MOLECULAR AND CELLULAR BIOLOGY, May 1989, p. 1850–1856 Vol. 9, No. 5
0270-7306/89/051850-07$02.00/0

Mutations, Duplication, and Deletion of Recombined Switch Regions Suggest a Role for DNA Replication in the Immunoglobulin Heavy-Chain Switch

WESLEY DUNNICK,[1]* MICHAEL WILSON,[1] AND JANET STAVNEZER[2]

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0620,[1] and Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655[2]

Received 21 November 1988/Accepted 28 January 1989

The heavy-chain switch from immunoglobulin M (IgM) expression to IgA expression is mediated by a recombination event between segments of DNA called switch regions. The switch regions lie two to six kilobases upstream of the μ and α constant region coding segments. Switch recombination to IgA expression results in a recombinant μ-α switch region upstream of the expressed α constant region gene. We have characterized the products of switch recombination by a lymphoma cell line, I.29. Two sets of molecular clones represent the expected products of simple μ to α switches. Five members of a third set of molecular clones share the same recombination site in both the μ and the α switch regions, implying that the five molecular clones were derived from a single switch recombination event. Surprisingly, the five clones fall into two sets of sequences, which differ from each other by several point mutations and small deletions. Duplication of switch region sequences are also found in these five molecular clones. An explanation for these data is that switch recombination involves DNA synthesis, which results in nucleotide substitutions, small deletions, and duplications.

Antigenic stimulation of immunoglobulin-expressing lymphocytes can result in a switch by individual cells from expression of immunoglobulin M (IgM) to expression of IgG, IgA, or IgE. The switch from IgM to IgA, for example, is a substitution of an α constant (C) region for a μ constant region in the immunoglobulin heavy chain. The I.29 mouse B-lymphoma cell line switches from IgM to IgA expression in vivo and in vitro (1, 21, 23). The genetic basis of the class switch in I.29 cells is a DNA rearrangement that moves the VDJ (variable-diversity joining segments) complex, encoding the variable region of the expressed heavy-chain gene, from physical association with the Cμ gene to physical association with the Cα gene (22, 23). The rearrangement is accomplished by a recombination event between segments of DNA (called Sμ and Sα regions) located between two and six kilobases upstream of the Cμ and Cα genes. This recombination event between S regions is similar to that observed in the vast majority of heavy-chain switches in other immunoglobulin-producing cells (3, 12, 14, 17).

S regions are composed of simple sequences imperfectly repeated in tandem. For example, the Sμ region is composed of the repeat unit GGGGT(GAGCT)*n*, where *n* is usually between one and seven (13, 17). The Sα region is composed of an 80-base-pair (bp) long repeat which is based on pentamers similar to those found in the Sμ region (3, 14). Switch recombination events in both the Sμ and the Sα regions have been shown to be scattered over most of the respective switch regions (3, 10, 12, 14). Most studies of switch recombination sites have focused on myeloma or hybridoma cells and have analyzed switch recombination sites of a clonal progeny cell. We have examined switch recombination sites from different cellular progeny of what is apparently a single switch recombination event. If the immediate DNA product of a switch recombination event included regions of base mismatches, one might expect two populations of DNA molecules, segregated into two populations of cells, following replication and cell division. We have found that the progeny of a single switch recombination can fall into two groups of very similar, but distinguishable, sequences.

* Corresponding author.

MATERIALS AND METHODS

All of the expressed α genes used in this study were *Bam*HI fragments cloned in bacteriophage vectors that include the expressed VDJ exon, the SμSα recombination site and part of the expressed Cα gene. The molecular clones Eα.J1, Eα.J2, Eα.J3, Eα.J4, Eα.J5, and Eα.J8 have been described in detail elsewhere (11, 22). Briefly, these molecular clones were derived from a population of I.29 cells which had been passaged in vivo five times and that was 77% IgA$^+$ and 17% IgM$^+$. The BF0.5 and BF0.6 expressed-α-gene clones were derived from a clonal cell line [BF0.3 (IgA$^+$)] that had switched in vivo and been cloned in vitro (11, 21). The 40.1 and the 41 series of molecular clones (41.1, 41.3, 41.4, 41.6, and 41.8) of expressed α genes were derived in a single experiment by induction of the I.29μ cell line (not cloned) in vitro. After 35 days of treatment with lipopolysaccharide and anti-idiotype (giving rise to 40.1) or lipopolysaccharide and anti-immunoglobulin (giving rise to the 41 series), approximately 95% of the cells were IgA$^+$. DNA was prepared from these cells, and molecular clones were derived. L47.α2 contained the germ line α gene cloned from the IgM.5P cells (22). The nonexpressed α gene in IgM.5P cells had the identical restriction map as the germ line α gene in liver cells from I/St mice (22 and data not shown).

Switch region subclones from the bacteriophage clones mentioned above were prepared in the Bluescript KS vector or in pSA801, which includes a *Sac*I fragment and about 150 bp of flanking sequence from bacteriophage lambda DNA in the *Bam*HI site of pBR322. pCα is a *Sac*I subclone of the Sα region from L47.α2 and has the same size *Sac*I fragment as the parent clone. *Sau*3A, *Bst*NI, or *Alu*I partial subclones were prepared in M13 and sequenced by the dideoxy method (18). Southern hybridization was performed as described elsewhere (11).

RESULTS

We prepared populations of I.29 cells that included IgA^+ cells which arose by a switch recombination event from I.29 (IgM^+) cells. From these cell populations or from cellular clones from these populations, we prepared molecular clones of the expressed α heavy-chain genes and determined the nucleotide sequence of the switch recombination sites.

BF0.5 and BF0.6 represent independent molecular clones derived from the clonal IgA^+ cell line BF0.3 and therefore represent the same gene. As expected, they have the same recombination site and the same sequence for 300 bp surrounding the recombination site (Fig. 1A). The 40.1 and the 41 series of molecular clones were derived from two cultures, in the same experiment, of I.29μ cells that had been induced to switch. The I.29μ cells used for this experiment had not been cloned; approximately 0.5% of the cells expressed IgA when the cultures were initiated. As presented in Fig. 1A, 40.1 and all of the 41 series of molecular clones also share the same switch recombination site. They also share the same sequence for 264 bp surrounding the recombination site, except for 41.6, which has a single base change 137 bp upstream of the recombination site (not shown).

The Eα.J series of molecular clones represents more complex recombination events. These six expressed α genes were cloned from a population of I.29 cells obtained by selecting for IgM^+ cells on the fluorescence-activated cell sorter and subsequent passage five times in mice. We first characterized the SμSα regions of *Sac*I plasmid subclones of the Eα.J phage clones by examination of complete *Sau*3A digests. Each of the six Eα.J subclones has a large (greater than 1,000 bp) *Sau*3A fragment that is variable in length. DNA sequencing demonstrated that these large fragments consist mostly of Sμ tandem repeats (data not shown). This variability in length is probably due to deletions of the highly repetitive Sμ region during growth in bacteriophage vectors (13, 17). Eα.J1, Eα.J3, and Eα.J5 are similar in that they share a 295-bp fragment not found in Eα.J2, Eα.J4, Eα.J8, or pCα (Eα.J1 is shown as an example in Fig. 2A, lane 4). Eα.J1 also has a 320-bp fragment similar to that found in the germ line Sα clone, pCα (Fig. 2A, lanes 2 and 4). Eα.J2 and Eα.J4 share 300-bp and 290-bp fragments, neither of which is found in any of the other subclones (Fig. 2A and data not shown). Eα.J8 has a unique 360-bp fragment. The *Sau*3A fragment composition of the molecular clones around the SμSα recombination sites is shown in Fig. 3.

Eα.J1, Eα.J2, Eα.J3, Eα.J4, and Eα.J5 share the same switch recombination site (Fig. 1B). The Sμ recombination site in Eα.J8 is upstream of the Sμ recombination sites of other Eα.J series clones, and the Sα recombination site is downstream of the Sα recombination site of the other Eα.J series clones. (The Eα.J8 sequence is contiguous between the two arrows in Fig. 1B.) The fact that five of the Eα.J series clones share a single recombination site suggests that they are derived from a single switch event in a single cell. Thus, the IgA^+ progeny of one switch event appears to dominate the cell population in passage 5. Since IgA^+ cells dominate the passage 5 population, they must have some growth advantage over the IgM^+ cells (22). IgA^+ cells arise at a low frequency from the IgM^+ precursors (21). The first cell that switches to α from μ does not have to compete with other IgA^+ cells for a significant time, and the progeny of that cell may dominate the final population. For reasons to be discussed below, it is likely that the molecular clone Eα.J8 is derived from progeny of the same switch event that gave rise to Eα.J1 through Eα.J5.

All of the Eα.J series clones have the germ line α *Sau*3A and *Pvu*II fragments downstream of the Sα *Sau*3A site designated S-2 in Fig. 3A. Hence, the switch recombination sites are in the 5′ end of the Sα region, 5′ of S-2. We compared the DNA sequence of the germ line Sα 320 fragment between S-1 and S-2 (Fig. 3A), the sequence of the Sα 270 fragment (Fig. 3A), and the sequences of the Eα.J1 through Eα.J5 switch recombination sites. The comparison suggests that the switch recombinations occur in the Sα 320 fragment and not in the Sα 270 fragment (see residues 301 to 345, Fig. 1B). An alternative interpretation of these data is that the recombination event occurred in the Sα 270 fragment and was accompanied by four point mutations that happened to coincide with the sequence of the Sα 320 fragment. We favor the former simpler interpretation.

Nevertheless, the sequences of the 290 and 295 fragments found in Eα.J1 through Eα.J5 are very similar to the germ line 320-bp fragment, except for a 25-bp deletion and a few single-base changes. The 290-bp fragments in Eα.J2 and Eα.J4 have an additional 5-bp deletion. Since the SμSα recombination event in the Eα.J series of clones interrupts the germ line 320-bp fragment and all restriction fragments downstream of the 320-bp fragment are in the germ line configuration, the 290 and 295 fragments appear to represent a duplication of germ line Sα sequences. To note this duplication, we have designated the new *Sau*3A site in clones Eα.J1 through Eα.J5 S-2′.

Clone Eα.J1 includes an additional 320-bp *Sau*3A fragment, the location of which on the Eα.J1 restriction map has not been determined. *Pvu*II and *Sau*3A fragment sizes suggest that it is 5′ of S-2. It represents another duplication of Sα sequences but differs from the germ line 320 fragment by eight base substitutions (data not shown).

It was possible, however, that the germ line α gene clone, L47.α2, had deleted some of the Sα sequences present in genomic DNA and that this could explain the apparent addition of Sα sequences in the Eα molecular clones. Given the DNA sequence data discussed above, the deletion would have to be at least 290 bp long to account for the extra fragment in Eα.J2 and Eα.J4. This possibility was investigated by comparing the size of Sα *Sac*I and *Sac*I-*Pvu*II fragments in cellular DNA from I.29μ cells and in the cloned germ line α gene. The *Sac*I and the *Sac*I-*Pvu*II fragments in the molecular clone were 80 to 100 bp smaller than the corresponding fragments in genomic DNA (Fig. 2B, lanes 5 through 8). This migration difference between the molecular clone and the corresponding genomic fragment may not represent a true difference in fragment length; it may result from artifactual migration of different samples. Nevertheless, even if the migration difference represents a 100-bp difference in fragment length, this deletion is much too small to account for duplications of 290 to 600 bp in the Eα.J series of clones.

Besides having additional Sα sequences relative to the germ line Sα region, the sequences of the Eα.J1 molecular clones also differed from the germ line Sα sequences. Eα.J1, Eα.J3, and Eα.J5 differ from the germ line Sα sequence by four single-base changes and the 25-bp deletion in the 360 bp 3′ of the SμSα recombination site. Eα.J2 and Eα.J4 differ from the germ line by five single-base changes, the 25-bp deletion, and the 5-bp deletion. Eα.J8 differs from the germ

```
                                  Sμ↓↓ Sα
A. 41.3/40.1     GAGCTGGGGTGAGCTGAGCTGGGCTGATCTGGAATGAGCTGGGATGAGCTGAGCTAGGCT

                             Sμ↓↓↓↓↓↓↓↓↓↓↓↓ Sα
   BFO.5/.6      GAGCTGAGCTGAGCTAGGGTGAGCTGAGCTAGAATGATCTGGAATGGGCTGAGCTAGGCT

                                  Sau3A-0
B. Eα.J2/J4      GAGCTGAGGTGAGCTAGGGTGAGCTGATCTGAGTTAGGGTGAGCTGAGCTGAGCTGGGGT  60
   Eα.J8         ------------------------------------------------------------

   Eα.J1/J3/J5   GAGCTGAGCTGAGCTGGGGTGAGCTGAGCTGGGGTGAGTTGAGCTGAGCTGACCTGAGCT 120
   Eα.J2/J4      -------A----------------------------------------------------
   Eα.J8         -------A----------------------------------------------------

   Eα.J1/J3/J5   GAGCTGAGCTGAGCTGGGGTGAGCTGGGGTGAGCTGAGCTGGGGTGAGCTGAGCTGAGCT 180
   Eα.J2/J4      -------C----------A-----------------------------------------
   Eα.J8         -------C----------A-----------------------------------------

   Eα.J1/J3/J5   GGGGTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGTT GGGTGAGCTGAGCT 240
   Eα.J2/J4      ------------------T-     -------------------- --------------
   Eα.J8         -------------T↓
                                                                       Sμ↓
   Eα.J1/J3/J5   GAGCTGAGCTGGGGTGAGATGAGCTGGGGTGAGCTGAGCTGAGCTGGGGTGAATTGGGGT 300
   Eα.J2/J4      -------------------------------------A----------------------

                 Sα                                      Sau3A-2'
   Sα 320        CGGCTGGTGTGAGCTGAGCTAGGCTGAGCTGAGCTAGAATGATCTGGGATGGGCTGAGCT 360
   Eα.J1/J3/J5   ---------------------------------T--------------------------
   Eα.J2/J4      ------------------------------------------------------------
   Sα 270        A----CA---------G--------------------------(end)

   Sα 320        AGGCTAAAATAGGCTGGGCTGGGCTGGTGTGAGCTGAGCTAGGCTGAGCTGAGGTGGAAT 420
   Eα.J1/J3/J5   -----------------------------------------------------C------
   Eα.J2/J4      ---------------------------------------------     ---C------

   Sα 320        GAGCTGGGATGGGCTGAGCTAGGCTGGAATAGGCTGGGCTGGGCTGGTGTGAGCTGAGCT 480
   Eα.J1/J3/J5   ------------------------------------------------------------
   Eα.J2/J4      ------------------------------------------------------------
   Eα.J8                            ↓----------------------------------------

   Sα 320        AGGCTGAGCTGAGCTGGAATGAGCTGGGATGGGCTGAGCTAGGCTGGAATAGGCTGGGCT 540
   Eα.J1/J3/J5   -----                         ------------------------------
   Eα.J2/J4      -----                         --------T---------------------
   Eα.J8         -----                         --------T---------------------

   Sα 320        GGGCTCATGTGAGCTGGGCTAGGCTGAGCTGAGCTGGAATGAGCTGGGATGAGCTGAGCT 600
   Eα.J1/J3/J5   ------------------------------------------------------------
   Eα.J2/J4      --------------------------------A---------------------------
   Eα.J8         --------------------------------A---------------------------
                                                                        Sau3A-2
   Sα 320        AGGCTGGAATAGGCTGGGCTCGGCTGGTGTGAGCTGAGCTAGGCTGAGCTGAGCTAGAATGATC
   Eα.J1/J3/J5   ---------------------------------G------------------A-----------
   Eα.J2/J4      ---------------------------------G------------------------------
   Eα.J8         ---------------------------------G------------------------------
```

FIG. 1. Nucleotide sequences near μ-α switch recombination sites. μ-α switch recombination sites are noted by arrows and are delimited by the residues which are clearly derived from Sμ and Sα. The recombination sites can be ambiguous, since one or more residues could be derived from either Sμ or Sα. (A) Nucleotide sequences for the molecular clones 41.3, 40.1, BF0.5, and BF0.6. The sequences for five other genomic clones, independently derived from the same culture as 41.3, are identical to 41.3. (B) Nucleotide sequences for the Eα.J series of molecular clones. ----, Identity to the upper sequence in each group of lines. Substitutions are indicated by the appropriate base abbreviation. Spaces indicate deletions relative to the upper sequence. For example, the sequence of Eα.J8 is contiguous from one arrow (residue 195) to the other arrow (residue 440). To retain the pentamer arrangement in Sμ, a space in the sequence has been introduced at residue 226. *Sau*3A sites are designated as in Fig. 3. Sα 320 and Sα 270 are germ line fragments identified in Fig. 3. Because some Sα sequences are duplicated in the Eα.J clones, some of the Sα 320 fragment sequences have been duplicated in this figure (residues 301 through 345). Only a small portion of the Sα 270 sequence is shown. The 300 fragment (see Fig. 2 and 3) extends from *Sau*3A-0 to *Sau*3A-2′ in the Eα.J2-Eα.J4 sequence. The 290 and 295 fragments (see Fig. 2 and 3) extend from *Sau*3A-2′ to *Sau*3A-2 in the Eα.J2-Eα.J4 and the Eα.J1-Eα.J3-Eα.J5 sequences, respectively.

line by four single-base changes and the 25-bp deletion. A few of these single-base changes might be an artifact of the comparison made, since we have compared the 290 and 295 fragments in Eα.J1 through Eα.J5 to the germ line 320 fragment, even though the 290 and 295 fragments might arise by duplication of other Sα sequences. A more valid comparison might eliminate some of the base changes relative to the germ line. Importantly, six differences (including the 5-bp deletion) in the Sα region between Eα.J1, Eα.J3, and Eα.J5 and Eα.J2, Eα.J4, and Eα.J8 would remain.

The sequence of germ line Sμ is not available in the region 5′ of the SμSα recombination site. However, the Eα.J1,

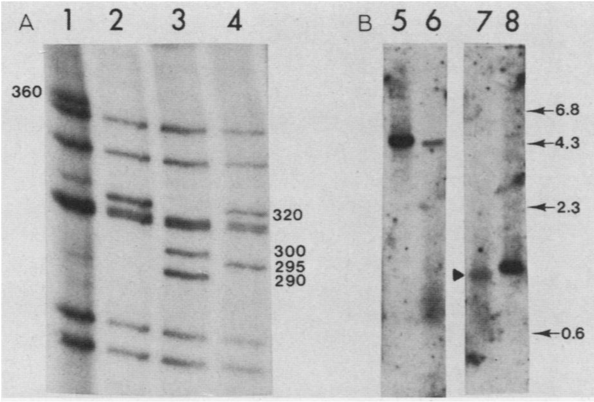

FIG. 2. Restriction fragment composition of μ-α recombined and α germ line switch regions. (A) *Sau*3A fragment composition. A 0.1-μg portion of Eα.J8 (lane 1), pCα (lane 2), Eα.J4 (lane 3), or Eα.J1 (lane 4) DNA was digested with *Sau*3A and end labeled with [α-^{32}P]dATP, by using the large fragment of DNA polymerase. The resulting DNA fragments were fractionated on a 6% polyacrylamide gel. Lengths of some fragments in bp are noted. Several fragments in lane 1, including the fragment at about 325 bp, are the result of end-labeling and are not observed by ethidium bromide staining. (B) Comparison of *Sac*I and *Sac*I-*Pvu*II fragments in genomic and cloned α genes. Eleven micrograms of genomic DNA from I.29μ cells (lanes 5 and 8) or 0.1 ng of L47.α2 DNA (lanes 6 and 7) were cut with *Sac*I (lanes 5 and 6) or *Sac*I and *Pvu*II (lanes 7 and 8), fractionated on a 1.2% agarose gel, and blotted onto Zetabind paper. The filter was hybridized with an RNA probe transcribed from the 320 Sα fragment cloned in a Bluescribe plasmid. These fragments were identical in size in bacteriophage clone L47.α2 and the plasmid subclone pCα (not shown).

Eα.J3, and Eα.J5 sequences differ from the Eα.J2 and Eα.J4 sequences by five single-base changes and a 5-bp deletion. Each of the differences must represent deviations from the germ line in one set of sequences or the other. (This does not imply that any sequence from the Eα.J series need be totally germ line or totally mutated; each molecular clone could be a mix of germ line residues at some of the differences and mutated residues at other differences.) Eα.J8 is similar to Eα.J2 and Eα.J4 in the Sμ sequences; it has a single-base difference from Eα.J2 and Eα.J4. In all of the base changes, transitions predominate. A single transversion (G to C) is observed.

An additional base change results in a *Sau*3A site in the Sμ portion of Eα.J2, Eα.J4, and Eα.J8. This *Sau*3A site, designated S-0 in Fig. 1B and 3B, is not found in Eα.J1, Eα.J3, Eα.J5, or in a 2.3-kilobase molecular clone of the Sμ region from I.29 cells. This novel *Sau*3A site results in a 300-bp fragment (Fig. 2A, lane 3); the corresponding fragment in Eα.J1, Eα.J3, and Eα.J5 is longer than 1,000 bp. The structure of Eα.J8 is the result of replacement of the 300- and 290-bp fragments in Eα.J2 and Eα.J4 by the 360-bp fragment.

DISCUSSION

We assume that molecular clones with identical switch recombination sites are derived from a single switch recombination event. No two independent switch sites have ever been shown to be identical at the sequence level (3, 12, 14, 16, 17, 24, 25). In every case studied to date the two *Igh* loci within a single cell are found in distinguishable, rearranged restriction enzyme fragments (2, 6, 8, 20, 25). Therefore, within a single cell, the two switch recombinations, even when occurring within the same switch region, occur at different S region locations. Thus, it appears that in generation of both the 40.1 and 41 series of molecular clones and the Eα.J molecular clones, cells deriving from a single switch recombination event dominated the population at the time of DNA preparation. Since the 40.1 and 41 series clones were derived from separate wells, the switched cell apparently existed before induction by lipopolysaccharide and anti-immunoglobulin antibodies. If the Eα.J1, Eα.J3, and Eα.J5 and the Eα.J2 and Eα.J4 clones arose by two switch events which both occurred at exactly the same Sμ and Sα site, they would also have to share the 25-bp deletion of Sα sequences in the 290 and 295 fragments and the apparent Sα sequence duplication. Although we provide no formal evidence to rule out independent switch recombination events occurring at the same Sμ and Sα sites, given the precedents in the literature, the derivation of the cell populations and the molecular clones, and the structure of the molecular clones, the more parsimonious interpretation would favor single recombination events giving rise to similar recombination sites.

One of the striking findings in the Eα.J molecular clones is the presence of two groups of sequences (Eα.J1, Eα.J3, and Eα.J5 and Eα.J2, Eα.J4, and Eα.J8) that are nearly identical within a group but differ between groups by several point mutations and deletions. It is unlikely that the point mutations are the result of cell culture or recombinant DNA artifacts. The sequences of BF0.5 and BF0.6, which were derived from a cloned IgA$^+$ cell line, were identical. Furthermore, the sequences of the 40.1 and 41 series clones, which appear to have been derived from a fortuitously clonal line, were virtually identical to each other. If artifactual mutations were occurring, we should have observed them in these molecular clones.

The point mutations are probably not related to the hypermutation mechanism associated with VDJ regions. They are located far from the VDJ complex, where somatic hypermutation is concentrated (4, 10, 15). Furthermore, somatic hypermutation is apparently not active in I.29 cells, as these molecular clones lack somatic mutations in their VDJ exons (11).

We presume that the Eα.J1 through Eα.J5 molecular clones arose from a single switch recombination event in a single precursor cell. That precursor cell divided many times, its progeny dominating the population obtained after passage 5. The five molecular clones were probably derived from the DNA of five different progeny cells. If the point mutations occurred before switch recombination in the precursor cell, the five Eα.J clones should have identical sequences. Since we observe two different sequences, the mutations cannot have occurred before the switch. If the point mutations occurred after the switch, the five Eα.J clones should each have a different sequence, because each molecular clone was presumably derived from a different progeny cell in the final cell population. Hence, we suggest that the mutations occurred at about the same time as the switch recombination event. Since the single cell that switched proliferated, the mutations were segregated into two groups in the final cell population, giving rise to two different sets of molecular clones.

As detailed in Results, it is formally possible that switch recombination occurred in the Sα 270 fragment and that the 290 and 295 fragments represent products of small deletions of the germ line Sα 320 fragment and not duplications. We favor the interpretation that the Sα 290 and 295 fragments represent duplications, with small deletions, of Sα se-

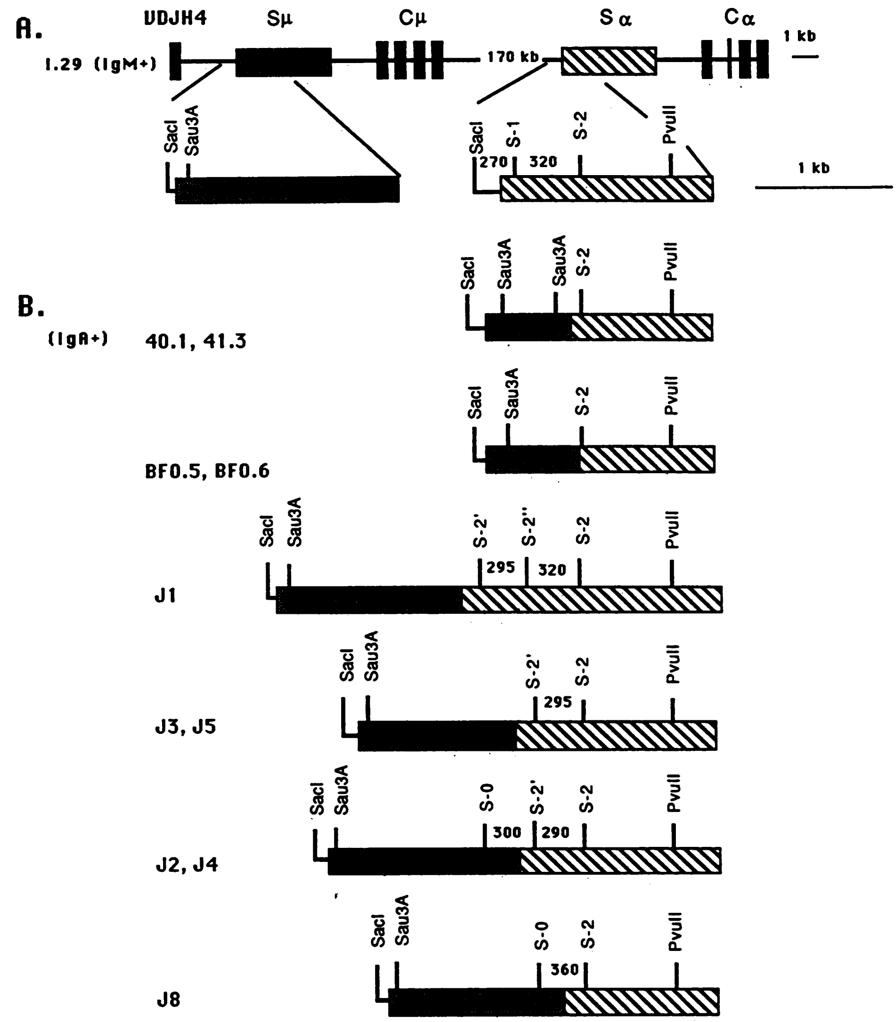

FIG. 3. Diagrams and restriction maps of the expressed immunoglobulin heavy-chain gene in I.29 cells and derived molecular clones. (A) The expressed μ heavy-chain gene and the unrearranged, downstream α constant region gene in I.29 (IgM^+) cells is shown. Expanded maps, with a few restriction enzyme cleavage sites, are shown for the Sμ (shaded) and Sα (hatched) regions. (B) Maps, with a few restriction enzyme cleavage sites, are shown for molecular clones derived from I.29 (IgA^+) cells. The portion of the restriction map shown includes the μ to α switch recombination site. We do not mean to imply an order of biological derivation by the order of presentation of the Eα.J series of molecular clones. S, *Sau*3A sites. For ease of discussion, some *Sau*3A sites are given additional designations: S-1, S-2, S-2', S-2", and S-0. Several *Sau*3A fragments are named according to their length.

quences. These duplications and deletions may have occurred during the propagation of IgA^+ cells or molecular clones. For example, the switch recombination site observed in Eα.J8 might be the result of an artifactual deletion of the original recombination site, which would be the same as that in the other Eα.J series clones. The Eα.J8 recombination site might be the product of some other internal deletion, unrelated to switch recombination. In fact, since Eα.J8 shares so many mutations with Eα.J2 and Eα.J4 and shares the 25-bp deletion with all the Eα.J series clones, it is very likely that it shares the original switch recombination site. We think that it is possible that the recombination site in Eα.J8 is the result of a secondary switch recombination. In support of this possibility, the one mutation relative to Eα.J2 and Eα.J4 might have arisen by such a secondary switch recombination.

The apparent duplications show some clonal variation, since the Eα.J1 molecular clone includes an additional 320-bp fragment. This might imply that the duplication process could take place independently of switching. It is also possible that Eα.J1 through Eα.J5 each had the additional 320-bp fragment after switching and that homologous recombination in the lymphoma cells or during growth of the bacteriophage clone eliminated the fragment. In this case, independent events in Eα.J2 through Eα.J4 would have to yield nearly identical products.

DNA synthesis during switch recombination could give rise to both the mutations and the duplications-deletions. This role of DNA synthesis, which has been previously suggested by others (9, 19), is illustrated in Fig. 4. The tandemly repeated nature of switch regions could give rise to slippage during DNA synthesis, resulting in duplication or deletion of sequences. In this communication, we have reported deletion and duplication in recombined switch regions. Deletions and duplications of nonrecombined switch regions are associated with activation of switch recombination in both I.29 and normal splenic B cells (7, 23). One might hypothesize that DNA synthesis during switch

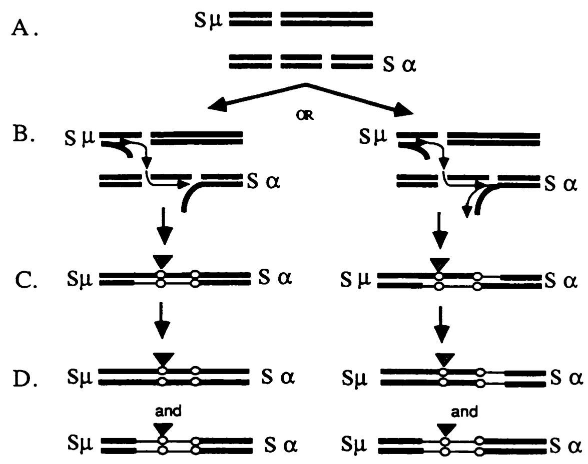

FIG. 4. Model for DNA synthesis in heavy-chain switch recombination. Existing DNA strands are symbolized by thick lines; newly synthesized strands are symbolized with thin lines and arrows noting the direction of synthesis. An early step in switch recombination is nicking of at least one strand of the switch region DNA double helix (A). As one strand melts away, DNA synthesis begins, by using the other strand as a template (B). This synthesis could initiate in either or both switch regions. DNA synthesis occurs in only the 5′ to 3′ direction. Hence, synthesis goes from left to right with the upper strand as a template and right to left with the lower strand as a template. At some point, the synthesis crosses over from the Sμ to the Sα sequences, accomplishing the heavy-chain switch recombination. Depending on the synthesis pattern of the switch regions and the resolution of the recombinational complex, duplication of the switch region sequences could result (C). Nicks in the DNA (○) now religated, and the switch recombination point (△) are noted. Errors during DNA synthesis could result in a heteroduplex with differences from the germ line sequence. Depending on how the heteroduplex was resolved or repaired, both germ line and mutated sequences could arise (D).

recombination may switch templates not only between donor and acceptor switch regions but also within a single switch region. The unusual composition of switch regions might also cause DNA synthesis to be error prone. Error-prone DNA synthesis in the switch would explain discrepancies previously observed between recombined and germ line switch regions (14, 24). Nucleotides not accounted for by either the donor or recipient switch region at switch recombination sites (25) might be explained by non-template-directed DNA synthesis. c-*myc* translocations often involve rearrangement with switch regions and may be a form of abberant heavy-chain switch recombination. Insertions, deletions, and a duplication have been observed in c-*myc* switch region recombination sites and have been interpreted similarly (5).

ACKNOWLEDGMENTS

We thank the members of our laboratories for helpful discussion and Sonia Sirlin and Jennifer Nietupski for excellent technical assistance.

This work was supported by Public Health Service grants CA-39068 (awarded by the National Cancer Institute to W.D.) and AI-23283 (awarded by the National Institute of Allergy and Infectious Disease to J.S.).

LITERATURE CITED

1. **Alberini, C., R. Biassoni, S. DeAmbrosis, D. Vismara, and R. Sitia.** 1987. Differentiation in the murine B cell lymphoma I.29: individual μ+ clones may be induced by lipopolysaccharide to both IgM secretion and isotype switching. Eur. J. Immunol. **17**:555–562.
2. **Borzillo, G. V., M. D. Cooper, H. Kubagawa, A. Landay, and P. D. Burrows.** 1987. Isotype switching in human B lymphocyte malignancies occurs by DNA deletion: evidence for nonspecific switch recombination. J. Immunol. **139**:1326–1335.
3. **Davis, M. M., S. K. Kim, and L. E. Hood.** 1980. DNA sequences mediating class switches in alpha-immunoglobulins. Science **209**:1360–1365.
4. **Gearhart, P. J., and D. F. Bogenhagen.** 1983. Clusters of point mutations are found exclusively around rearranged antibody variable genes. Proc. Natl. Acad. Sci. USA **80**:3439–3443.
5. **Gerondakis, S., S. Cory, and J. M. Adams.** 1984. Translocation of the *myc* cellular oncogene to the immunoglobulin heavy chain locus in murine plasmacytomas is an imprecise reciprocal exchange. Cell **36**:973–982.
6. **Hummel, M., J. K. Berry, and W. Dunnick.** 1987. Switch region content of hybridomas: the two spleen cell *Igh* loci tend to rearrange to the same isotype. J. Immunol. **138**:3539–3548.
7. **Hurwitz, J. L., and J. J. Cebra.** 1982. Rearrangements between the immunoglobulin heavy chain gene JH and Cμ regions accompany normal B lymphocyte differentiation *in vitro*. Nature (London) **299**:742–744.
8. **Katzenberg, D. R., and B. K. Birshstein.** 1988. Sites of switch recombination in IgG2b- and IgG2a-producing hybridomas. J. Immunol. **140**:3219–3227.
9. **Kenter, A. L., and J. V. Watson.** 1987. Cell cycle kinetics model of LPS-stimulated spleen cells correlates switch region rearrangements with S phase. J. Immunol. Methods **97**:111–117.
10. **Kim, S., M. Davis, E. Sinn, P. Patten, and L. Hood.** 1981. Antibody diversity: somatic hypermutation of rearranged VH genes. Cell **27**:573–581.
11. **Klein, D., J. Nietupski, S. Sirlin, and J. Stavnezer.** 1988. I.29 lymphoma cells express a non-mutated immunoglobulin heavy chain variable region gene before and after heavy chain switch. J. Immunol. **140**:1676–1684.
12. **Marcu, K. B., R. B. Lang, L. W. Stanton, and L. J. Harris.** 1982. A model for the molecular requirements of immunoglobulin heavy chain class switching. Nature (London) **298**:87–89.
13. **Nikaido, T., S. Nakai, and T. Honjo.** 1981. Switch region of Ig Cμ gene is composed of simple tandem repetitive sequences. Nature (London) **292**:845–848.
14. **Obata, M., T. Kataoka, S. Nakai, H. Yamagishi, N. Takahashi, Y. Yamakami-Kataoka, T. Nikaido, A. Shimizu, and T. Honjo.** 1981. Structure of a rearranged γ1 chain gene and its implication to immunoglobulin class-switch mechanism. Proc. Natl. Acad. Sci. USA **78**:2437–2441.
15. **Pech, M., J. Hochtl, H. Schnell, and H. G. Zachua.** 1981. Differences between germline and rearranged immunoglobulin V coding sequences suggest a localized mutation mechanism. Nature (London) **291**:668–671.
16. **Petrini, J., B. Shell, M. Hummel, and W. Dunnick.** 1987. The immunoglobulin heavy chain switch: structural features of γ1 recombinant switch regions. J. Immunol. **138**:1940–1946.
17. **Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa.** 1980. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy chain genes. Nature (London) **286**:676–683.
18. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
19. **Severinson, E., S. Bergstedt-Lindquist, W. Van der Roo, and C. Fernandez.** 1982. Characterization of the IgG response induced by polyclonal B cell activators. Immunol. Rev. **67**:73–85.
20. **Siekevitz, M., C. Kocks, K. Rajewsky, and R. Dildrop.** 1987. Analysis of somatic mutation and class switching in naive and memory B cells generating adoptive primary and secondary responses. Cell **48**:757–770.
21. **Sitia, R., A. Rubartelli, and U. Hammerling.** 1981. Expression of two immunoglobulin isotypes, IgM and IgA, with identical idiotype in the B cell lymphoma I.29. J. Immunol. **127**:1388–1394.

22. **Stavnezer, J., K. B. Marcu, S. Sirlin, B. Alhadeff, and U. Hammerling.** 1982. Rearrangements and deletions of immunoglobulin heavy chain genes in the double-producing B cell lymphoma I.29. Mol. Cell. Biol. **2**:1002–1013.
23. **Stavnezer, J., S. Sirlin, and J. Abbott.** 1985. Induction of immunoglobulin isotype switching in cultured I.29 lymphoma cells. J. Exp. Med. **161**:577–601.
24. **Szurek, P., J. Petrini, and W. Dunnick.** 1985. Complete nucleotide sequence of the murine $\gamma 3$ switch region and analysis of switch recombination sites in two $\gamma 3$-expressing hybridomas. J. Immunol. **135**:620–626.
25. **Winter, E., U. Krawinkel, and A. Radbruch.** 1987. Directed Ig class switch recombination in activated murine B cells. EMBO J. **6**:1663–1671.