is proposed to have been generated by a single USCE event, whereas the more frequent PFC classes II and III require at least two recombination events (Figure 6, B and C, respectively). While these models might seem intuitively unappealing, it is important to point out that multiple, concerted recombination events have been suggested previously to explain mitotic recombination in Saccharomyces cerevisiae (Goldin et al. 1986; Rothstein et al. 1987; Kupiec and Petes 1988; Bethke and Golin 1994) and Arabidopsis (Assaad and Signer 1992), as well as gene targeting in S. cerevisiae (Orr-Weaver et al. 1981), Kluyveromyces lactis (Rossolini et al. 1992), and mammalian cells (P. Ng and M. D. Baker 1999). Furthermore, the pathways proposed in Figure 6, B and C, to explain recombination between direct repeats in the same chromosome, generate a DNA circle that integrates into the bacterial chromosome through a region of shared homology. If the direct repeats have been copied either as two replicons or as a replication bubble, direct-repeat recombination followed by circle integration into one of the two repeat copies will generate a gene triplication (Romero and Palacios 1997) as proposed for the \( \mu \)-gene amplification in the present study in Figure 6, B and C. Strikingly, in the study by Mahan and Roth (1988), the frequency of recombinants generated by the two recombination events of circle excision and circle integration was virtually identical to the frequency of recombinants generated by only a single crossover event, a result similar to the present study, where the frequencies of the class I–III PFC were not enormously different. Of further significance, the efficiency of circle reintegration approached 100% when the amount of shared homology was \( \sim3-4\) kb (Mahan and Roth 1988). In the present study, 4 kb of perfect \( \mu \)-region homology was available at position 1 for circle recapture in generating the class II PFC. In the case of the class III PFC, circle integration into the recipient chromosome may have occurred anywhere within the 0.1–10 Mb of perfect sequence identity located between the donor wild-type and recipient mutant igm482 \( \mu \)-region, perhaps explaining the slightly higher frequency of class III PFC.

While the generation of the class I–III PFC can be explained by the pathways in Figure 6, A–C, respectively, are the results consistent with any other interpretation? As illustrated in Figure 2, B–D, and summarized in Table 1, PFC classes I–III cannot be accounted for by homologous recombination between the single copy donor and recipient \( \mu \)-region in other configurations. However, what if the parental E9 hybridoma was heterogeneous, composed of a subpopulation of cells bearing an intrachromosomal duplication of the donor wild-type and/or recipient mutant igm482 \( \mu \)-region? Such an E9 subpopulation might be present at a low frequency, thus precluding detection by FISH or Southern blot analysis of E9 and its subclones. Thus, might PFC classes I–III be generated from such an E9 subpopulation by simple homologous recombination mechanisms of gene conversion and/or single reciprocal crossover? First, one would have to make the unlikely assumption that all 99 E9 PFC were generated exclusively from the putative subpopulation bearing the duplication. Even if true, a second problem remains, that being the fact that simple recombination events do not readily account for PFC classes I–III even if an extra copy of the donor wild-type \( \mu \)-region and/or recipient mutant igm482 chromosomal \( \mu \)-gene were present on the same chromosome. For example, in the case of a donor and/or recipient duplication in which the donor resides 5 and in direct orientation, it can be deduced from examination of Figure 2A that gene conversion by itself is incapable of generating E9 PFC classes I–III. Furthermore, neither intrachromatid single reciprocal crossover nor USCE between donor and recipient \( \mu \)-regions could account for the 21.5- and 7.3-kb reciprocal \( \mu \)-region crossover products that were present on the same chromosome.
in the class II PFC. Also, these mechanisms cannot account for the particular C\textsubscript{\textmu} region triplication found in the class II PFC that does not include the 16.8-kb wild-type donor C\textsubscript{\textmu} region fragment. Similar arguments can be made for duplications in which the donor wild-type C\textsubscript{\textmu} region might reside 3' and in direct orientation. What of putative donor/recipient C\textsubscript{\textmu} region duplications in which the donor resides in an inverted orientation? A duplication involving the donor alone has the difficulty of being unable to account for the synthesis of both the wild-type and mutant \(\mu\)-heavy chains in the PFC by simple conversion or crossover mechanisms. However, in the case in which an inverted donor was linked to a duplicated recipient mutant igm482 \(\mu\)-gene, a single intrachromosomal crossover (inversion) event between donor and recipient C\textsubscript{\textmu} regions would generate both the 21.5- and 7.3-kb EcoRI \(\mu\)-gene fragments that, together with the remaining 12.5-kb EcoRI fragment bearing the mutant igm482 \(\mu\)-gene, would account for the C\textsubscript{\textmu} region triplication found in the class II PFC. In addition, a simple intrachromosomal gene conversion between the donor and recipient C\textsubscript{\textmu} region could generate the class III PFC. To generate the class I PFC, an unequal crossover between sister chromatids would need to occur generating a dicentric chromosome and an acentric fragment. Insertion of the acentric fragment into the dicentric chromosome near the original C\textsubscript{\textmu} fragments followed by cell division might then lead to a daughter cell receiving the C\textsubscript{\textmu} fragments present in the class I PFC. As this event would be expected to occur only rarely, it might explain the lower frequency of the class I PFC. However, the above mechanism presents difficulties as random breakage of the dicentric chromosome, followed by segregation to daughter cells, would be expected to lead to differences in the intensities of the 12.5- and 16.8-kb EcoRI C\textsubscript{\textmu} bands that were not observed in the class I PFC. Also, as indicated above, for this mechanism to be correct, all PFC would have to have been generated exclusively by inversion between donor and recipient C\textsubscript{\textmu} regions in an E9 subpopulation bearing the putative duplication. This seems unlikely as no PFC class bearing only the 21.5- and 7.3-kb EcoRI C\textsubscript{\textmu} fragments was recovered as would be expected from the bulk of the E9 population if the single copy donor and recipient C\textsubscript{\textmu} region were inverted (as depicted in Figure 2, C and D).

Is the generation of E9 PFC classes I–III consistent with previously described mechanisms of gene amplification? Studies in mammalian cells have suggested several mechanisms that might be involved in gene amplification (Schmike 1982; Stark and Wahl 1984; Hamelin et al. 1991; Smit et al. 1992; Toledo et al. 1992; Ma et al. 1993): (i) amplification involving multiple rounds of DNA replication in a single cell cycle, followed by integration of the amplified copies as a tandem array into the chromosome; (ii) deletion of the locus from the chromosome, followed by its amplification and maintenance as an episome; (iii) a conservative transposition mechanism in which extra copies of the particular locus involved are generated by rolling circle replication but the original chromosomal locus remains intact; (iv) cycles of chromosome bridging, breakage, and fusion; and (v) a USCE mechanism that generates an initial duplication of the gene and that might be followed by additional rounds of unequal crossing over.

Several characteristics of the PFC examined in this study are not explained by gene amplification mechanisms (i–iv). These mechanisms do not account for (i) the presence of the single reciprocal recombination products in the PFC, namely, the 21.5-kb and/or 7.3-kb EcoRI C\textsubscript{\textmu} region fragments; (ii) the retention of all C\textsubscript{\textmu} sequences on a single hybridoma chromosome; and (iii) the production of both wild-type and mutant \(\mu\)-chains in the PFC. In contrast, USCE mechanism (v) is more relevant in that it can explain the C\textsubscript{\textmu} region triplication found in the class I PFC (Figure 6A) and with slight modification, PFC classes II and III as illustrated in Figure 6, B and C, respectively. To summarize, although other explanations cannot be ruled out completely, the evidence is consistent with the proposal that the parental E9 hybridoma bears a single copy donor wild-type C\textsubscript{\textmu} region that is in direct orientation, 5' of the recipient mutant igm482 C\textsubscript{\textmu} region, and that E9 PFC classes I–III can be explained by the models proposed in Figure 6, A–C, respectively.

Two mechanisms can be postulated to account for the occurrence of a second crossover in explaining recombination between the well-separated homologous C\textsubscript{\textmu} regions in the hybridomas. In one mechanism, the unequal pairing between homologous donor and recipient C\textsubscript{\textmu} regions might have resulted in the coincident alignment of DNA sequences not normally juxtaposed in this region of the chromosome. If the aligned sequences shared sufficient homology and underwent crossover, they could account for the generation of the class II and class III PFC. A second mechanism postulates that homologous pairing or homologous recombination might have resulted in stress strong enough to snap the extensively misaligned sister chromatid fiber. If stress was a factor, it might also have been relieved through cleavage of the sister chromatid by a DNA topoisomerase. In either case, DNA breakage, end-joining, and integration of a large, circular chromosomal segment into the recipient chromatid might have generated the G418\textsuperscript{R} class II and III PFC.

Examination of the relative level of Sp6 wild-type and mutant igm482 \(\mu\)-chains secreted by the PFC revealed that in some, the levels were similar while in others, the levels varied. This suggested differences in the expression of the wild-type and mutant igm482 \(\mu\)-genes, which, in turn, might reflect differences in the recombinant \(\mu\)-gene structures in the PFC. It is interesting that the class I PFC, produced by USCE between the misaligned C\textsubscript{\textmu} regions at position 1 (Figure 6A), had the same
pattern of μ-chain synthesis, the level of the mutant igm482 μ-chain was slightly higher than the wild-type Sp6 μ-chain. Thus, unequal crossover at position 1 may have resulted in the removal of a regulatory element located 3’ of the 12.5-kb EcoRI fragment encoding the endogenous Cμ region that was required for high level expression of the recombinant 21.5-kb EcoRI μ-gene in the PFC. The data suggested that the PFC in classes II and III were generated by single reciprocal crossovers that integrated various-sized circular chromosomal DNA segments from the donor sister chromatid. In generating the DNA circle, a regulatory element(s) may have been introduced (or removed) affecting μ-gene expression in the various PFC.

We thank Zong Mei Zhang for technical assistance. This research was supported by operating grants from the Medical Research Council and the Natural Sciences and Engineering Research Council of Canada to M.D.B.

LITERATURE CITED


Intrachromosomal Homologous Recombination


Communicating editor: C. Kozak