UNEQUAL SISTER CHROMATID EXCHANGE

A Mechanism Affecting Ig Gene Arrangement and Expression

By SHERMAINE A. TILLEY and BARBARA K. BIRSHTEIN

From the Department of Cell Biology, Albert Einstein College of Medicine, Bronx,
New York 10461

Though the phenomenon of sister chromatid exchange (SCE)[1] has now been known for over thirty years (1–3), its biological role(s) remains poorly defined (see ref. 4). The greatest number of observations of SCE have come from cytogenetic studies using agents such as [3H]thymidine or 5-bromodeoxyuridine to label sister chromatids differentially and observe their exchange. Such studies demonstrate a marked increase in SCE after mutagenesis of somatic cells with certain agents (5–7), or in cells from patients with Bloom's syndrome (8), a disorder characterized by a unique type of chromosomal instability. These observations suggest that SCE is, in some way, involved in normal processes of DNA repair (5, 6).

There is also genetic evidence for unequal SCE in germ line cells of *Drosophila melanogaster*. In studies by Ritossa (9) and Tartof (10), unequal SCE was induced by mutations (e.g. *bobbed*) impairing ribosomal RNA (rRNA) expression, and resulted in a genetically stable increase or decrease in rRNA gene copy number. Those flies showing a stable increase in rRNA gene copy number were restored to the wild type phenotype.

Unequal SCE may also be the mechanism of DNA rearrangements that alter gene expression in mammalian cells of the B lymphoid lineage. Such cells undergo a DNA rearrangement during differentiation in vivo or in vitro known as the Ig heavy (H) chain class switch (reviewed in refs. 11 and 12). In this type of DNA rearrangement, a previously assembled H chain variable region (V_H) gene is rearranged from the region 5' of the expressed H chain constant region (C_H) gene to the region 5' of another C_H gene to be expressed. There is evidence that this usually occurs on a single chromosome and in an order corresponding to the 5' to 3' order of C_H genes along the chromosome (although skips may occur). The loss or deletion of C_H genes upstream (5') of the one expressed after an H chain class switch has often been interpreted to mean that the switch occurs by looping out and deletion of DNA on a single chromatid or chromosome. Other studies show that the 5' to 3' order of C_H genes may be altered before or during switching, and that switching involves an asymmetric cell division: these results support SCE as the mechanism of class switching.

[1] *Abbreviations used in this paper:* C, constant region of Ig; H, heavy chain of Ig; NTS, nontranscribed spacer; rRNA, ribosomal RNA; SCE, sister chromatid exchange; V, variable region of Ig.

We have been studying the arrangement and rearrangement of γ2b and γ2a Ig C_H genes in a mouse myeloma cell line producing a γ2b H chain (MPC11), and variants of this line obtained after mutagenesis. Two types of variants examined are γ2b → γ2a H chain class switch variants, which have undergone DNA rearrangements similar to those observed by others (13, 14) in such cells, and hybrid γ2b-γ2a H chain producers, which are the result of γ2b-γ2a gene recombination (15). In the course of examining the latter variants, we were surprised to find that the single-copy, expressed γ2b gene of MPC11 had undergone recombination with one of two different γ2a genes originally present in the MPC11 cell line. Similarly, a more recent study (14 and L. A. Eckhardt, Columbia University, New York, personal communication) shows that γ2b → γ2a class switch variants of MPC11 have undergone recombination between the 5' flanking region of the single-copy, expressed γ2b gene of MPC11 and that of one or the other γ2a genes mentioned. These γ2a genes differ by restriction maps in their flanking regions, but not in their coding regions. Since BALB/c mice (from which MPC11 was derived) have only a single γ2a gene per haploid genome (16), we proposed (15) that either mitotic recombination (between homologues) occurred in the generation of some γ2b → γ2a class switch variants and some hybrid γ2b-γ2a H chain–producing variants, or tandem γ2a genes existed downstream of the γ2b gene of MPC11. Here, we demonstrate that the latter is indeed the case, that these genes were apparently generated by spontaneous, unequal SCE, and that these γ2a genes undergo copy number variation in MPC11 variants derived by mutagenesis. This copy number variation is the result of mutagen-enhanced unequal SCE. Implications for the role of unequal SCE in general and in the H chain class switch are discussed.

Materials and Methods

Cell Lines. The MPC11 DNA used in these studies was isolated from 45.6.2.4 clone of the cultured cell line originally derived from the MPC11 (γ2b, κ) BALB/c tumor (17). Additional nonmutagenized clones of the MPC11 cell line were used in one experiment; these are 45.6.2.4A (recently subcloned from 45.6.2.4 by L. A. Eckhardt), 45.6 and 45.6.3.2 (18), 45.6.2.4 R152$^2_1$ (isolated by Dr. B. Diamond, Albert Einstein College of Medicine), and 4T001.1 (a drug-marked cell which is thioguanine- and ouabain-resistant) (19). These clones appear identical to each other and to 45.6.2.4 with respect to IgH chain production.

Mutagenesis of the MPC11 cell line with 1CR-191 or Melphalan has generated variants that are short γ2b H chain producers (M311, 4.68.13.9, 11.19E5.7, 4.68.135F5.5), hybrid γ2b-γ2a H chain producers (9.7.1, 11.19.2, 11.19.3, 4.68.66, 4.68.110, 16), and γ2b → γ2a H chain class switch variants (9.9.2.1, 9.9.1.6.7, 11.8, M319.2, M224) (18, 20–26, and G. Gilmore and B. Birshtein, Albert Einstein College of Medicine, unpublished results).

Probes. γ2a- and γ2b-specific probes were prepared by isolating Sst I fragments containing only C_H3 domain sequences from the genes. The γ2a-C_H3 probe is either the 312-basepair (bp) Sst I fragment, containing most of the γ2a C_H3 domain from pG2a-10-21 (27), or this fragment subcloned into pDH24 (28), a pBR322-derived vector with ~60 bp of DNA containing two Sst I sites and multiple Xba I sites inserted into the plasmid Eco RI site. The γ2b-C_H3 probe is the 312-bp Sst I fragment containing most of the γ2b C_H3 domain from pγ2b(11)[7] (29).

The γ2a probe (crosshybridizing with γ2b gene sequences) is either the Pst I fragment of ~1 kilobasepair (kbp) containing most of the γ2a C_H region from pG2a-10-21 (27), or

the Pst I-Sst I fragment of 511 bp representing the 5' half of the Pst I fragment just described.

Probe pγ2b RI.4 is the 7 kbp Eco RI fragment containing the γ2b gene isolated from a recombinant phage, RBL 216 (30), and subcloned into pBR325 by L. A. Eckhardt (see Fig. 1 *C*).

Genomic Southern Blot Analysis. DNA isolation and digestion, agarose gel electrophoresis, blotting to nitrocellulose, and hybridization to ^{32}P-labeled nick-translated probes were as described by Eckhardt et al. (13).

Heteroduplex Analysis. Charon 4 phage clones Ch.M.Ig.γ2a-9 and Ch.M.Ig.ε-12 (16) were grown in the K802 strain of *E. coli*, and the phage DNA was isolated essentially as described by F. R. Blattner (University of Wisconsin, Madison) in the protocol that accompanies the Charon λ phages. Heteroduplexes were formed using a modification (31) of the formamide technique described by Davis et al. (32). Under these conditions, completely heteroduplexed regions of DNA are ≥63% homologous (33). In one reaction, phage DNA from Ch.M.Ig.γ2a-9 and Ch.M.Ig.ε-12 were denatured and reannealed, and in a separate reaction, pBR322 DNA linearized with Hind III was denatured and reannealed to itself. The two reactions were combined before spreading the DNA on grids: the pBR322 DNA provided an internal size marker for both single-stranded and double-stranded DNA. Electron micrographs were taken with a JEOL 100CX electron microscope at magnifications ranging from 5,000 to 20,000×, and images were enlarged an additional 3×. DNA lengths were measured with a graphics calculator, Numonics Model 237.

Results

Tandem γ2a Genes Downstream of the Expressed γ2b Gene of MPC11 Have Apparently Arisen by Spontaneous, Unequal SCE. Lang et al. (30) first showed that there are two different γ2a gene forms in the MPC11 cell line based on the fact that a germline-size (22 kbp) Kpn I fragment and a non-germline-size (14 kbp) Kpn I fragment of MPC11 DNA hybridized strongly to a γ2a probe. Using a similar probe (which crosshybridizes with γ2b gene sequences) and a variety of other restriction enzymes (Bam HI, Hind III, Eco RI, and Bgl I), however, we detected only fragments of germline-size attributable to γ2a gene hybridization in MPC11. The apparent discrepancy between these results will be discussed further on.

We then prepared a γ2a-specific probe (γ2a-C$_{H}$3, see Materials and Methods) to map the chromosomal location of the two MPC11 γ2a gene forms by Southern blot analysis. In Fig. 1 *A*, results of hybridization of γ2a-C$_{H}$3 to MPC11 and liver DNA digested with Eco RI, Kpn I/Eco RI, Kpn I, or Bgl I are shown. Note that there are two bands in each MPC11 track, one of which is identical to germline (liver) in size, and one of which is not. Assuming that the restriction fragments of germline size (fragments a–c, and g) derive from one γ2a gene form, and the fragments unique to MPC11 (fragments d–f) derive from the other γ2a gene form, we can construct the restriction map of these forms shown in Fig. 1 *C*, third line from the top (Hind III data in Fig. 1 *B* and *C* will be discussed further on). This map places the two γ2a gene forms in tandem downstream of the expressed γ2b gene of MPC11: the γ2-specific restriction fragments unique to MPC11 derive from the more 5' γ2a gene form, and those of germline size from the more 3' γ2a gene form. The upper part of Fig. 1 *C* shows how the γ2a gene duplication in MPC11 could have arisen by unequal SCE following precise pairing of the single γ2a gene on one chromatid with the expressed γ2b gene on the other chromatid.
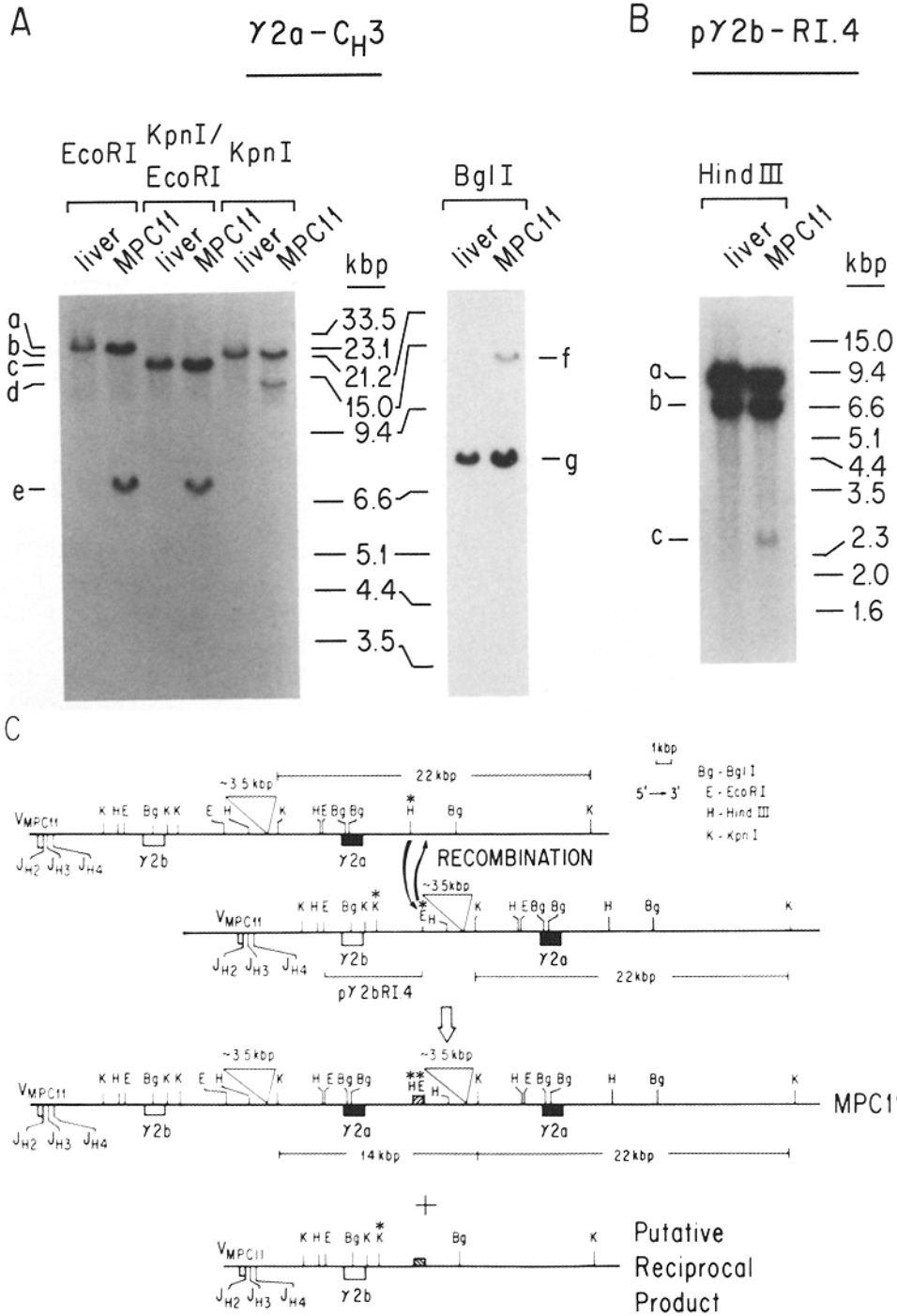
We were aided in our construction of this map by two important types of observations. First, we knew that the deletion of ~3.5 kbp shown 5′ of γ2a genes in Fig. 1 C is unique to the expressed chromosome of MPC11 (14). The other copy of chromosome 12 in MPC11 has undergone a reciprocal translocation with chromosome 15 via recombination between c-myc and $S_{\gamma 2a}$ sequences (34). Because of this deletion, the distance between the expressed γ2b gene and the γ2a gene downstream of it is 12.3 kbp, rather than the germline distance of 15.8 kbp (14). Furthermore, the distance between the γ2a gene form unique to MPC11 and its downstream γ2a gene is also 12.3 kbp, as determined by the size of the γ2a-specific restriction fragments unique to MPC11 (14 kbp Kpn I and 13.5 kbp Bgl I fragments, d and f in Fig. 1A, and a 13.2 kbp Xho I fragment, data not shown). These results implied that the tandem γ2a genes have the ~3.5 kbp deletion between them. Since the deletion is unique to the expressed chromosome, the tandem γ2a genes must be on this chromosome.

Second, the fact that we were able to map the two γ2a genes at the same distance apart as the expressed γ2b gene and its downstream γ2a gene suggested that the γ2a gene duplication must have arisen by a process of unequal crossing over after precise pairing of γ2b and γ2a genes, as shown in Fig. 1 C. This model was made even more attractive by the knowledge that γ2b and γ2a genes are highly homologous to each other (35), and that their 3′ flanking sequences, out to a distance of some 3 kbp from the genes, form a complete heteroduplex when reannealed in the form of phage clones and examined in the electron microscope (36).

The model further explained why only Kpn I, of the enzymes previously tested, allowed us to detect the newly created non-germline γ2a gene form of MPC11 by hybridization with γ2a probes that crosshybridize with γ2b gene sequences. For example, the Bgl I fragment containing the 3′ end of the non-germline γ2a gene form (13.5 kbp) (Fig. 1, A and C) is identical in size to that containing the 3′ end of the expressed γ2b gene of MPC11 (14, 30). This fragment would, therefore, have been attributed to the expressed γ2b gene in previous studies, while, in fact, it represented both the expressed γ2b gene and the non-germline γ2a gene. As the model shows, all the enzymes we used, with the exception of Kpn I, yield a restriction fragment size for the non-germline γ2a gene form which is either identical to that of the germ line γ2a gene form or to that of the expressed γ2b gene.

FIGURE 1.   Demonstration of tandem γ2a genes in MPC11 by genomic Southern blot analysis, and a model showing their formation by unequal SCE. (A) Eco RI, Kpn I/Eco RI, Kpn I, and Bgl I digests of MPC11 vs. germline (liver) DNA hybridized with a γ2a-specific probe (γ2a-C_H3). (B) Hind III digests of MPC11 and liver DNA hybridized with pγ2b RI.4, a probe containing γ2b gene coding and flanking sequences (see C). (C) Restriction map of MPC11 tandem γ2a genes apparently generated by unequal SCE as shown. Most of the restriction sites around the expressed γ2b gene and the germ line γ2a gene form (22 kbp Kpn I fragment) of MPC11 were originally mapped by Lang et al. (30). The latter also found a non-germline γ2a gene form (14 kbp Kpn I fragment) in MPC11, but did not map its location. The deletion of ~3.5 kbp was mapped by Eckhardt and Birshtein (14). The crosshatched regions on the maps of MPC11 and the putative reciprocal product denote the site of recombination between chromatids (novel joint). The presence or absence of the asterisked Kpn I, Hind III, and Eco RI sites in MPC11 versus the recombining chromatids were used to determine the boundaries of this site.

Confirmation of the model was obtained by mapping a novel joint resulting from the γ2a gene duplication as follows. First of all, note that the γ2a gene form unique to MPC11 (the more 5' form) is contained in an Eco RI fragment of 7 kbp (fragment e in Fig. 1 A), which is identical in size to the Eco RI fragment containing the expressed γ2b gene of MPC11 (30). Since the 5' flanking Eco RI sites of the γ2b and γ2a genes are at the same distance from the genes (data not shown), this finding implies that the newly created γ2a gene form in MPC11 acquired its 3' flanking Eco RI site from the expressed γ2b gene via the mechanism of precise pairing and recombination shown in Fig. 1 C. The acquired Eco RI site is indicated by an asterisk on the recombining chromatid and the product. The fact that the 7 kbp Eco RI fragment, representing the newly created γ2a gene form, is not cut by Kpn I (see Kpn I/Eco RI track of MPC11 DNA in Fig. 1 A) placed the site of recombination in the region between the γ2b 3' flanking Kpn I site and the Eco RI site just discussed (both indicated by an asterisk, Fig. 1 C).

The site of recombination was further delimited by showing that the newly created γ2a gene form in MPC11 retained the 3' flanking Hind III site from the γ2a gene. As shown on the MPC11 map in Fig. 1 C, retention of this site (also indicated by an asterisk) should create a new Hind III fragment spanning the crosshatched region or novel joint. This Hind III fragment should be 2.5 kbp in length, and should hybridize to the probe, pγ2b RI.4, which was derived from the γ2b gene and flanking regions as shown in Fig. 1 C. Fig. 1 B shows that, in addition to strongly detecting germline-size γ2b and γ2a genes in liver and MPC11 DNA (fragments a and b, respectively), probe pγ2b RI.4 detects the predicted Hind III fragment (fragment c) in MPC11, but not in liver (germline) DNA. The novel joint resulting from the γ2a gene duplication in MPC11 is, thus, localized to a region of ~800 bp existing some 3.5 kbp 3' of the γ2a gene form created by the duplication.

The 3' flanking region of the γ2b gene, extending from the asterisked Kpn I site (Fig. 1 C) to within ~300 bp of the asterisked Eco RI site, is at least moderately homologous to a nontranscribed spacer (NTS) region of the tandemly repeated mouse rRNA genes (37). Sequences homologous to the NTS region are dispersed throughout the mouse genome, including the flanking regions of C_μ and C_α genes. The function of these sequences is unknown, but they could have played a role in the γ2a gene duplication in MPC11, especially since NTS-homologous sequences probably exist in the 3' flanking region of the γ2a gene as well, by virtue of its high homology to that of the γ2b gene (see below).

The Recombining Regions Involved in the γ2a Gene Duplication in MPC11 Are Highly Homologous.   As in classical models (38, 39) of unequal crossing over in germline cells, we have invoked homology between the γ2b and γ2a genes and their 3' flanking regions as the basis for unequal pairing and precise recombination between sister chromatids in MPC11. In fact, though a heteroduplexing study (36) shows the 3' flanking regions of γ2b and γ2a genes to be highly homologous out to ~500 bp 5' of the 800 bp region containing the novel joint in MPC11 (crosshatched area, Fig. 1 C), the precise regions of germ line DNA that contributed to the recombination breakpoint (novel joint) in MPC11 have not been sequenced or assessed for degree of homology.

To address the question of whether these germline regions are highly homologous, we have formed heteroduplexes of the relevant 3' flanking regions of γ2b and γ2a genes with each other, and viewed them by electron microscopy. Fig. 2C shows the germline configurations of γ2b and γ2a genes on the top and bottom lines; the γ2b gene on the top line is aligned with the γ2a gene on the bottom line, and the sequences that contributed to the novel joint in MPC11 are depicted as an open, vertical box extending across all the lines. The Charon 4 phage clones of germline DNA derived from the regions shown on the top and bottom lines (Fig. 2C) are depicted on the second and fourth lines, respectively (γ2a-9 and ε-12) (16). The genomic DNA inserts in these clones are oriented in the same direction with respect to the Charon 4 arms, facilitating analysis of the heteroduplex formed between γ2a-9 and ε-12 DNA. Fig. 2A shows a representative electron micrograph of such a heteroduplex, with an interpretive drawing in Fig. 2B. The average of measurements from this molecule and ten others are given in the Fig. 2 legend, and are represented by the map in the middle of Fig. 2C (γ2a-9/ε-12 heteroduplex). Moving from left to right (5' → 3') on this map and on the molecule depicted in Fig. 2B, we see first a complete duplex of the short arm of Charon 4, followed by a short, single-stranded loop (indicated by an arrow in Fig. 2B). The loop results from the fact that the ε-12 genomic DNA insert extends ~800 bp further 5' than the γ2a-9 insert as they are aligned. Following this single-stranded loop, we enter a region of high homology which is completely duplexed through the region containing sequences contributing to the novel joint (open vertical box). Immediately 3' of the novel joint, we encounter a region of low homology represented by two single-stranded loops of slightly different lengths; the length difference is attributable to the extension of the γ2a-9 insert ~900 bp further 3' than the ε-12 insert. The remainder of the molecule represents the duplexed long arm of Charon 4 DNA.

In summary, the results corroborate those of Nakai et al. (36) and further demonstrate that the two germline sequences contributing to the novel joint in MPC11 form a complete heteroduplex when denatured and reannealed; the two regions must, therefore, be highly homologous (33). This result was confirmed by hybridization of the appropriate germline sequences to each other under stringent conditions. From consideration of our hybridization conditions, we can conclude that the germline sequences recombining to form the novel joint in MPC11 are ≥75% homologous to each other. This result then extends the analogy of unequal crossing over to generate tandem γ2a genes in MPC11 to unequal crossing over in germline cells, which is thought to be facilitated by homology of the recombining regions (38, 39). It is also interesting that the actual site of recombination (novel joint) is located at the boundary of regions of high and low homology (Fig. 2). This is consistent with the concept of double-strand branch migration proceeding through the region of high homology to the boundary of the region of low homology where it is blocked, allowing recombination to occur (40).

γ2a Genes on the Expressed Chromosome of MPC11 Undergo Further Copy Number Variation by Unequal SCE in Mutagenized Cells Expressing Altered H Chains. In Fig. 3, we show the results of hybridization with a γ2a probe to Kpn I digests of DNA from MPC11 and several variants of this cell line in Ig H chain production.
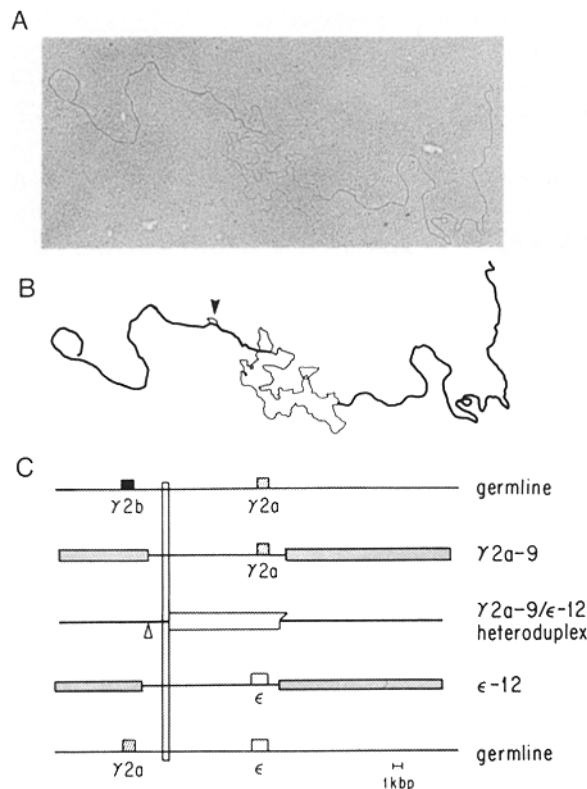
FIGURE 2.   Demonstration of marked homology between the recombining regions of germ-line DNA involved in the γ2a gene duplication in MPC11 by heteroduplex analysis. (A) A representative electron micrograph (39,000 ×) of a heteroduplex formed between γ2a-9 and ε-12 DNA (see C). (B) Interpretive drawing of the heteroduplex shown in (A). The arrow orients this drawing to the map in C: the short single-stranded loop it indicates is depicted in C by a small triangle. (C) Derivation of γ2a-9 and ε-12 from genomic DNA and the summary of measurements on 11 γ2a-9/ε-12 heteroduplexed molecules. γ2a-9 and ε-12 are Charon 4 phage clones of genomic DNA derived from the regions shown on the top and bottom lines, respectively (16). The Charon 4 arms of γ2a-9 and ε-12 are indicated by stippled boxes. The regions of germline DNA contributing to the novel joint in MPC11 are indicated by an open, vertical box extending across all the lines. The measurements of γ2a-9/ε-12 heteroduplexes are summarized on the middle line by a thick line indicating duplexed regions, and thin lines extending above or below the level of the thick line indicating single-stranded loops. Moving from 5′ to 3′, the average lengths of these regions ± SD in kbp are: short arm of Charon 4 DNA, 10.81 ± 0.32; single-stranded loop, 0.80 ± 0.04; double-stranded region of insert DNA, 2.19 ± 0.13; longer single-stranded loop, 14.50 ± 0.32; shorter single-stranded loop, 13.59 ± 0.28; long arm of Charon 4 DNA, 19.64 ± 0.54.

The variants were isolated after mutagenesis of MPC11 with ICR-191 or Mel-phalan (see Materials and Methods). Of particular interest is the copy number variation of the non-germline (14 kbp, fragment d) γ2a gene form in the variants. For example, note that 9.7.1 and M319.2 have a greater copy number of this form than MPC11, while 9.9.1.6.7 and M224 have deleted this form. In contrast, the germline γ2a gene form (22 kbp, fragment b) remains constant in copy number from variant to variant. The copy number variation of the non-germline (14 kbp) γ2a germ form is not restricted to any one type of variant: 9.7.1 is

a hybrid γ2b-γ2a H chain producer, and 9.9.1.6.7, M319.2, and M224 are γ2b → γ2a H chain class switch variants. Also, variants derived by either 1CR-191 or Melphalan mutagenesis show the γ2a gene copy number variation: 9.7.1 and 9.9.1.6.7 were derived by 1CR-191 mutagenesis and M319.2 and M224 by Melphalan mutagenesis. Fig. 3B shows that γ2a gene copy number variation occurs at a lower frequency in MPC11 cells that have not been mutagenized. As compared to 9 out of 12 (~75%) independent MPC11 variants showing the γ2a gene copy number variation (see Fig. 3 legend), only one (4T001.1) of six independent MPC11 clones shows the copy number variation (i.e., modest amplification). Since 4T001.1 was derived by selection for thioguanine and ouabain resistance (19), its appropriateness as a clone to include in analysis of the spontaneous level of γ2a gene copy number variation in MPC11 is questionable. With this consideration, then, we can conclude that the frequency of copy number variation is ≤16% in nonmutagenized MPC11 cells.

In Fig. 4, we show that a model of unequal SCE accounts precisely for the γ2a gene copy number variation in MPC11 variants. This SCE may be facilitated by unequal pairing of the tandem γ2a genes to create a stretch of ~13 kbp of duplexed DNA in which the strands are 100% homologous (indicated by dashed lines in Fig. 4). Recombination within this region yields one chromatid with two copies of the non-germline γ2a gene form (14 kbp Kpn I fragment), and another chromatid which has deleted this form. Segregation of these two chromatids into daughter cells upon cell division yields two independent variants with γ2a gene copy number variation. Note that the copy number of the germline γ2a gene form (22 kbp Kpn I fragment) remains constant (one per chromatid) throughout this process. If recombination occurred more than a few kbp 5' or 3' of the boundaries shown in Fig. 4, new Kpn I or Eco RI fragments would be created, which would be detectable with a γ2a probe in MPC11 variants. Thus far, no such fragments have been detected. We do not, therefore, believe that there are any special features of the non-germline γ2a gene form that cause its copy number variation rather than the germline γ2a gene form, but rather that the results are a direct consequence of pairing the two highly homologous regions of DNA shown in Fig. 4 and recombining between them.

The process of γ2a gene copy number variation has apparently continued for several generations following mutagenesis in most of the MPC11 variants (Fig. 3). Variant 16 has multiple copies of the non-germline γ2a gene form, 11.19.2 has less than one copy of this form, presumably due to deletion of the gene in a subpopulation of the 11.19.2 cells after cloning, and the secondary variants, 9.9.1.6.7 and 9.9.2.1, have a different γ2a gene copy number than the primary variant, 9.7.1, from which they arose spontaneously. This observation implies that a stable increase in the frequency of unequal SCE around the γ2a genes has been induced in MPC11 variants, presumably by mutagenesis. The mutagen, thus, appears to act as a differentiation or inducer signal for unequal SCE in the C_H locus.

γ2a Gene Copy Number Variation Occurs Independently of γ2a Gene Rearrangements Resulting in Altered H Chain Expression. One of the most attractive features of the model shown in Fig. 4 is that it accounts for both an increase and a decrease in γ2a gene copy number by one simple mechanism. If one only had to

account for an increased γ2a gene copy number in MPC11 variants, other models such as gene amplification by re-replication might be proposed. For this reason, we felt it was important to demonstrate unambiguously that deletion of the non-germline γ2a gene form could occur independently of gene rearrangements resulting in altered H chain expression. We were particularly concerned about this point, since most of the MPC11 variants that show deletion of the non-germline γ2a gene form (14 kbp Kpn I fragment) are hybrid γ2b-γ2a H chain producers or γ2b → γ2a class switch variants that may have deleted this form as a consequence of recombination of the germline γ2a gene form (22 kbp Kpn I fragment) or its 5' flanking sequences with the γ2b gene or its 5' flanking sequences. On the other hand, these variants may have initially deleted the non-germline γ2a gene form by copy number variation as shown in Fig. 4, then used the germline γ2a gene form for recombination with the γ2b gene. One could not tell the order of these events in such variants, however.

To address this point, we chose for further study a variant, 4.68.13.9, that

---

FIGURE 3. (A) Genomic Southern blots of Kpn I-digested MPC11 and variant DNA hybridized with a γ2a probe showing copy number variation of the non-germline γ2a gene form (fragment d, 14 kbp) in the variants. Fragments b and h represent the germline γ2a gene form and γ2b genes, respectively. Fragments a, c, and e–g are the result of γ2b and γ2a gene rearrangements associated with altered H chain expression in the variants. The first six lanes in the right hand panel were published previously (15).[2] The frequency of copy number variation was evaluated as follows. We assumed that each independent variant had the opportunity to undergo copy number variation, with the exception of the group of 4.68 variants, which all derived from a common precursor variant whose non-germline γ2a gene form was apparently deleted. This group is counted as one independent variant, to yield a total of 12 independent variants examined (including 9.9.2.1, Fig. 3B). Since the 11.19 group of variants derived from a common variant precursor as well, and since 11.19E5.7 and 11.19.3 appear identical to each other in terms of γ2a gene copy number, we count only two of these three variants as having undergone copy number variation. Variants 11.8 and M311 appear identical to MPC11, and presumably have not undergone copy number variation. Finally, though 9.9.2.1 appears similar, if not identical, to MPC11 (shown in Fig. 3B), we assume it has undergone copy number variation, since it arose directly from 9.7.1, which has markedly amplified γ2a gene copy number. Variant 9.9.2.1 has, thus, undergone deletion of the non-germline γ2a gene form with respect to its precursor variant. In summary, 9 of 12 independent variants show copy number variation. (B) Genomic Southern blot showing γ2a gene copy number variation in only one (4T001.1) of six independent clones of the MPC11 cell line (9.9.2.1 is a variant, see above) whose DNA was digested with Kpn I and hybridized with a γ2a probe. Fragments b, d, and h are identified above.

---

[2] In hybrid γ2b-γ2a H chain–producing variants 11.19.3, 11.19.2, and 16, the non-germline γ2a gene form, represented by fragment d, has undergone recombination with the expressed γ2b gene of MPC11 to yield a hybrid gene represented by fragment e, whereas in hybrid H chain–producing variants 4.68.66 and 4.68.110, the germline γ2a gene form, represented by fragment b, has undergone recombination with the γ2b gene to yield a hybrid gene represented by a fragment ~2 kbp smaller than fragment b. This smaller fragment is not seen here, but is detectable on blots of Kpn I-digested DNA hybridized with a probe recognizing γ2b gene 5' flanking sequences (15). We had originally considered the possibility of gene conversion in hybrid γ2b-γ2a gene formation in these variants, since at least one copy of whichever γ2a gene form was used for recombination in any given variant was retained. We now know that multiple copies of the non-germline γ2a gene form can be generated by copy number variation, and that the germline γ2a gene form exists on two different chromosomes. Therefore, it is not necessary to invoke gene conversion in hybrid gene formation, though it cannot be formally excluded.
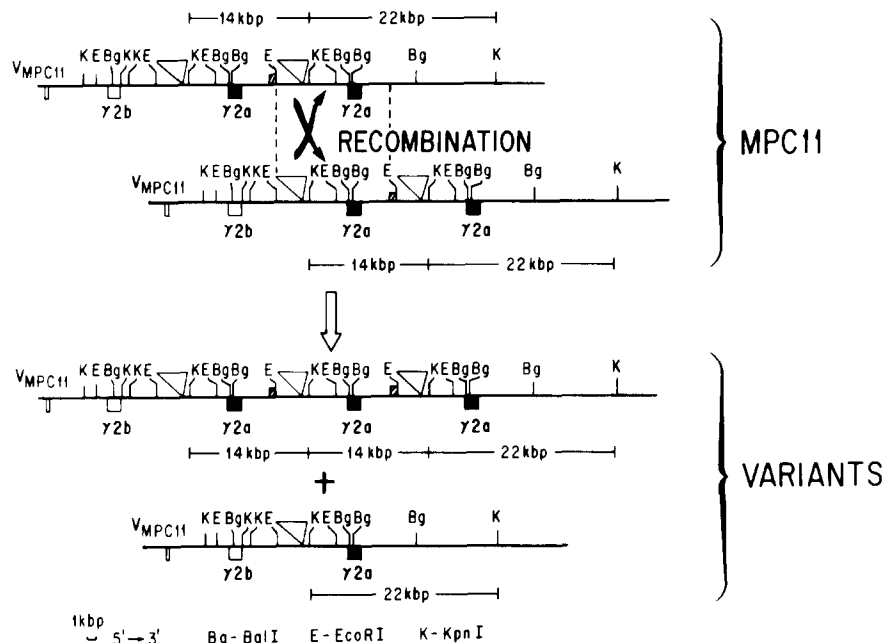
FIGURE 4. A model of unequal SCE accounting precisely for the γ2a gene copy number variation in MPC11 variants. The model is based on misalignment of sister chromatids from MPC11, so that the more 3' γ2a gene on one chromatid pairs with the more 5' γ2a gene on the other chromatid. This results in formation of a duplex of ~13 kbp of 100% homologous sequences (whose boundaries are indicated by dashed lines) within which recombination may occur. The crosshatched boxes are novel joints from the γ2a gene duplication in MPC11, and the triangles represent deletions originally present in MPC11 (see Fig. 1C).

produces a short γ2b H chain as a consequence of a simple frameshift mutation at the 3' end of the C_H2 domain of the expressed γ2b gene (26). Fig. 3A shows that 4.68.13.9 has deleted the 14 kbp Kpn I fragment representing the non-germline γ2a gene form (fragment d). Yet no additional γ2a or γ2b gene rearrangements on the expressed chromosome are seen in 4.68.13.9 by Southern blot analysis of 4.68.13.9 DNA compared to MPC11 DNA cut with Bgl II, Xba I, Eco RI, Bam HI (41), and Hha I (S. Tilley and B. Birshtein, data not shown). We, therefore, predict from our model that the map of the expressed H chain chromosome of 4.68.13.9 should be identical to the map at the very bottom of Fig. 4. By hybridization with γ2a- and γ2b-specific probes to Bgl I-digested 4.68.13.9, MPC11, and liver DNA, we have confirmed that this is, indeed, the case. These results show that γ2a gene deletion via copy number variation can occur independently of gene rearrangements resulting in altered H chain expression.

Discussion

Unequal SCE as the Mechanism of γ2a Gene Duplication in MPC11.   Tandem γ2a genes downstream of the expressed γ2b gene of MPC11 have apparently been generated by spontaneous, unequal SCE. The only other simple mechanisms accounting for this γ2a gene duplication are mitotic recombination between

homologous chromosomes or re-replication of DNA. Since an identical deletion (of ~3.5 kbp) appears 5' of each of the γ2a genes on the expressed chromosome in MPC11, mitotic recombination can only account for the gene duplication if one invokes identical independent deletions occurring 5' of the γ2a genes on two separate chromosomes before recombination, or 5' of each γ2a gene after recombination. This is, of course, much less likely than the deletion occurring once on a single chromosome and then being duplicated by unequal SCE. Re-replication of DNA has been invoked to explain the developmentally regulated amplification of rRNA genes of the *Xenopus* oocyte (42, 43), and chorion genes of the *Drosophila melanogaster* ovary (44, 45), as well as the amplification of dihydrofolate reductase genes of Chinese hamster ovary cells selected for methotrexate resistance (46). This re-replication apparently initiates at specific replicon origins that are normally used only once during a single round of DNA replication. The result is marked amplification of large stretches of DNA ($\geq$40 kbp) (47). It is highly unlikely that the γ2a gene duplication in MPC11 arose by DNA re-replication, since a stretch of only ~13 kbp of DNA was duplicated (and not amplified further). Also, studies on the time of replication of C_H genes during S phase are consistent with an origin of replication near C_α, from which $C_{\gamma2a}$ is replicated (48). However, most of the DNA between $C_{\gamma2a}$ and C_α does not appear to be duplicated in MPC11.

Our earliest clones of the MPC11 cell line show the γ2a gene duplication, indicating that the event occurred before or during establishment of the cell line. We strongly suspect that the event occurred either in a B cell precursor of MPC11 or in MPC11 itself rather than in the germline, since the ~3.5 kbp deletion 5' of γ2a must have occurred before the γ2a gene duplication, and such deletions (which include switch region sequences) have been described in other cells of the B lymphoid lineage (49), but not in germline cells of individuals within a particular mouse strain. While there is genetic evidence for unequal SCE in the germline of *Drosophila* carrying a mutation in the rRNA genes (10), there has been little, if any, convincing evidence for spontaneous (i.e., not mutagen-induced) SCE in somatic cells of any organism. Our data on the tandem γ2a genes of MPC11 may, therefore, be the best evidence for spontaneous, unequal SCE in somatic cells to date.

γ2a Gene Copy Number Variation in MPC11 Variants Also Occurs by Unequal SCE. After the initial, spontaneous γ2a gene duplication in MPC11, variants of this line were obtained by mutagenesis with ICR-191 or Melphalan, and selection for altered H chain expression. In addition to the selected phenotype, these variants showed a striking variation in γ2a gene copy number such that amplification or deletion of the non-germline γ2a gene form is seen in different variants. Both this observation and the fact that no new restriction fragments hybridizing with γ2b or γ2a probes are generated in the variants via copy number variation are strongly indicative of unequal SCE as the basis of this phenomenon. In fact, we cannot distinguish copy number variation mechanistically from the initial γ2a gene duplication. Both appear to result from precise pairing and reciprocal recombination between highly homologous regions, as in classical models of unequal crossing over (38, 39).

Mechanistic Similarity of Unequal SCE in Somatic Cells to Unequal Crossing Over

in Germline Cells.   The unequal crossing over that was apparently responsible for the γ2a gene duplication in MPC11 and γ2a gene copy number variation in MPC11 variants bears a remarkable resemblance to that which has been invoked to explain the duplication and deletion of homologous genes within multigene families during evolution (38, 39). A more recent evolutionary event is the γ2a gene duplication in certain wild mouse strains (50, 51). The array of a single γ2b gene with downstream tandem γ2a genes in these mice is very similar to that seen in MPC11 (but without the ~3.5 kbp deletion 5' of the γ2a genes). Yet the γ2a gene duplication in wild mice was a germline rather than somatic event. Recent studies have also indicated that unequal crossing over occurs at a high frequency in the C_H cluster of the human germline (52). Thus, whatever the physiological role of unequal SCE in somatic cells may be, what we learn about this mechanism should illuminate the important, analogous germline events as well.

High Frequency of Unequal SCE in the C_H Locus as Observed in MPC11 Variants.   9 out of 12 (~75%) independent variants of MPC11 show γ2a gene copy number variation via unequal SCE. Even if we exclude two variants (i.e., 9.9.1.6.7 and M224, Fig. 3) in which the deletion of the non-germline γ2a gene form may have occurred as a consequence of recombination of the more 3' (germline) γ2a gene form with γ2b (see Results), we still have 7 out of 12, or ~58% of variants showing copy number variation. This is to be compared with the highest reported mutation frequencies at a single locus after mutagenesis, which are on the order of a few percent (18).

It is clear that the frequency of γ2a gene copy number variation is at least fourfold greater in MPC11 variants than in the parental cell line (MPC11). Compared to a minimum of ~58% of the variants that show copy number variation, ≤16% of the independent, non-mutagenized clones of MPC11 show copy number variation. Since we have not examined mutagenized MPC11 cells that retain the parental phenotype of Ig H chain production (i.e., do not become variant in this property through mutagenesis), we cannot rigorously exclude the possibility that becoming variant in Ig H chain production, rather than having been mutagenized, is the key to enhanced copy number variation in MPC11 variants. We can only point out that there is a precedent for the enhancement of SCE (by which copy number variation apparently occurred) by mutagens (5–7).

The most conservative explanation for the high frequency of γ2a gene copy number variation in MPC11 variants is that it is a simple consequence of mutagenizing tandem genes that are virtually identical, and which lie near a highly expressed locus (in this case, the γ2b gene). Preliminary results of probing the α- and β-globin loci in MPC11 variants for copy number variation are negative (L. Kuhn, X.-D. Fan, and B. Birshtein, unpublished results), suggesting, at least, that highly homologous tandem genes that are presumably not near a highly expressed locus in MPC11 are not affected by mutagenesis in the same way as the tandem γ2a genes.

The high frequency of γ2a gene copy number variation in MPC11 variants may, in fact, reveal a genetic property(ies) unique to the Ig C_H locus. Since we have examined only six independent, nonmutagenized clones of the MPC11 cell

line for copy number variation thus far, we do not yet know whether the spontaneous level of this event is particularly high in the C_H locus of these cells. Whatever the spontaneous frequency of copy number variation may be, it is possible that the C_H locus in such cells is particularly susceptible to perturbations such as mutagenesis. In this case, the mutagens could be mimicking a physiological signal that specifically induces unequal SCE in this locus.

Possible Roles of Unequal SCE in Ig Gene Expression. Likely roles of unequal SCE in the C_H locus would be to influence or effect the H chain class switch. One way in which unequal SCE can influence the class switch is through C_H gene copy number variation, as illustrated by the effect of spontaneous $\gamma 2a$ gene duplication (via unequal SCE) on switching in MPC11. The MPC11 cell line undergoes class switch recombination between the 5' flanking region of the expressed $\gamma 2b$ gene and that of either of the two $\gamma 2a$ gene forms in tandem downstream (14 and L. A. Eckhardt, personal communication). In relating this observation to class switching in vivo, it should be noted that having multiple copies of a C_H gene (such as $C_{\gamma 2a}$ in MPC11) might prejudice the cell to switch to that isotype or to maintain production of that isotype, rather than switching to C_H genes further downstream. This would be particularly true in situations where C_H genes are used sequentially (rather than where C_H genes are skipped) in the class switch, as is the case in certain in vitro systems (53). Perhaps of greater physiological significance would be the effect of deletion of a C_H gene or genes (such as $C_{\gamma 2a}$ in the putative reciprocal product of unequal SCE, Fig. 1 C) on the class switch. Such a deletion would not only prevent expression of the gene(s) deleted, but could enhance the frequency of switches to C_H genes 3' of the one deleted, or commit the cell to production of a particular C_H gene on the 3' side of the deletion. This is an especially interesting concept because few studies have completely characterized the intervening DNA in cells before class switching; C_H genes may actually be deleted before a switch recombination event.

Unequal SCE has, itself, been proposed as a mechanism by which class switching can occur (reviewed in refs. 11 and 12). The consequences of switching in MPC11 by this mechanism are illustrated in Fig. 5. While a recent study concludes that unequal SCE is not the mechanism of class switching in a pre-B cell line (54), it is not yet clear whether this observation will extend to other cell lines of the B-lymphoid lineage, or to B cells in vivo. It is of interest that Melphalan, which increases the level of SCE in human cells (55–58), enhances the frequency of class switch variants from MPC11 (20) and the level of unequal SCE, as evidenced by $\gamma 2a$ gene copy number variation. Ultraviolet light also increases SCE (59, 60) and promotes H chain class switching in human lymphoblastoid cells (61).

Yet another explanation for the high frequency of unequal SCE in the C_H locus of B lymphoid cells is that it is a consequence or by-product of the genetic variability at this locus. Myeloma cell lines such as MPC11 give rise to variants in Ig H chain production at frequencies orders of magnitude above mutations at other loci (62), and a pre-B cell line exhibits hypermutability at this locus, as well (63). This genetic variability, which is thought to play an important role in Ig gene expression, may induce or involve repair enzymes whose activities promote unequal SCE. The involvement of such enzymes in unequal SCE (as assessed by
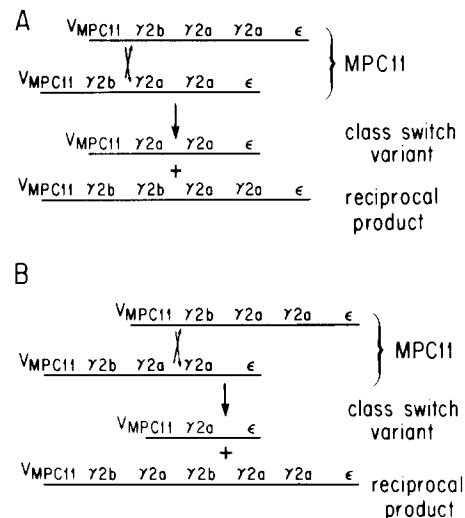
FIGURE 5.   Model showing how the γ2b → γ2a class switch variants of MPC11 could have been generated by unequal SCE, resulting in the switch to either of the tandem γ2a genes of MPC11 (A or B). By analogy, the hybrid γ2b-γ2a H chain–producing variants of MPC11 could have been generated by this mechanism as well, via recombination between the expressed γ2b gene and either of the tandem γ2a genes. In fact, our data do not allow us to exclude looping out as the mechanism of class switching or hybrid gene formation in the MPC11 system. Furthermore, though we have discussed only mechanisms involving intrachromosomal recombination here, mitotic recombination remains a formal possibility as the mechanism of generating those variants in which the germ line γ2a gene form was used, since a germ line γ2a gene form also exists on the t(12;15) chromosomal translocation in MPC11 (34). However, there is evidence for intrachromosomal rather than interchromosomal recombination in the H chain class switch (reviewed in ref. 11), and mitotic recombination is generally considered a highly infrequent event in somatic cells.

rRNA gene copy number variation) has been documented in *Drosophila* (64, and R. S. Hawley, Albert Einstein College of Medicine, personal communication).

In summary, our results suggest a possible relationship of unequal SCE to the H chain class switch and/or to the somatic variability of Ig genes, to which the class switch contributes. This relationship(s) could involve the sharing of certain DNA structural intermediates which might be acted upon to result in either unequal SCE or the class switch. Alternatively, these processes may share enzymes in common. The definition of the precise relationship of unequal SCE to Ig gene rearrangements affecting expression should lead to a fuller understanding of both of these important somatic processes.

Summary

Two γ2a gene forms in the MPC11 mouse myeloma cell line (γ2b, κ) have been localized to the expressed H chain chromosome, where they exist in tandem downstream of the expressed γ2b gene. The γ2a gene duplication has apparently occurred by spontaneous, unequal sister chromatid exchange (SCE) in a B lymphoid precursor of MPC11 or in MPC11 itself. It is especially significant that either of the tandem γ2a gene forms may be used in the class switch from γ2b to γ2a production occurring in the MPC11 line, indicating that heavy chain

constant region (C_H) gene duplication (or deletion) may be a mechanism normally impinging on C_H gene expression. The $\gamma 2a$ genes of MPC11 undergo further copy number variation via unequal SCE in variants of this line derived by mutagenesis and selection for altered H chain production. The remarkable frequency with which copy number variation occurs in these cells suggests that unequal SCE may play a physiological role in C_H gene arrangement and expression.

References

1. McClintock, B. 1941. Spontaneous alterations in chromosome size and form in *Zea Mays. Cold Spring Harbor Symp. Quant. Biol.* 9:72.
2. Schwartz, D. 1953. Evidence for sister-strand crossing-over in maize. *Genetics.* 38:251.
3. Taylor, J. H. 1958. Sister chromatid exchanges in tritium-labeled chromosomes. *Genetics.* 43:515.
4. German, J. 1982. Biological role for chromatid exchange in mammalian somatic cells? *In* Gene Amplification. R. T. Schimke, editor. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 307–312.
5. Kato, H. 1974. Induction of sister chromatid exchanges by chemical mutagens and its possible relevance to DNA repair. *Exp. Cell Res.* 85:239.
6. Perry, P., and H. J. Evans. 1975. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature (Lond.).* 258:121.
7. Carrano, A. V., L. H. Thompson, P. A. Lindl, and J. L. Minkler. 1978. Sister chromatid exchange as an indicator of mutagenesis. *Nature (Lond.).* 271:551.
8. Chaganti, R. S. K., S. Schonberg, and J. German. 1974. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc. Natl. Acad. Sci. USA.* 71:4508.
9. Ritossa, F. M. 1968. Unstable redundancy of genes for ribosomal RNA. *Proc. Natl. Acad. Sci. USA.* 60:509.
10. Tartof, K. D. 1974. Unequal mitotic sister chromatid exchange as the mechanism of ribosomal RNA gene magnification. *Proc. Natl. Acad. Sci. USA.* 71:1272.
11. Honjo, T., S. Nakai, Y. Nishida, T. Kataoka, Y. Yamawaki-Kataoka, N. Takahashi, M. Obata, A. Shimizu, Y. Yaoita, T. Nikaido, and N. Ishida. 1981. Rearrangements of immunoglobulin genes during differentiation and evolution. *Immunol. Rev.* 59:33.
12. Cebra, J. J., J. L. Komisar, and P. A. Schweitzer. 1984. C_H isotype 'switching' during normal B-lymphocyte development. *Ann. Rev. Immunol.* 2:493.
13. Eckhardt, L. A., S. A. Tilley, R. B. Lang, K. B. Marcu, and B. K. Birshtein. 1982. DNA rearrangements in MPC-11 immunoglobulin heavy chain class-switch variants. *Proc. Natl. Acad. Sci. USA.* 79:3006.
14. Eckhardt, L. A., and B. K. Birshtein. 1985. Analysis of independent immunoglobulin class-switch events occurring in a single myeloma cell line. *Molec. Cell. Biol.* 5:856.
15. Tilley, S. A., L. A. Eckhardt, K. B. Marcu, and B. K. Birshtein. 1983. Hybrid $\gamma 2b$-$\gamma 2a$ genes expressed in myeloma variants: evidence for homologous recombination. *Proc. Natl. Acad. Sci. USA.* 80:6967.
16. Shimizu, A., N. Takahashi, Y. Yamawaki-Kataoka, Y. Nishida, T. Kataoka, and T.

Honjo. 1981. Ordering of mouse immunoglobulin heavy chain genes by molecular cloning. *Nature (Lond.)*. 289:149.

17. Laskov, R., and M. D. Scharff. 1970. Synthesis, assembly, and secretion of gamma globulin by mouse myeloma cells. I. Adaptation of the Merwin plasma cell tumor-11 to culture, cloning and characterization of gamma globulin subunits. *J. Exp. Med.* 131:515.

18. Birshtein, B. K., J.-L. Preud'homme, and M. D. Scharff. 1974. Variants of mouse myeloma cells that produce short immunoglobulin heavy chains. *Proc. Natl. Acad. Sci. USA.* 71:3478.

19. Margulies, D. H., W. M. Kuehl, and M. D. Scharff. 1976. Somatic cell hybridization of mouse myeloma cells. *Cell.* 8:405.

20. Preud'homme, J.-L., B. K. Birshtein, and M. D. Scharff. 1975. Variants of a mouse myeloma cell line that synthesize immunoglobulin heavy chains having an altered serotype. *Proc. Natl. Acad. Sci. USA.* 72:1427.

21. Koskimies, S., and B. K. Birshtein. 1976. Primary and secondary variants in immunoglobulin heavy chain production. *Nature (Lond.)*. 264:480.

22. Francus, T., B. Dharmgrongartama, R. Campbell, M. D. Scharff, and B. K. Birshtein. 1978. IgG2a-producing variants of an IgG2b-producing mouse myeloma cell line. *J. Exp. Med.* 147:1535.

23. Francus, T., and B. K. Birshtein. 1978. An IgG2a-producing variant of an IgG2b-producing mouse myeloma cell line. Structural studies in the Fc region of parent and variant heavy chains. *Biochemistry.* 17:4324.

24. Kenter, A. L., and B. K. Birshtein. 1979. Genetic mechanism accounting for precise immunoglobulin domain deletion in a variant of MPC 11 myeloma cells. *Science (Wash. DC)*. 206:1307.

25. Birshtein, B. K., R. Campbell, and M. L. Greenberg. 1980. A γ2b-γ2a hybrid immunoglobulin heavy chain produced by a variant of the MPC11 mouse myeloma cell line. *Biochemistry.* 19:1730.

26. Kenter, A. L., T. Warren, D. Shields, and B. K. Birshtein. 1984. Mouse myeloma cells that make short immunoglobulin heavy chains: pleiotropic effects on glycosylation and chain assembly. *J. Cell Biol.* 98:2215.

27. Auffray, C., R. Nageotte, B. Chambraud, and F. Rougeon. 1980. Mouse immunoglobulin genes: a bacterial plasmid containing the entire coding sequence for a pre-γ2a heavy chain. *Nucleic Acids Res.* 8:1231.

28. Hare, D. L., and J. R. Sadler. 1980. *Recomb. DNA Tech. Bull.* 2:4.

29. Tucker, P. W., K. B. Marcu, J. L. Slightom, and F. R. Blattner. 1979. Structure of the constant and 3' untranslated regions of the murine γ2b heavy chain messenger RNA. *Science (Wash. DC)*. 206:1299.

30. Lang, R. B., L. W. Stanton, and K. B. Marcu. 1982. On immunoglobulin heavy chain gene switching: two γ2b genes are rearranged via switch sequences in MPC-11 cells but only one is expressed. *Nucleic Acids Res.* 10:611.

31. Yamagishi, H., H. Inokuchi, and H. Ozeki. 1976. Excision and duplication of su3+-transducing fragments carried by bacteriophage ϕ80. I. Novel structure of ϕ80sus2psu3+ DNA molecule. *J. Virol.* 18:1016.

32. Davis, R. W., M. Simon, and N. Davidson. 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. *Methods Enzymol.* 21D:413.

33. Sharp, P. A., M.-T. Hsu, E. Ohtsubo, and N. Davidson. 1972. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. I. Structure of F-prime factors. *J. Mol. Biol.* 71:471.

34. Stanton, L. W., J.-Q. Yang, L. A. Eckhardt, L. J. Harris, B. K. Birshtein, and K. B.

Marcu. 1984. Products of a reciprocal chromosome translocation involving the c-myc gene in a murine plasmacytoma. *Proc. Natl. Acad. Sci. USA.* 81:829.

35. Yamawaki-Kataoka, Y., T. Miyata, and T. Honjo. 1981. The complete nucleotide sequence of mouse immunoglobulin γ2a gene and evolution of heavy chain genes: further evidence for intervening sequence-mediated domain transfer. *Nucleic Acids Res.* 9:1365.

36. Nakai, S., V. Oi, L. A. Herzenberg, H. Yamagishi, and T. Honjo. 1982. Nucleotide sequences encoding membrane domains are conserved among immunoglobulin gamma subclass genes. *Biomedical Res.* 3:37.

37. Marcu, K. B., N. Arnheim, J. Banerji, N. A. Penncavage, P. Seperack, R. Lang, R. Miesfeld, L. Harris, and R. Greenberg. 1981. Studies on the nature and germ-line stability of DNA sequences flanking the mouse immunoglobulin heavy-chain constant-region genes. *Cold Spring Harbor Symp. Quant. Biol.* 45:899.

38. Smith, G. P. 1974. Unequal crossover and the evolution of multigene families. *Cold Spring Harbor Symp. Quant. Biol.* 38:507.

39. Hood, L., J. H. Campbell, and S. C. R. Elgin. 1975. The organization, expression, and evolution of antibody genes and other multigene families. *Ann. Rev. Genet.* 9:305.

40. Radding, C. M. 1978. Genetic recombination: strand transfer and mismatch repair. *Ann. Rev. Biochem.* 47:847.

41. Kenter, A. L. 1982. Genetic mechanisms affecting immunoglobulin gene expression. Ph.D. thesis. Albert Einstein College of Medicine, Bronx, NY. 133 pp.

42. Brown, D. D., and A. W. Blackler. 1972. Gene amplification proceeds by a chromosome copy mechanism. *J. Mol. Biol.* 63:75.

43. Hourcade, D., D. Dressler, and J. Wolfson. 1974. The nucleolus and the rolling circle. *Cold Spring Harbor Symp. Quant. Biol.* 38:537.

44. Spradling, A. C. 1981. The organization and amplification of two chromosomal domains containing Drosophila chorion genes. *Cell.* 27:193.

45. Osheim, Y. N., and O. L. Miller, Jr. 1983. Novel amplification and transcriptional activity of chorion genes in Drosophila melanogaster follicle cells. *Cell.* 33:543.

46. Mariani, B. D., and R. T. Schimke. 1984. Gene amplification in a single cell cycle in Chinese hamster ovary cells. *J. Biol. Chem.* 259:1901.

47. Roberts, J. M., L. B. Buck, and R. Axel. 1983. A structure for amplified DNA. *Cell.* 33:53.

48. Braunstein, J. D., D. Schulze, T. DelGiudice, A. Furst, and C. L. Schildkraut. 1982. The temporal order of replication of murine immunoglobulin heavy chain constant region sequences corresponds to their linear order in the genome. *Nucleic Acids Res.* 10:6887.

49. Stavnezer, J., S. Sirlin, and J. Abbott. 1985. Induction of immunoglobulin isotype switching in cultured I.29 B lymphoma cells; characterization of the accompanying rearrangements of heavy chain genes. *J. Exp. Med.* 161:577.

50. Shimizu, A., Y. Hamaguchi, Y. Yaoita, K. Moriwaki, K. Kondo, and T. Honjo. 1982. Japanese wild mouse, *Mus musculus molossinus,* has duplicated immunoglobulin γ2a genes. *Nature (Lond.).* 298:82.

51. Fukui, K., Y. Hamaguchi, A. Shimizu, S. Nakai, K. Moriwaki, C. H. Wang, and T. Honjo. 1984. Duplicated immunoglobulin gamma 2a genes in wild mice. *J. Mol. Cell. Immunol.* 1:321.

52. Migone, N., S. Oliviero, G. de Lange, D. L. Delacroix, D. Boschis, F. Altruda, L. Silengo, M. DeMarchi, and A. O. Carbonara. 1984. Multiple gene deletions within the human immunoglobulin heavy-chain cluster. *Proc. Natl. Acad. Sci. USA.* 81:5811.

53. Beyreuther, K., J. Bovens, R. Dildrop, H. Dorff, T. Geske, B. Liesegang, C. Muller, M. S. Neuberger, A. Radbruch, K. Rajewsky, F. Sablitzky, P. H. Schreier, and S.

Zaiss. 1981. Isolation and characterization of class switch variants of meyloma and hybridoma cells. *In* Immunoglobulin Idiotypes. C. Janeway, E. E. Sercarz, H. Wigzell, C. F. Fox, editors. Academic Press, NY. 229–244.

54. Wabl, M., J. Meyer, G. Beck-Engeser, M. Tenkhoff, and P. D. Burrows. 1985. Critical test of a sister chromatid exchange model for the immunoglobulin heavy-chain class switch. *Nature (Lond.).* 313:687.

55. Lambert, B., U. Ringborg, E. Harper, and A. Lindblad. 1978. Sister chromatid exchanges in lymphocyte cultures of patients receiving chemotherapy for malignant disorders. *Cancer Treat. Rep.* 62:1413.

56. Schuette, B. P., M. Rabinovitz, and D. T. Vistica. 1980. A comparative cytogenetic study of melphalan-sensitive and -resistant murine L1210 leukemia cells. *Cancer Lett.* 8:335.

57. Ringborg, U., B. Lambert, R. Lewensohn, and I. Turesson. 1981. DNA-damage in human cells treated with the closely related alkylating agents peptichemio, m-L-sarcolysin and melphalan. *Eur. J. Cancer & Clin. Oncol.* 17:991.

58. Einhorn, N., G. Eklund, S. Franzen, B. Lambert, J. Lindsten, and S. Soderhall. 1982. Late side effects of chemotherapy in ovarian carcinoma. A cytogenetic, hematologic, and statistical study. *Cancer (Phila.).* 49:2234.

59. Kato, H. 1973. Induction of sister chromatid exchanges by UV light and its inhibition by caffeine. *Exp. Cell Res.* 82:383.

60. Rommelaere, J., M. Susskind, and M. Errera. 1973. Chromosome and chromatid exchanges in Chinese hamster cells. *Chromosoma (Berl.).* 41:243.

61. Rosen, A., and G. Klein. 1983. UV light-induced immunoglobulin heavy-chain class switch in a human lymphoblastoid cell line. *Nature (Lond.).* 306:189.

62. Baumal, R., B. K. Birshtein, P. Coffino, and M. D. Scharff. 1973. Mutations in immunoglobulin-producing mouse myeloma cells. *Science (Wash. DC).* 182:164.

63. Wabl, M., P. D. Burrows, A. von Gabain, and C. Steinberg. 1985. Hypermutation at the immunoglobulin heavy chain locus in a pre-B cell line. *Proc. Natl. Acad. Sci. USA.* 82:479.

64. Hawley, R. S., and K. D. Tartof. 1983. The effect of *mei-41* on rDNA redundancy in Drosophila melanogaster. *Genetics.* 104:63.