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DNA between variable and joining gene segments of immunoglobulin κ light chain is frequently retained in cells that rearrange the κ locus

(antibody/light chain genes/gene rearrangement/sister chromatid exchange)

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ABSTRACT **A systematic analysis of the fate of the DNA between κ chain variable (V_κ) and joining (J_κ) genes in cells that have rearranged κ loci was carried out. The DNA from a variety of κ-producing plasmacytomas, λ-producing hybridomas, and κ-expressing lymphocytes was digested, fractionated by size, and analyzed with two probes containing sequences 5′ of J_κ. In 13 of 28 plasmacytomas examined the rearrangement of V_κ and J_κ appears to be accompanied by loss of DNA upstream of J_κ. However, in the rest of the plasmacytomas one or more upstream sequences are retained in a new context. In 9 of 12 λ-producing hybridomas (which frequently rearrange both κ loci) one or more upstream segments were detected. These unique fragments were probably generated by a recombination event near or at the J_κ region. The extent to which the region between V and J is maintained in κ-expressing lymphocytes was also measured. Most (76%) of the region upstream of J_κ is retained in the population, even though 68% of the κ loci are rearranged. In order to explain how these upstream elements occur in some, but not all, cell lines, and the significant occurrence in the lymphocyte population, we propose a model in which a step in V–J joining involves mitotic recombination by unequal sister chromatid exchange.**

The formation of antibody genes requires site-specific translocations of DNA that bring together the segments encoding the variable (V) and joining (J) regions of light chains and V, diversity (D), and J regions of heavy chains (1–5). Little is known of the mechanism of joining, but certain features of these gene segments must be considered in any model: The gene segments of each family are linked (6, 7), and the order is generally assumed to be *V–J–C*. The translocation of a gene segment occurs mainly, if not exclusively, within one homologous chromosome (8, 9). The gene segments that are joined are flanked by two inverted repeat sequences separated by a stretch of about 11 or 22 nucleotide pairs (4, 5). It is believed that these flanking sequences could form a stem structure that brings the gene segments together (2, 3) and could serve as recognition sites for enzymes that act to join them (4, 5).

The outcome of gene segment joining is the deletion of DNA between the recombined gene segments (1, 10, 11). In the case of the MPC11 plasmacytoma, which has rearranged V gene segments at both κ light chain loci, regions of DNA between *V* and *J* from both chromosomes were not detected (10). This finding suggests that, after gene segment joining, the intervening DNA is deleted and lost from the genome of the cell. In the plasmacytoma designated T, however, a unique fragment in a new context has been characterized, which appears to be a region of DNA between V_κ and J_κ gene segments (12). The sequence of this fragment indicates it may have been generated as a product of a *V–J* fusion. This observation indicates that the DNA between a V_κ and J_κ segment is not necessarily degraded after joining. These apparently conflicting data indicate that the mechanism of *V–J* fusion may be more complex than hitherto assumed. Therefore, we have undertaken a systematic analysis of the fate of DNA between V_κ and J_κ genes in cells that have rearranged κ loci. We find that such DNA is commonly found in a new context both in plasmacytomas and in hybridomas and is present to a significant degree in populations of lymphocytes expressing κ chain. That this DNA is maintained in the lymphocyte population could mean that the intervening DNA is in the form of an episomal element (12). Alternatively, a more attractive possibility, which is consistent with our data, is that these elements could be retained if the *V–J* joining process involves sister chromatid exchange.

MATERIALS AND METHODS

All plasmacytomas used in this study express κ light chains of the V_κ21 group, with the exception of PC613 and PC2567, which express the V_κ27 and V_κ26 groups, respectively (ref. 13; unpublished). The κ-bearing B lymphocytes were purified from BALB/c mouse spleen cells. Suspensions of spleen cells were depleted of T cells and sorted for κ-bearing cells, using a fluorescence-activated cell sorter as described (14). The λ-producing hybridomas are the same as used in our earlier survey (14). DNA was isolated from B lymphocytes, plasmacytomas, λ hybridomas, or 12- to 14-day mouse embryos (15). Purified NZB mouse embryo DNA was digested with *Bam*HI, and the 12.7-kilobase pair (kb) fragment containing C_κ was inserted into λ phage Charon 28 (unpublished). From this clone a 2-kb *Xba* I fragment (designated U_{Xba}) was isolated from a low-temperature-melting agarose (Sigma) gel. The plasmid pEC_κ was constructed by inserting a 6.5-kb *Eco*RI/*Bam*HI fragment containing C_κ into pBR322 (14). The physical maps of these probes are shown at the bottom of Fig. 1. DNA samples of genomic DNA (usually 10 μg) were digested to completion with the appropriate restriction enzyme (Bethesda Research Laboratories), electrophoresed through 0.7% agarose, transferred to nitrocellulose paper (Schleicher & Schuell), and hybridized to ^{32}P-labeled DNA probes. After a final wash in 30 mM NaCl/3 mM sodium citrate/0.1% sodium dodecyl sulfate for 1 hr at 65°C, filters were exposed to x-ray film at −70°C.

RESULTS

The κ light chain genes in mouse plasmacytomas and hybridomas are found in three forms: as unrearranged genes, κ^0,

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Abbreviations: C, constant; V, variable; J, joining; D, diversity; U, upstream DNA 5′ of J_κ region; kb, kilobase pair(s); uSCE, unequal sister chromatid exchange.

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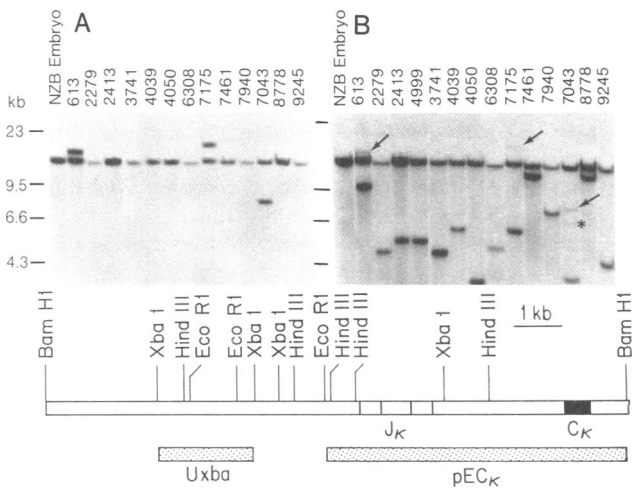

FIG. 1. Detection of upstream κ segments in plasmacytomas in which one κ locus is rearranged (κ^+) and one is germ line (κ^0). Southern blots of *Bam*HI-digested DNA from plasmacytomas were hybridized with the U_{Xba} (*A*) and pEC_κ (*B*) probes. The more intense bands in *B* are the C_κ-containing restriction fragments that correspond to those that hybridize to a probe specific solely for the C_κ region (unpublished). The fainter bands designated with arrows in *B* are attributable to the 5′ region of the pEC_κ probe and correspond to the same unique fragments seen with U_{Xba} in *A*. The band denoted with an asterisk is detected with the pEC_κ probe but not with the U_{Xba} probe.

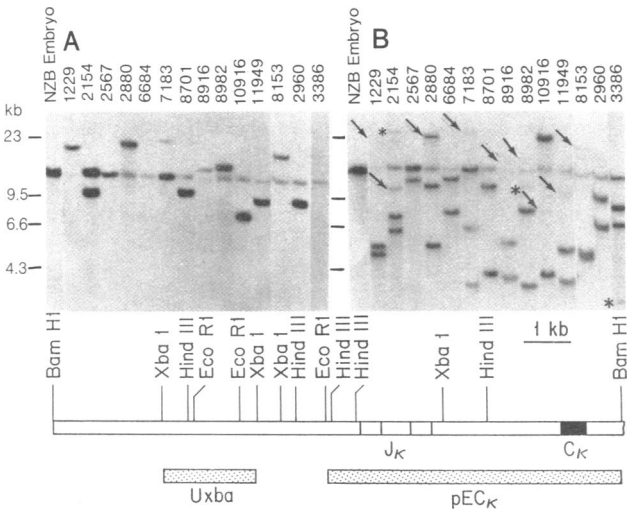

FIG. 2. Detection of upstream κ segments in plasmacytomas in which both κ loci are rearranged (κ^+/κ^-). Southern blots of *Bam*HI-digested DNA from plasmacytomas were hybridized with the U_{Xba} (*A*) and pEC_κ (*B*) probes. The two intense bands in each sample in *B* are the C_κ-containing restriction fragments and correspond to those that hybridize to a probe specific solely for the C_κ region (unpublished). The fainter bands designated with arrows are attributable to the 5′ region of the pEC_κ probe and correspond to the same unique fragments seen with U_{Xba} in *A*. The bands denoted with asterisks are detected with the pEC_κ probe but not with the U_{Xba} probe.

productively rearranged genes, κ^+, or nonproductively rearranged genes, κ^- (16). One result of a DNA rearrangement of either the κ^+ or κ^- type is the acquisition of new restriction sites to the 5′ side of the *J* region, brought in by the *V* segment (17). This process has been observed in Southern blot hybridization experiments as a change in the size of DNA fragments containing J_κ, C_κ, or both relative to their size in embryo DNA (18). We have examined the structural changes resulting from the DNA rearrangments, with particular emphasis on the DNA between the recombining *V* and *J* segments, using two probes containing sequences 5′ of J_κ. (The region 5′ of J_κ will be referred to as the upstream region and designated U.) The probe designated U_{Xba} in Fig. 1 contains the DNA that is 2 kb upstream of J_1. In the germ-line configuration this region is within a 12.7-kb *Bam*HI restriction fragment. Because the U_{Xba} sequence lies upstream of the *J* segment but downstream of the *V* gene locus it should be deleted from the DNA strand in which a *V–J* join has occurred. A second probe, pEC_κ, contains about 800 bases 5′ of J_1, which also should be deleted in any *V–J* joining event. Because the pEC_κ probe also includes the entire J_κ and C_κ region (Fig. 1), this probe can be used to examine these loci as well.

Upstream Elements in Plasmacytomas and Hybridomas. Using U_{Xba} as a hybridization probe for Southern blots of *Bam*HI-digested DNA, we examined a variety of κ-producing plasmacytomas (Figs. 1*A* and 2*A*) and λ-producing hybridomas (Fig. 3). These cell lines are grouped into categories based on analyses with other probes (ref. 18 and Figs. 1*B* and 2*B*). One category consists of plasmacytomas in which one κ locus is productively rearranged and the other remains in the germ-line configuration (κ^+/κ^0). As shown by the pEC_κ probe (Fig. 1*B*), this is detected in each example as two C_κ-containing fragments, one that corresponds to the germ-line size (12.7 kb) and one that is characteristic of the V_κ21 group (unpublished). As expected, U_{Xba} hybridizes to the 12.7-kb fragment originating from the germ-line allele, but in three examples an additional, unique fragment was detected (Fig. 1*A*). These fragments are also detected by the pEC_κ probe (arrows), as discussed below.

A second category consists of plasmacytomas of the type in which both κ alleles have undergone rearrangement (κ^+/κ^-), detected by the pEC_κ probe as two new C_κ-containing *Bam*HI fragments (Fig. 2*B*). Despite the fact that both κ alleles are rearranged, 12 out of 14 samples examined contained one or more fragments that hybridized to the U_{Xba} probe (Fig. 2*A*). Bands at the 12.7-kb germ-line position are observed in these examples, but they are generally only about 10% as intense as the rearranged fragments detected with either the U_{Xba} probe or the pEC_κ probe. We interpret these faint bands as being de-

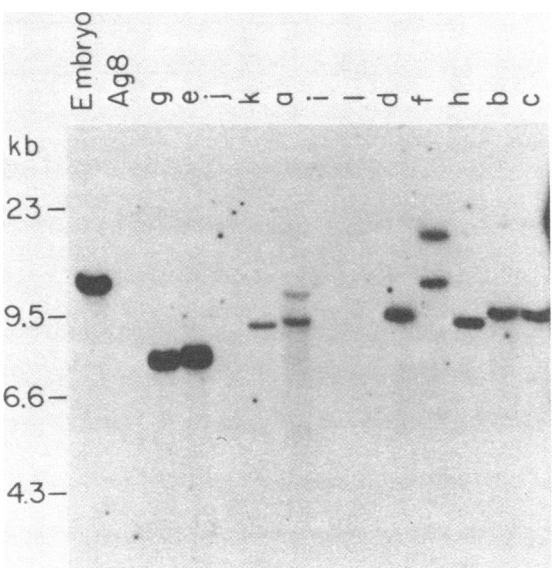

FIG. 3. Detection of upstream κ segments in λ-producing hybridomas. The letter designations are the same as used in an earlier study (14). Except for hybridoma f, which has one germ-line κ allele, all hybridomas in this survey have rearranged both κ alleles. The Ag8 in lane 2 is the fusion parent of the λ-producing spleen cells. The bands in lanes g and e appear as doublets in a shorter exposure.

rived from host tissue, which normally contaminates these plasmacytomas grown *in vivo*. In a few examples—e.g., 2154, 7183, and 2567—bands of significant intensity at the 12.7-kb position are observed. Because both the C_κ loci and the J_κ loci (18) are rearranged in these examples we believe these may represent a unique form of the upstream DNA, but of a size that coincides with the germ-line form.

A third category consists of λ-producing hybridomas. As previously shown (14), the κ loci of λ plasmacytomas and hybridomas are usually, but not always, rearranged (κ^-/κ^- or κ^-/κ^0). We examined 12 λ-producing hybridomas for the region upstream of the J_κ segments by probing *Bam*HI-digested hybridoma DNA with the U_{Xba} probe. As shown in Fig. 3, the U_{Xba} sequence is found in a unique fragment in 9 out of 12 λ-producing hybridomas. Four examples show two different hybridizing fragments (a, e, f, and g). Only one of these, hybridoma f [previously shown to be of the κ^-/κ^0 type (14)], exhibits the 12.7-kb germ-line fragment.

Because the pEC_κ probe extends about 800 bases 5′ of the J_κ region, this probe also hybridizes to DNA between V_κ and J_κ. The additional bands indicated by arrows in Figs. 1*B* and 2*B* are unique fragments not associated with the expressed J_κ or C_κ genes. These fragments, however, coincide in size with the fragments seen by the U_{Xba} probe, indicating that the upstream elements detected by U_{Xba} are generated by events near or in the J_κ locus. The intensity of these new fragments seen by pEC_κ varies, although they are generally less intense than the C_κ-containing fragment. This is as expected, because the rearranged C_κ fragment would hybridize to 70–80% of the available probe, whereas, depending on the number of *J* segments remaining on the 5′ side of the *V–J* join, the new fragment would hybridize to only 10–30% of the available pEC_κ probe.

The pEC_κ probe also reveals an additional fragment in four cases (designated with an asterisk in Figs. 1*B* and 2*B*). These fragments are associated with neither the C_κ region nor the upstream region that includes U_{Xba}. In addition, these fragments are not associated with the intervening J_κ-C_κ region (18). It is likely that these fragments include part of the J_κ region that, as discussed below, may be generated by further rearrangements in upstream elements.

Upstream DNA in Lymphocytes Expressing κ Chain. The amount of DNA upstream of J_κ was estimated in a κ-specific lymphocyte population. The strategy for this analysis was similar to the method previously used for assessing the extent of rearrangement at the J_κ locus in κ^+ splenic lymphocytes (14). This study took advantage of the fact that the J_κ and C_κ regions can be separated by *Hin*dIII digestion to yield two fragments, both of which hybridize to the pEC_κ probe (see Fig. 4*A*). Rearrangements in J_κ will alter the context of the 2.9-kb J_κ *Hin*dIII fragment but will not affect the 4.4-kb C_κ *Hin*dIII fragment. Hence only the germ-line J_κ fragment will be depleted by rearrangements, and comparison of the amount of germ-line J_κ fragment to the C_κ fragment provides a measure of the extent of rearrangement in κ^+ lymphocytes. Similarly, by using the U_{Xba} probe we were able to measure the decrease of the upstream *Hin*dIII fragment (see Fig. 4 *Upper Left*) in κ-bearing lymphocytes relative to the same fragment in embryo DNA. We could compare this with the decrease in intensity of the J_κ region by including a pEC_κ probe. Although the two probes differed in size and specific activity, the intensity data could be normalized by comparing all values to the intensity of the C_κ-containing *Hin*dIII fragment. Fig. 4 *Lower Left* shows the three fragments of interest, obtained by hybridizing various amounts of embryo and κ-bearing B cell DNA (1–10 μg) with a mixture of pEC_κ and U_{Xba} probes. A plot of the intensity of the J_κ band versus that of the C_κ band in embryo and B cells (Fig. 4 *Right*) confirms the earlier observation that the *J/C* ratio in B cells was 32% of that found in embryo DNA (14). In contrast, the ratio of the

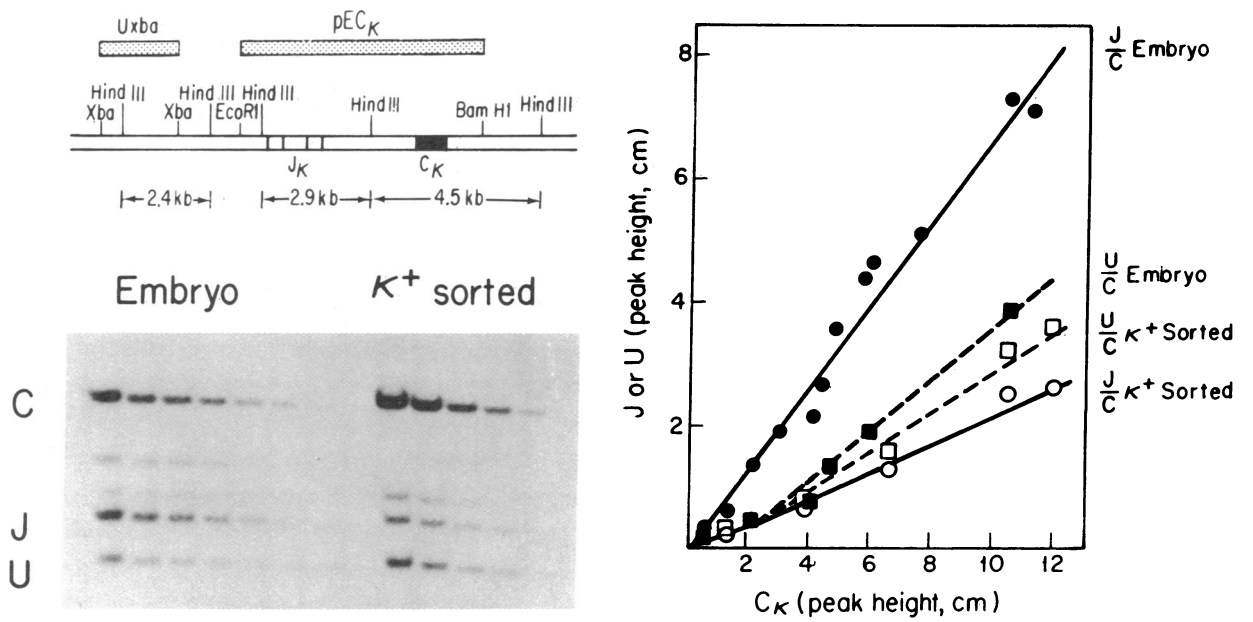

FIG. 4. Comparison of the extent of κ upstream deletion with J_κ rearrangement in κ-bearing lymphocytes. (*Upper Left*) Map of the two probes, pEC_κ and U_{Xba}, and the *Hin*dIII fragments to which they hybridize. (*Lower Left*) Southern blot of *Hin*dIII-digested DNA (1–10 μg) from embryo or κ-bearing lymphocytes was probed with ^{32}P-labeled U_{Xba} and pEC_κ. Filters were exposed to x-ray film at −70°C, using an intensifying screen for various periods; the one shown was exposed for 12 hr. The faint band above the *J* band is from the 3.1-kb *Hin*dIII fragment that overlaps the 5′ region of the U_{Xba} probe. The source of the faint band below the *C* band is unknown. (*Right*) Graphical estimation of the extent of J_κ rearrangement and κ upstream deletion in κ^+ lymphocytes. Densitometer tracings from the Southern blot shown in *Lower Left* were performed and the peak heights of *J* or the upstream (U) segment were plotted against C_κ peak heights. Two tracings were performed on each track after two exposures in which the intensities were within the linear portion of the film dose–response curve. Except for the *J/C* embryo, only the data points from a 12-hr exposure are shown. A least-squares fit of all the data gave slopes with at least 0.99 correlation. The slope of *J/C* in lymphocytes is 32% of that in embryo, and the slope of *U/C* in lymphocytes is 76% of that in embryo.

upstream region, seen by U_{Xba}, to C_κ (*U/C*, Fig. 4 *Right*) in B cells is 76% of that found in embryo DNA. Thus, despite the fact that 2/3 of the J_κ regions are rearranged, only 1/4 of the regions upstream of J_κ are lost. Clearly, rearrangements in the J_κ region are not generally accompanied by loss of DNA between the V_κ and J_κ regions.

DISCUSSION

Our survey of plasmacytomas and hybridomas has shown that elements upstream of J_κ are retained in a new context in the DNA. In many of the κ-producing plasmacytomas we detected one or more unique DNA fragments that hybridized to probes specific for the region between V_κ and J_κ. The extent to which this region is maintained in the κ^+ lymphocyte population has also been measured. Most (76%) of the region upstream of J_κ is retained, even though 68% of the loci are rearranged. The amount of upstream DNA recovered may underestimate the amount actually present in the total lymphocyte population. The B lymphocytes that we have analyzed include only the population bearing a κ^+ allele. If the upstream elements are not restricted to their cell of origin (ref. 12, and see below), they may be retained in other lymphocytes that do not express κ. A case in point is the κ^-/κ^0 and κ^-/κ^- lymphocytes that express λ chain. In 9 of 12 λ-producing hybridomas one or more upstream elements were found.

In 13 of 28 plasmacytomas we have surveyed, the rearrangement of V_κ and J_κ appears to be accompanied by loss of DNA upstream of J_κ. These are analogous to the MPC11 plasmacytoma described by Seidman *et al.* (10). However, in the other examples, one or more upstream sequences are retained in a new context. Thus, a simple deletion/loss model cannot adequately explain the data for the majority of plasmacytomas analyzed. To account for the persistence of the upstream segments in the plasmacytomas, Steinmetz *et al.* (12) have considered that the DNA formed in a stem loop structure could be excised, the 3′ end of the recombining *V* segment and 5′ end of the *J* segment ligated, and the intervening fragment maintained as an extrachromosomal element or reintegrated into the DNA. This suggestion is based on their observation and characterization of a unique upstream element in the plasmacytoma T (12). The sequence of this fragment includes the joining repeat sequence to the 5′ side of the J_1 segment followed by the joining repeat sequence typically found to the 3′ side of unrearranged V_κ genes. However, in contrast to what would be predicted by this model, the T fragment sequence bears no relationship to either of the sequences of the *V* and *J* segments joined in the T plasmacytoma.

The unique fragments containing upstream sequences that we have detected in plasmacytomas and hybridomas with the U_{Xba} and pEC_κ probes are likely to be analogous to the T fragment, in that they were probably generated by a recombination event near or at the J_κ region. In order to explain how these upstream elements occur in some, but not all, cell lines, we propose a model in which a step in *V–J* joining involves mitotic recombination by unequal sister chromatid exchange (uSCE). Fig. 5 shows how uSCE can produce a *V–J* fusion and generate upstream segments in a new context in the DNA. Only one chromosome is shown; the other, homologous, chromosome could either remain unrearranged or undergo uSCE. The reciprocal of the *V* to *J* fusion links the sequence 5′ of *J* and 3′ of the recombining *V*. Thus, one of the chromatids contains a rearranged κ locus while the other retains the upstream element in both the unrearranged configuration (U^0) and a new context (U′). Each chromatid segregates with one of the chromatids from the homologous chromosome. If the *V–J* join creates a functional κ light chain gene, and segregates with a nonrearranged allele, then the genotype of that cell would be κ^+/κ^0U^0. This series of events will lead to the genotype of the majority of plasmacytomas in Fig. 1, which contain the upstream segment only in the germ-line context.

The segregant containing the upstream segment in a new context still contains all the appropriate signals for further chro-

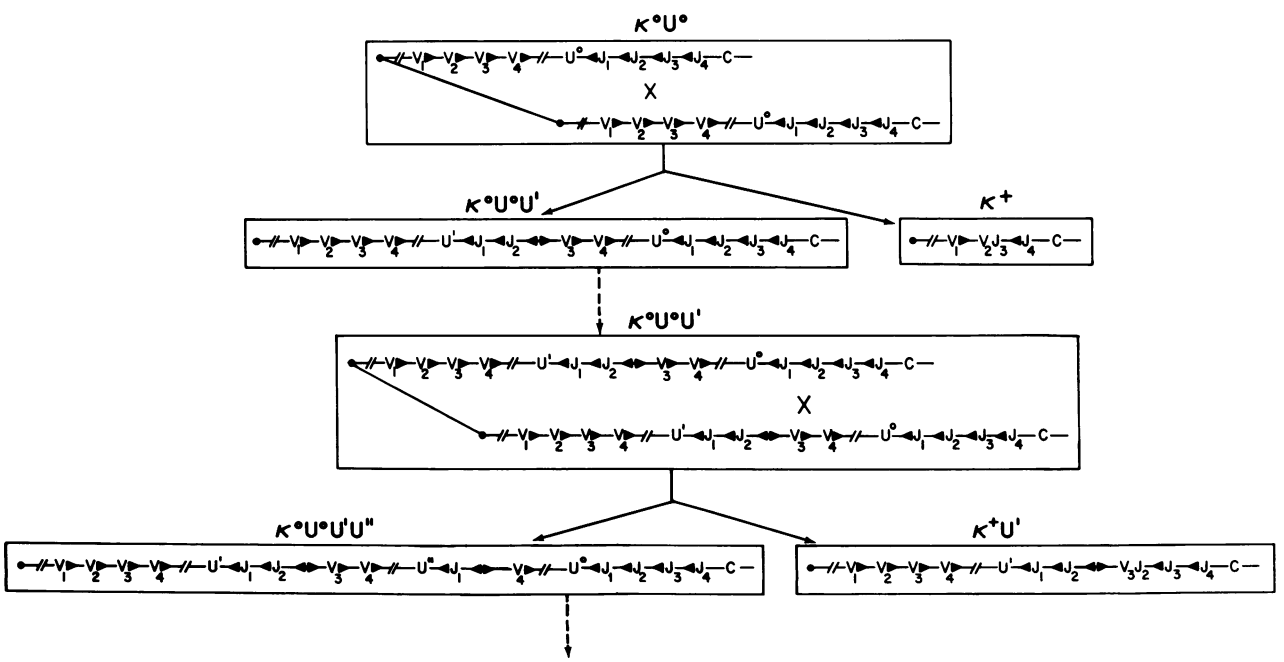

FIG. 5. A model of κ light chain gene translocation by unequal sister chromatid exchange (uSCE). A chromatid pair (κ^0U^0) is shown to undergo an unequal exchange to join *V* to *J*. Solid triangles indicate the inverted repeat sequences that flank all *J* segments on the 5′ side and all *V* segments on the 3′ side. The reciprocal of the *V* to *J* fusion links these sequences 5′ of *J* and 3′ of the recombining *V*. Thus, one of the chromatids contains a rearranged κ locus while the other retains the upstream element both in the unrearranged configuration (U^0) and in a new context (U′). Each chromatid will segregate with one chromatid from the homologous chromosome. A subsequent replication and unequal exchange can generate a chromatid with a *V–J* fusion that retains the U′. Other possible results of uSCE are discussed in the text.

matid exchange. One of a number of possibilities is shown in Fig. 5. In this case, uSCE is shown to generate a *V–J* fusion on the chromatid containing the U′ generated by the previous event. If the other chromosome contributes a germ-line allele, then the genotype of the cell would be κ^+U'/κ^0U^0. (Note that an indistinguishable genotype would result if the κ^0 chromosome containing a U′ segregated with a κ^+ generated on the homologous chromosome—i.e., κ^+/κ^0U^0U'.) Three examples in Fig. 1 (613, 7175, and 7043) would be of this kind. The retention of upstream elements in the other categories of cell lines surveyed, κ^+/κ^- (Fig. 2), $\kappa^-/\kappa^-(\lambda^+)$, or $\kappa^0/\kappa^-(\lambda^+)$ (Fig. 3), can also be explained by this model. These depend on observations of allelic and isotypic exclusion, in which the silent allele(s) of the κ locus is aberrantly rearranged (κ^-). By substituting a κ^- rearrangement as the initial event in Fig. 5, upstream elements in a new context can be generated and retained in cells that have rearranged both κ loci. These kinds of examples are frequently observed in the plasmacytomas shown in Fig. 2 and the λ-producing hybridomas in Fig. 3. Moreover, several cell lines exhibit two non-germ-line fragments that hybridize to the upstream U_{Xba} probe. If both rearranged chromosomes contain an upstream segment in a new context or if a chromatid containing an upstream segment in a new context is the reciprocal of a second *V–J* fusion, it would be possible to generate a cell with multiple copies of unique upstream segments. This extension of uSCE to include κ^- rearrangements applies only if these are generated by the same mechanism that generates κ^+. This is the case for certain κ^- rearrangements (10, 19). However, there may be other types of recombination events that give rise to aberrant rearrangements and displaced upstream elements that do not involve segregation. Such events might contribute to the high frequency of U′ detected in the κ^+/κ^- and κ^-/κ^- cell lines.

Four fragments hybridized to the pEC_κ probe but were not detected by the U_{Xba} probe. If a retained upstream segment present in a new context within the *V* locus contains any J_κ segments (i.e., those 5′ of the J_κ involved in the prior *V–J* joining event), it may contain the necessary signals for a subsequent *V–J* fusion. The result would be a fusion that would delete the region 5′ of the *J* used in the second joining event but retain the region 3′ to the *J*. A unique segment would thus be generated, which would contain part of the *J* region but lack the upstream region.

uSCE is a model that can accommodate our observations as well as the observations of Seidman *et al.* (10) and Steinmetz *et al.* (12). Because the reciprocal of the chromatid exchange segregates away from the chromatid containing the *V–J* join, κ-producing lymphocytes may sometimes lack any rearranged upstream fragment, as in the case of the MPC11 plasmacytoma (10) and the 12 examples seen in our survey. Furthermore, in the lymphocytes containing rearranged upstream segments, these segments would have arisen from a prior event and would not be related to the *V* or *J* segments joined in the cell. This would explain why the sequence of the unique fragment in the T plasmacytoma (12) is unrelated to the joined *V* or *J* segments in that cell line. Therefore, because segregation is an inherent property of uSCE, the unrelatedness of the T sequence could be predicted, whereas a simple deletion/insertion model would have to be modified to account for segregation.

If uSCE plays a role in *V* gene rearrangement, this must represent only one of a series of events. In order to produce a precise *V–J* fusion the recombination must be site specific. The site specificity may be directed by the recognition of the inverted repeat sequences that characteristically flank the *V* and *J* segments. An additional important element of a model employing uSCE is a requirement for DNA replication. In a rapidly dividing pre-B cell population there would be ample opportunity for sister chromatid exchange during and following each DNA replication cycle. However, when a productive rearrangement of a light chain locus occurs, the pre-B cell develops into a quiescent B cell, presumably arrested in a prereplicative phase, and uSCE would not occur. Thus, one would expect a substantial decrease in *V–J* recombination frequency after the productive rearrangement of a light chain gene. Such a decrease has recently been invoked in order to account for observations related to allelic exclusion (14, 16).

Recombination between chromatids has also recently been invoked to explain certain aspects of heavy chain class switching (20). The *V–D–J* (or *V–J*) and class switching events of somatic recombination are clearly distinct: they involve entirely dissimilar DNA sequence signals and occur at quite different stages of lymphocyte development. However, they may share some mechanistic features in that both are site specific and both may employ uSCE. In neither case is it clear that exchange between chromatids is the obligate pathway to the exclusion of intrachromosomal recombination. Conceivably, the mechanisms that juxtapose recombination sites in both events may be unable to distinguish between sister chromatids held together in a replication complex, resulting sometimes in interchromatid, sometimes intrachromatid, recombination.

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1. Hozumi, N. & Tonegawa, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3628–3632.
2. Lenhard-Schuller, R., Hohn, B., Brack, C., Hirama, M. & Tonegawa, S. (1978) *Proc. Natl. Acad. Sci. USA* **74**, 3171–3175.
3. Max, E. E., Seidman, J. G. & Leder, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3450–3454.
4. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19**, 981–992.
5. Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. (1979) *Nature (London)* **280**, 288–294.
6. Weigert, M. & Potter, M. (1977) *Immunogenetics* **5**, 491–524.
7. Mage, R., Lieberman, R., Potter, M. & Terry, W. D. (1973) in *The Antigens*, ed. Sela, M. (Academic, New York), Vol. 1, pp. 299–326.
8. Kindt, T. J., Mandy, W. J. & Todd, C. W. (1970) *Biochemistry* **9**, 2028–2032.
9. Landucci-Tosi, S., Mage, R. G. & Dubiski, S. (1970) *J. Immunol.* **104**, 641–647.
10. Seidman, J. G., Nau, M. M., Norman, B., Kwan, S. -P., Scharff, M. & Leder, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6022–6026.
11. Sakano, H., Kurosawa, Y., Weigert, M. & Tonegawa, S. (1981) *Nature (London)* **290**, 562–565.
12. Steinmetz, M., Altenburger, W. & Zachau, H. G. (1980) *Nucleic Acids Res.* **8**, 1709–1720.
13. Weigert, M., Gatmaitan, L., Loh, E., Schilling, J. & Hood, L. (1978) *Nature (London)* **276**, 785–790.
14. Coleclough, C., Perry, R. P., Karjalainen, K. & Weigert, M. (1981) *Nature (London)* **290**, 372–378.
15. Perry, R. P., Kelley, D. E., Schibler, U., Heubner, K. & Croce, C. M. (1979) *J. Cell. Physiol.* **98**, 553–560.
16. Perry, R. P., Coleclough, C. & Weigert, M. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 925–933.
17. Tonegawa, S., Hozumi, N., Matthyssens, G. & Schuller, R. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41**, 877–889.
18. Perry, R. P., Kelley, D. E., Coleclough, C., Seidman, J. G., Leder, P., Tonegawa, S., Matthyssens, G. & Weigert, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1937–1941.
19. Max, E. E., Seidman, J. G., Miller, H. & Leder, P. (1980) *Cell* **21**, 793–799.
20. Obata, M., Kataoka, T., Nakai, S., Yamagishi, H., Takahashi, N., Yamawaki-Kataoka, Y., Nikaido, T., Shimizu, A. & Honjo, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2437–2441.