Asymmetric mutation around the recombination break point of immunoglobulin class switch sequences on extrachromosomal substrates

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Received February 2, 1996; Revised and Accepted April 9, 1996

ABSTRACT

Junctions at class switch recombination sites in the genome are characterized by a unique sequence feature. Nucleotide substitutions and small deletions are common on either of the two sides of the switch junction, but not on both together. We have previously reported an extrachromosomal substrate assay system for analyzing the recombination of class switch sequences. Here we have sequenced nine junctions on each side of the break point and compared these to 17 recombination junctions of control substrates from the same cells. Five of the nine switch recombination junctions have nucleotide substitutions and deletions, with multiple nucleotide changes being more common. Furthermore, mutations were found only on a single side of the junction, just as for the recombination of switch sequences in the genome. In contrast, only one of 17 control substrate junctions had a mutation, and this was a single nucleotide insertion. This difference is highly significant (P < 0.00007) and indicates that the fundamental recombination mechanism is likely to be similar for switch sequences in the chromosome and on minichromosome substrates.

INTRODUCTION

There are two types of DNA recombination that are universal in the production of immunoglobulins in mammals. The first involves assembly of the variable domain exon by V(D)J recombination (1). The second involves appending the heavy chain variable domain exon to a variety of different constant domains. This change from IgM and IgD to IgG, IgA or IgE is called class switch recombination and occurs at switch regions located just upstream of each of the constant domains (2–5). An understanding of the physiological mechanism of switch recombination is important because we would like to understand how this process fails in human disease. The switch regions are involved in translocations in various lymphoid malignancies. The c-myc gene translocates to the Ig switch regions in all sporadic Burkitt’s lymphomas; in ~20% of diffuse, large cell non-Hodgkin’s lymphomas; in all L3-type acute lymphoblastic lymphomas and in a large fraction of HIV-associated non-Hodgkin’s lymphomas (6).

In the classification of DNA recombination reactions, class switch is unusual because the target zone is distinctive for its extensive length and for its repetitive nature. Because of these features, the term regionally specific recombination better describes switch recombination than the term site-specific recombination (7). The switch sequences are 1–10 kb long and the recombination crossover points can be anywhere within them (2). The switch regions are highly repetitive and G-rich on the non-template strand. The repeat lengths vary from 20 to 80 nt. The upstream or donor switch region is Sµ. The downstream or acceptor switch region can be any of SYβ, γ1, γ2b, γ2a, ε or α in mouse and any of SYβ, γ1, α1, γ2, γ4, ε or α2 in human, in that physical order along the chromosome. The downstream break points are usually within the switch regions. In contrast, only ~60% of the Sµ switch region break points are within it; 30% are upstream and 10% are downstream by best estimates (3).

A distinguishing feature or signature of switch recombination is difficult to identify, i.e. it is difficult to find characteristics that unambiguously identify a recombination event as having been mediated by switch recombination activity rather than by non-homologous DNA end joining at a random break site. Approximately 60% of the donor and acceptor switch break point sequences show one or more nucleotides of homology between the two ends at the recombination junction such that these few nucleotides could be assigned to either participating DNA end (3), but this feature does not distinguish switch recombination from non-homologous end joining (8). An undetermined fraction of the recombination events at switch regions involve nucleotide mutations in the vicinity of the recombination crossover (3,9,10). Interestingly, in the cases examined to date, the mutations are always on one side or the other of the crossover point, but not on both sides. This feature, the temporal association with transcription (11–14) and the switch sequence dependence are the most distinguishing features of switch recombination that have been noted thus far (3).

Explanation of switch recombination within a chromosomal context is the ultimate goal, however, the study of genomic switch...
recombination events is not suitable for all purposes of analysis because cloning each recombination junction is time consuming. Determination of the rate of recombination is often impossible and manipulation of processes such as transcription and replication must be indirect. Recently, we (15) and others (16–18) have described an extrachromosomal switch sequence substrate assay that recapitulates many of the features of switch recombination in the genome. These switch sequence-bearing minichromosomes recombined at high frequency in murine mature B cell lines, in some but not all pre-B cell lines and in two plasmacytoma lines. In the B lineage cell lines that scored positive, a control substrate with non-switch sequences in place of switch sequences recombined at levels that were typically 50-fold lower. The recombination was transcription- and switch region orientation-dependent; transcription in the physiological orientation stimulated recombination, but transcription in the reverse direction did not.

Here we have analyzed the primary structural features of recombinants generated in the minichromosome substrate assay. We have examined the degree of targeting of the recombination to the switch regions and the sequences of recombinants. We find that truncated versions of the switch regions show some evidence of targeting, and they show the same propensity for nucleotide mutation on one side of the recombination crossover point as has been described for genomic switch recombination events. This adds an important parallel between the recombination of switch sequences extrachromosomally and within the context of the chromosome. Because the extrachromosomal assay can permit determination of the recombination rate and the mutation frequencies, it may permit distinction among models for how switch recombination occurs.

MATERIALS AND METHODS

Cell lines and plasmids

The cell lines used in this study (1-8 and Bal17) and their growth conditions have been described previously (15).

pGD244 and pGD209 are the switch region and control plasmids, which have also been described previously (15). In brief, we have bypassed tissue-specific transcription from the promoters by the use of constitutive promoters, as outlined in Figure 1. These promoters are positioned immediately upstream of positions A and B, the sites where test sequences [switch (µ or γ3) or eukaryotic control (E1 or E2) DNA segments] are inserted.

In the control substrates, positions A and B contain portions of the RAG-1 and -2 genes (oriented in an antisense direction) (pGD209). The SV/µk and hCMV promoters function well in a wide variety of cells and are active in each of the cell lines used in this study. In order to facilitate the analysis of recombinants, a second prokaryotic selection marker was inserted upstream of the promoter for region A. By selecting for recombinants which preserve both the β-lactamase (bla) and kanamycin (kan) resistance genes, we can readily observe a more restricted group of recombinants by selecting against recombination events extending upstream of region A of the plasmid. The prokaryotic origin of replication begins ~400 bp downstream of site B, limiting the deletions from extending a large distance downstream of this. The majority of these recombinants will have junctions lying within or close to regions A and B. Because class switch recombination in the genome also includes larger deletions which extend outside the switch regions, we were interested in using this assay system to examine a broader target region. Positive selection of recombinants with ampicillin alone allows identification of recombinants with junctions further upstream of Sµ. Transformation of switch sequence-bearing substrates, such as pGD244, directly into Escherichia coli without passing the plasmid through eukaryotic cells results in a 0.01% (10^-3) or lower background of white colonies.

Recombination assay

Recombination substrates were transfected into eukaryotic cells using either hypotonic DEAE–Dextran (19) or electroporation (15). The transfected cells were plated into 100 mm dishes at a density of 3 \times 10^5 cells/ml in complete medium. Covalently closed, circular DNA molecules were recovered by alkaline lysis at the indicated times and digested with an amount of DpnI that was in 10-fold excess over that specified as needed to cleave at the appropriate sites in DNA (New England Biolabs). Therefore, the plasmid DNA that is recovered includes only those molecules which have entered the nucleus and undergone at least one round of replication (20). The digested DNA was electroporated into strain MLB7070 containing an amber mutation in the β-galactosidase gene. The transformed bacteria were plated onto X-gal/IPTG (US Biochemical) LB plates containing either ampicillin (100 μg/ml) or ampicillin and kanamycin (25 μg/ml). The substrate molecules give rise to blue colonies and any recombinant molecules which have deleted supF will give rise to blue colonies on X-gal plates. Switch or control sequences are placed in regions A and B, which flank supF. The substrates are transfected into murine hematopoietic cell lines, incubated for specified times and the plasmid DNA is harvested and transformed into E.coli for genetic assay of product (bottom line). Recombination is scored by the deletion of supF and is reported as the change in ratio of replicated recombinant product molecules (white colonies) to total replicated molecules (blue plus white colonies) from 24 to 48 h post-transfection. All values are reported as the average slope (with SE) of at least three independent transfections (see Materials and Methods). The kan and bla segments are the kanamycin (Kn) and ampicillin (Ap) resistance genes, respectively. The polyoma large T antigen and polyoma origin (polyoma T, ori) allow replication of the substrate in murine cells. Between the polyoma large T and the kanamycin gene is the gastrin transcriptional terminator (filled rectangle). SV/µk and hCMV are constitutive eukaryotic promoters oriented in the direction of regions A and B. Ori is the prokaryotic origin of pBR322. Arrows represent the direction of transcription.

Figure 1. Extrachromosomal substrate structure. In the substrate (top line), the tRNA gene, supF, complements an amber mutation in the E.coli strain MLB7070, giving rise to blue colonies on X-gal plates. Switch or control sequences are placed in regions A and B, which flank supF. The substrates are transfected into murine hematopoietic cell lines, incubated for specified times and the plasmid DNA is harvested and transformed into E.coli for genetic assay of product (bottom line). Recombination is scored by the deletion of supF and is reported as the change in ratio of replicated recombinant product molecules (white colonies) to total replicated molecules (blue plus white colonies) from 24 to 48 h post-transfection. All values are reported as the average slope (with SE) of at least three independent transfections (see Materials and Methods). The kan and bla segments are the kanamycin (Kn) and ampicillin (Ap) resistance genes, respectively. The polyoma large T antigen and polyoma origin (polyoma T, ori) allow replication of the substrate in murine cells. Between the polyoma large T and the kanamycin gene is the gastrin transcriptional terminator (filled rectangle). SV/µk and hCMV are constitutive eukaryotic promoters oriented in the direction of regions A and B. Ori is the prokaryotic origin of pBR322. Arrows represent the direction of transcription.
rise to white colonies (15). The ratio of white colonies to the total colonies therefore represents the percentage of recombination for that substrate. Recombination values ($R$) are the percentage of recombination per hour from 24 to 48 h post-transfection. $R$ values are given for recombinants selected on ampicillin ($R_{Ap}$)- or ampicillin–kanamycin ($R_{ApKn}$)-containing plates. The number of blue colonies arising from transformation of $E. coli$ by the substrate plasmids is the same using either ampicillin or ampicillin–kanamycin plates. Each plasmid was transfected in at least three independent experiments with multiple replicates for each time point in each experiment and the average $R$ value ± SE is given.

DNA sequence analysis

We used manual Saenger sequencing and automated fluorescent sequencing with an Applied Biosystems Model 373 to analyze recombinant plasmid molecules on both DNA strands around the recombination point.

RESULTS

Distribution of recombination break points relative to the switch regions

The switch- and control sequence-bearing substrate structures have been described previously (15). Truncated segments of the $S_\mu$ and $S_\gamma 3$ regions are positioned downstream of constitutive promoters in the switch substrate (pGD244) (Fig. 1). In the control substrates, these same positions contain segments of eukaryotic (pGD209) DNA in place of the switch regions. Recombinant plasmids can be cloned by transformation into $E. coli$ and can be selected on ampicillin–kanamycin plates as detailed in Materials and Methods.

We have sequenced the junctions of recombinants selected in this way. In order to be certain of the sequence of the starting substrates, we fully resequenced both the $S_\mu$ and $S_\gamma 3$ regions. They were nearly identical to the published sequences (21,22), except for the site of deletion within $S_\mu$. As the work of others has shown (3), this indicated that the propagation of the switch regions in plasmids did not result in a high spontaneous mutation rate.

We then randomly selected members of the AmpKan pool of recombination products for detailed analysis. The ratio of recombinants in the AmpKan pool divided by that in the broader Amp pool was ~0.45. This ratio primarily reflects the targeting efficiency of region A in the substrates (Fig. 1), which for pGD244 is the truncated $S_\mu$ segment. This compares to a targeting efficiency of ~0.6 in the chromosome for full-length $S_\mu$ (3). Sequence analysis of the recombinants was preceded by first estimating the approximate location of the break point by restriction digestion. For all nine junctions sequenced, we examined at least 72 nt on each side (typically >200 nt), except for recombinant GAD8313, which had >200 nt on one side but only 35 nt on the other.

The location of the $S_\mu$ and $S_\gamma 3$ break points in each of the nine recombinants is shown in Figure 2. As is seen in the genome, there does not appear to be any targeting to a specific portion of the switch sequences, i.e. the process is apparently only regionally specific. The distribution of recombinants is relatively well constrained to the switch sequences. However, one of the break points on the $S_\mu$ side occurs 423 bp upstream of the $S_\mu$ region and one occurs 24 bp downstream. It should be noted that the $S_\mu$ segment used here is only 1.2 kb long, whereas the actual switch region within the genome is more than twice this length. In the course of cloning this segment (23), it appears that it underwent an internal deletion. Hence, it may no longer be as active in targeting as the full-length version in the genome. In addition, there are protein binding sites upstream and downstream of the switch regions which may be important in constraining the process more fully (24–26) and these are not included in this study.

Sequences of switch recombinant junctions

The sequences surrounding the break point in the nine switch recombinants are shown in Figure 3. Five showed mutations. All are point mutations except one, which is a 4 bp deletion. Point mutations and deletions are both seen at genomic switch recombination junctions (3). In parallel with genomic switch recombination events, all five recombinants with mutations had the mutations only on one side of the junction. In some, the
Figure 3. Switch substrate recombination junction sequences. Sequences of nine different recombination products from the switch substrate pGD244 are shown. The sequence is organized as if one were reading the $\Sigma_f$ side of the plasmid until the double-slash mark, which is the break point. The sequence of the recombinant continues at the double-slash mark on the $\Sigma_h$ side (the next line of sequence below). The sequences in parentheses after $\Sigma_f$ and before $\Sigma_h$ are the edges of the deleted portion of the plasmid; the analogous portions in genomic switch rearrangements would be deleted circles, whereas the plasmid remaining after recombination corresponds to the chromosome. The sequences in parentheses are provided so that the reader can determine what sequences are immediately on the other side of each break point (arbitrarily 10 bp here). We display $\sim 72$ bp for the $\Sigma_f$ and $\Sigma_h$ sequences on each side of the recombination point. The bold letters in the line of sequence are sites of mutation. The base specified above or below that line is the base found in the recombinant, whereas the base in the line of sequence is found in the original substrate. An X above the line (GAD8312) means that the mutation is a deletion of those nucleotides. Underlining indicates nucleotides that are homologous on the $\Sigma_f$ and $\Sigma_h$ sides of the sequence. Hence, one cannot determine if they derived from the side shown (arbitrarily taken to be the $\Sigma_f$ side) or rather from the start of the $\Sigma_h$ sequence.

Recombinants GAD8271 and 8272 are from the cell line 18-81 and all of the others (8223, 8306, 8307, 8308, 8312, 8313 and 8314) are from Bal17. The bottom two recombinants have junctional additions (italic). In recombinant GAD8313, the junctional addition derives from elsewhere within the plasmid at a site between $\Sigma_f$ and $\Sigma_h$ that is just downstream of the $\Sigma_f$ region by 230 nt. This 15 bp insertion is inverted relative to both the sequences of $\Sigma_f$ and $\Sigma_h$.

Six of the nine had junctions in which the nucleotides could be assigned to either the donor or acceptor switch regions (underlined in Fig. 3). These are short regions (usually 1–4 bp) of terminal homology between the two recombinant ends (sometimes referred to as microhomology). Such evidence of short terminal homology use is a common finding in genomic switch recombination events (3). This is also seen in non-homologous recombination events (8) and in some specialized types of recombination, such as V(D)J recombination, that involve DNA end joining in the final stages (27).

Two of the nine sequenced recombinants have junctional additions (italic). In recombinant GAD8313, the junctional addition derives from elsewhere within the plasmid at a site between $\Sigma_f$ and $\Sigma_h$ that is just downstream of the $\Sigma_f$ region by 230 nt. This 15 bp insertion is inverted relative to both the sequences of $\Sigma_f$ and $\Sigma_h$.

mutations were on the $\Sigma_f$ side, while in others, on the $\Sigma_h$ side. The most distant mutation in this collection was 96 bp away.

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Two of the nine sequenced recombinants had junctional additions (Fig. 3). The origin of these additions is unclear. Junctional insertions of uncertain origin are not uncommon in genomic switch recombination (5). One of the two insertions here (GAD8307) is palindromic over part of its length (compare tctagc at the start of the insert with gccta near the end of the addition). Junctional additions are also seen in non-homologous DNA end joining (8).

The recombination break points are shown relative to the consensus $\Sigma_f$ and $\Sigma_h$ repeat sequences in Figures 4 and 5. As with the large scale distribution (Fig. 2), there does not appear to be any obvious preference for a specific site of recombination. This is similar to the chromosomal switch events.

Sequences of control recombinant junctions

The control substrates did not recombine at nearly as high a level as the switch substrates in the same cell lines. Nevertheless, a low level of recombination was detectable. We were interested in determining the frequency of mutations among the recombinants
Figure 4. Break points within $\mu$ relative to the consensus repeat sequence. When homology between the donor and acceptor switch region is at the junction, the crossover point can be anywhere within a range. This is indicated by a double arrow for that recombinant. The consensus sequence is shown for $\mu$. Throughout the repetitive region of the $\mu$ region, the repeats show some degree of variation from the consensus. However, these variations are sufficiently infrequent for $\mu$ (in contrast to the acceptor switch regions) that all of the break points shown here can be perfectly aligned within the consensus.

Figure 5. Break points within $\gamma_3$ relative to the consensus repeat sequence. As in Figure 4, when homology between the donor and acceptor switch region is at the junction, the crossover point can be anywhere within a range. This is indicated by a double arrow for that recombinant. The consensus sequence is shown for $\gamma_3$ based on the most frequent nucleotide at each position within the repeat. Throughout the repetitive region of the $\gamma_3$ region, the repeats show variations from the consensus. Because of the extent of the variations, the consensus in its exact form never appears once throughout the entire $\gamma_3$ region (T.Wilson and M.R.Lieber, unpublished results). Within the consensus, the break points are demarcated at the corresponding position within the consensus; however, one will not see an exact correspondence between the break point sequence in Figure 3 and the region marked in Figure 5. This discrepancy is entirely due to the variations from the consensus in the repeats of $\gamma_3$. For the same reason, the ranges for crossovers within regions of homology (double arrowheads) will not match well with the corresponding recombinant in Figure 4.

Figure 6. Control substrate background recombination sequences. The control substrate, pGD209, recombines at a rate that is >50-fold lower than pGD244. The low level of recombinants that are produced were sequenced to determine if there is a qualitative difference in their sequence in addition to the 50-fold quantitative difference. Seventeen recombinant junctions are shown for 16 different plasmids. Nomenclature is identical to Figure 3. As indicated in the Figure 3 legend, italic means junctional addition. Bold upper case means mutation (only the last product, pJL3054); the nucleotide in the product is shown above the original sequence and in this case (pJL3054) the mutation is an insertion. Underlined means homology usage. Bold double-slashes indicate the recombination break point on the A ($\mu$ control) or B site ($\gamma_3$ control) sides. Five of the events are inversions. These events score in the genetic detection assay if there is also a deletion or an inversional interruption of the supF site. The side which is inverted relative to the rest of the plasmid is indicated by the designation Inversion. In only one (pJL3044/pJL3059) of the five inversions were both break points located and sequenced.

DISCUSSION

Comparison of switch and control recombinant sequences

Our finding that five out of nine switch recombinant junctions carry mutations on either side of the switch junction, whereas only one out of 17 control junctions was mutated, is highly significant ($P < 0.00007$, where the $P$ value was calculated according to the binomial distribution using an expected frequency of 1/17). The finding that mutations are present on one
Figure 7. Error-prone synthesis model for mutations in switch recombination. The thin lines represent the donor switch region (usually Sµ) and the thick lines represent any acceptor switch region, such as Sγ3. This model proposes that the DNA synthesis necessary to fill gaps is error prone. The mutations are symbolized as lower case x if they are on the strand continued from the donor side and upper case X if they are on the strand continued from the acceptor side. A necessary feature of this model is that the mismatches generated by the error-prone DNA synthesis are not corrected by the time of replicative DNA synthesis (S phase). When this mismatched region of the genome undergoes DNA replication, the sequence of the top strand (and its complementary newly synthesized strand) will have an apparent recombination junction at the start of the dashed region (the portion that was filled-in by error-prone DNA synthesis). All of the mutations will appear to be only to the right of the recombination junction. In contrast, the sequence of the bottom strand (and its complementary newly synthesized strand) will have an apparent recombination junction at the same position, but the mutations will be at different positions and all to the left of the apparent recombination point. The bracket along the lower strand in the top duplex indicates a small region of microhomology, which may be present early in the end joining process. This serves to prime the DNA synthesis.

The mutations consist of three transversions, four transitions and one 4 bp deletion. In one recent sequence analysis of junctions where both the starting (genomic) and recombinant sequences are documented, the ratio of transversion to total substitutions was 41% (9). This mixture of both types of substitutions within the minichromosome substrates is similar to that seen in the genome. The ratio of deletional mutations to total mutations in genomic switch recombination is ~0.07 (9). This ratio is similar to that seen with the minichromosome assay here (1/9 or 0.11). The one mutation that occurred in a control substrate recombinant was an insertion, whereas the mutations within the switch sequences were substitutions and deletions in the chromosome and in the minichromosome substrates. Insertions in switch recombinants (chromosomal and minichromosomal) are almost always at the junction between the two ends, rather than within one end or the other.

All of the switch recombinant mutations here were within 100 bp of the junction. Most mutations at chromosomal switch junctions are also within 100 bp (3). There was no apparent preponderance of crossover sites within any particular portion of the Sµ or Sγ3 repeats (Figs 4 and 5).

The fraction of minichromosome recombinant junctions with evidence of terminal homology (microhomology) usage is 0.67 (six of nine junctions). In genomic switch events, this fraction is 0.61 (3). For terminal microhomologies of length 1 or 2 nt, the statistical significance is uncertain, as has been pointed out for genomic switch recombination events (3). However, a subset of both genomic (3) and extrachromosomal switch junctions (Fig. 3, GAD8312 and 8273) have terminal microhomologies that are sufficiently long to be highly statistically significant. For genomic switch recombination junctions, microhomologies of length 4 nt or more occur in 15% of junctions, which is 10 times the frequency expected based on chance (3). This feature is also seen for general DNA end joining (alternatively termed non-homologous or illegitimate recombination). Not all DNA end joining events utilize terminal microhomology, but a subset show a statistically significant use of such short tracts in the alignment of the ends (8). It is important to distinguish the terminal microhomology described here from the long tracts (typically hundreds of base pairs) of homology as used in homologous recombination (28). Based on genomic switch recombination (3), there is no indication that switch recombination employs alignment over long stretches (>10 bp) of nucleotide sequence between donor and acceptor sequences. The separability of homologous recombination from non-homologous DNA end joining (in which terminal microhomology can be seen) is clear from genetic and biochemical studies (28).

Models for the mutational asymmetry around switch recombination break points

Dunnick et al. (9) have previously proposed a model based on error-prone DNA synthesis (Fig. 7). The most error-prone
polymerase known in eukaryotes is polβ, which makes ~1 nt incorporation error every 3000 nt (29). It is not known how much new DNA synthesis occurs around the break points. For comparison purposes, if we estimate that there are no more than 200 bp of new synthesis on each side of the junction, then the minimum mutation rate is 11/(400 × 5) = 0.00300 mutations/bp; for non-switch sequence substrates it is 1/(400 × 17) = 0.00015 mutations/bp. The mutation rate at switch junctions analyzed here (1 in 333) is notably higher than the polβ error rate (1 in 3000).

One can devise models that do not rely on error-prone DNA synthesis. In one that we call the error-prone repair model, error-prone repair is stimulated by non-B DNA recombination intermediates (Fig. 8). Recent work by others indicates that non-B DNA configurations are targeted for transcription-coupled nucleotide excision repair, but in an error-prone manner (30). These non-B DNA configurations may represent pause sites at which reiterative rounds of excision repair might introduce mutations. It is interesting to note that for uncertain reasons, these mutations have also been noted only on one side of the non-B DNA configuration (30). Reban and Griffin (31) have reported for Sµ and we have reported for Sγ3, Sγ2a and Sµ (32) that these switch regions acquire a non-B DNA configuration upon transcription in the physiological direction. This unusual configuration consists of a stable RNA–DNA hybrid. We believe that this configuration may be the active substrate for synopsis of the two sides and for cleavage. We have incorporated these concepts into the error-prone repair model (Fig. 8).

It is important to note that the mutations that occur are not restricted to the repetitive region. In three of the five switch recombinants with mutations, the mutations are located outside the repetitive region [400 bp upstream of the Sµ segment (GAD8271); 24 bp downstream of Sµ (GAD8313); 900 bp downstream of Sγ3 segment (GAD8306)]. These mutations are in regions of the substrate that represent unique sequences. In genomic recombination of Ig class switch sequences, asymmetric mutations are also found in cases where the break point is outside the repetitive region and in the unique sequence region (3). Because of these mutations in the unique sequence regions, error-prone synthesis models cannot invoke the error-prone nature of the polymerase as being induced by the repetitive nature of the sequences. Mutations attributable to error-prone repair induced by non-B DNA configurations have been noted at distances of >60 bp away from the site of the non-B configuration; deletions in such substrates can be >100 bp away (30). The mutations in non-repetitive DNA are another similarity between minichromosomal and chromosomal switch sequence recombination.

Regardless of the mechanism by which the mutations arise, in the events here they can occur on either the upstream (Sµ) side or on the downstream (Sγ3) side. This indicates that there is no discrimination between the two sides. If the mutations always occurred on the Sµ segment side, then there would have to be some way in which these DNA ends (after the initial cleavages) were distinguished. This is not the case. Therefore, the two DNA ends, Sµ and Sγ3, have the potential to be handled equivalently. However, as part of the recombination mechanism, in any one recombination event, one side may be distinguished from the other such that the mutations predominate on one side.

The results presented in this study indicate that the extrachromosomal switch recombination assay is characterized by many features thought to be a hallmark of chromosomal switch recombination and, therefore, appears to be a suitable system to study mechanistic features relevant to chromosomal class switch recombination. The advantages of this approach are that one can quantitate the rate of recombination over specific time intervals. One can readily alter the sequences and determine the effect on recombination. One can also metabolically manipulate transcription and replication. The cloning of specific recombination junctions is achieved with fewer manipulations than when cloning from a phage library and with fewer problems than are encountered when using PCR through repetitive DNA. Therefore, this additional approach may be very helpful in assessing mechanistic issues in class switch recombination (33).

ACKNOWLEDGEMENTS

G.A.D. was supported by PHS grant 5T32CA09302. This work was supported by NIH grants to M.R.L., who is a Leukemia Society of America Scholar. G.A.D. and J.L. contributed equally to this work and either can be designated as initial author.

REFERENCES