MOLECULAR AND CELLULAR BIOLOGY, Apr. 1987, p. 1364–1370
0270-7306/87/041364-07$02.00/0

Vol. 7, No. 4

Interruption of Two Immunoglobulin Heavy-Chain Switch Regions in Murine Plasmacytoma P3.26Bu4 by Insertion of Retroviruslike Element ETn

BRITON SHELL, PAUL SZUREK,† AND WESLEY DUNNICK*

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0620

Received 9 September 1986/Accepted 16 December 1986

A number of moderately reiterated murine genetic elements have been shown to have structures like those of retroviral proviruses. These elements are thought to be transposons, although little evidence of their transposability exists. Two members of one of these families of reiterated elements, the ETn family, have inserted into separate immunoglobulin heavy-chain switch regions in the plasmacytoma P3.26Bu4. Switch regions are those DNA segments associated with each immunoglobulin heavy-chain gene in which the somatic recombinations that accompany the heavy-chain switch occur. This role in somatic recombination may be relevant to the ETn insertions into the switch regions in P3.26Bu4 DNA. P3.26Bu4 and a number of other B-lineage cells contain ETn transcripts.

Transposable elements were first identified because of their ability to participate in genetic rearrangements. One class of eucaryotic transposable elements is known to be structurally analogous to proviral forms of retroviruses. These elements are moderately reiterated in their host genomes, and transcripts of many of them are associated with viruslike particles in the cytoplasm of host cells (for reviews, see references 14 and 39). Reverse transcriptase activity is also often associated with these particles (15, 41, 48), and one such element, yeast Ty, is known to transpose through an RNA intermediate (4). Murine reiterated elements having retroviral structures include IAP (9), GLN (22), VL30 (24), MuRRs (37), Mys (46), and ETn (23). Of these, only IAP (for intracisternal A particle) has been shown to be a mobile element, having interrupted known genes in several cell lines (6, 8, 16, 18, 49); the other mouse elements are termed transposons on the basis of their structure and of polymorphisms between mouse strains or species that suggest movement in evolutionary time.

ETn was first discovered as a teratocarcinoma-specific mRNA by Brulet and co-workers (5). The element is present in approximately 1,000 copies in the mouse genome. Genomic ETn clones have structural features highly reminiscent of retroviruses, as do their transcripts found in teratocarcinoma cells (23). Brulet et al. have shown that transcription of ETn takes place in teratocarcinomas but not in a number of more differentiated cell lines, including retinoic acid-induced derivatives of teratocarcinomas (5).

Immunoglobulin genes undergo two types of somatic rearrangement. The first, which occurs in developing B cells, is the assembly of both heavy- and light-chain variable-region gene segments (reviewed in reference 45). The second occurs when a B cell which expresses surface immunoglobulin M (IgM) differentiates into a plasma cell that secretes immunoglobulin of a different class. This heavy-chain switch rearrangement is a deletion of the μ constant-region gene and its replacement with a γ, ε, or α constant-region gene from downstream (10, 11, 20, 35). The deletion is accomplished by recombination between switch regions, 2 to 10-kilobase (kb) stretches of tandemly repeated DNA sequence that lie upstream of each constant-region gene; the resulting recombinant switch region is located in the intron between the assembled variable-region gene and the first constant-region exon.

Using the Southern hybridization technique and cloned switch region probes, we have discovered unexpected rearrangements of switch regions in the plasmacytoma P3.26Bu4. Two of these rearrangements are insertions of members of the ETn family; one of the insertions is into the joining segment-constant-region (J-C) intron of the expressed γ1 gene. Transcription of ETn occurs in plasmacytomas and in mouse spleen and may be a common feature of B-lineage cells.

MATERIALS AND METHODS

P3 is a derivative of the murine plasmacytoma MOPC21 isolated by Potter (34) and was adapted to culture by Horibata and Harris (21). The subclone being carried in our laboratory, P3.26Bu4 (27), was given to us by M. Scharff. The hybridoma 606, which secretes antibody directed against herpes simplex virus glycoprotein, was made by using P3X63Ag8.653 as the fusion partner and was a gift of J. Glorioso (19). High-molecular-weight DNAs from cell lines were made as described (43). DNA from kidneys and livers of BALB/c mice was used to represent germ line DNA and prepared in the same manner.

Recombinant DNA molecules used in Southern analysis and library screening were all pBR322 or pBR325 derivatives and included the following. The Sγ1 probe was pγ1/EH10.0, which includes the γ1 switch region (S) and some 5′-flanking sequences (28). The Sγ2a probe was pSγ2a-1, the *Eco*RI fragment containing Sγ2a, and was a gift of K. Marcu, as was the Sα probe p64-101, the *Eco*RI-*Hin*dIII fragment containing most of the α switch region (44). The derivation of the pSγ2a-1 subclones used as probes is described in the legend to Fig. 1.

For hybridization analysis, high-molecular-weight or cloned DNAs were digested with commercially obtained restriction enzymes and, after fractionation on 0.8% agarose gels, transferred to nitrocellulose filters by the method of

* Corresponding author.
† Present address: Department of Microbiology, University of Illinois, Urbana, IL 61801.

Southern (41). Filters were prehybridized in 1× Denhardt solution (0.2% each Ficoll, polyvinylpyrrolidone, and bovine serum albumin, 0.1% sodium dodecyl sulfate) at 6× SSC (1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate), hybridized in the same solution plus radiolabeled probe, and washed sequentially with the prehybridization solution, 6× SSC–0.1% sodium dodecyl sulfate, and 2× SSC–0.1% sodium dodecyl sulfate. All steps were carried out at 68°C. Probes were radiolabeled by nick translation (36).

In preparation for genomic cloning, P3.26Bu4 DNA was digested to completion with *Eco*RI and fractionated on 10 to 40% sucrose gradients; 6- to 10-kb fractions were ligated into Charon 4a arms (3), and recombinant libraries were screened by the method of Benton and Davis (2) with Sγ1 (pγ1/EH10.0) and Sγ2a (pSγ2a-1) probes. Sγ1 clone γM8.5 and Sγ2a clone γM72 were grown in liquid culture and restriction mapped by best-fit analysis of single- and double-digestion products. The 1.8- and 1.0-kb *Hin*dIII fragments and the 2.8-kb *Hin*dIII-*Eco*RI fragment of γM8.5 were subcloned into pBR325 and used as hybridization probes in Southern analysis of γM72.

Restriction or random sonicated fragments of cloned DNAs were subcloned into M13mp8, M13mp18, or M13mp19 and sequenced by the dideoxy chain termination method (44). Homology between unknown sequences and ETn was revealed by a search of the GENBANK DNA sequence library, using the BIONET system of programs.

RNA was prepared from whole P3.26Bu4 and 606 cells by the guanidinium-cesium chloride method (7). Cytoplasmic 3T3 RNA was a gift of M. Imperiale and was prepared by lysing cells with Nonidet P-40 and removing nuclei by centrifugation, followed by phenol extraction (31). Polyadenylated species were selected from these RNAs on an oligo(dt) column. Samples for hybridization analysis were heated to 68°C and spotted onto nitrocellulose filters (previously wetted in 2× SSC) that were henceforth treated as described above for Southern hybridization filters. The Northern blot filter was a gift of J. F. Mushinski and was prepared as described (29). This filter was handled in the same manner as the Southern hybridization filters.

RESULTS

IgG1-producing plasmacytoma P3.26Bu4 has unexpected rearrangements of switch region-containing DNA fragments. According to the commonly accepted deletion model, switches to γ1 heavy-chain production are accomplished by recombination between the μ and γ1 switch regions of a single chromosome. Sequences lying between these switch regions, including Cμ and Cγ3, are deleted, while downstream sequences (including γ2b, γ2a, ε, and α) are left intact. Southern analysis with probes for downstream switch regions should therefore reveal germ line hybridization patterns. This was not the case in the IgG1 plasmacytoma P3.26Bu4, however (Fig. 1A). A probe containing Sγ2a (probe A in Fig. 1A) hybridized the germ line-sized (4.8 kb) Sγ2a-containing *Eco*RI fragment (and cross-hybridized with the germ line Sγ2b-containing fragment) in both germ line and P3.26Bu4 DNAs (lanes 1 and 2). In addition, P3.26Bu4 contained 8.4- and 2.4-kb *Eco*RI fragments that hybridized with this probe (lane 2). The simplest explanation for these results is an insertion of 6 kb, including an *Eco*RI site, into one of the germ line Sγ2a alleles of P3.26Bu4. This was confirmed in Southern hybridization experiments with subcloned probes B (lanes 3 to 5) and C (lanes 6 to 9), which represent the 3′ and 5′ portions, respectively, of the original Sγ2a probe A. Of the rearranged *Eco*RI fragments, probe B hybridized only with the 2.4-kb and probe C hybridized only with the 8.4-kb fragment (Fig. 1A, compare P3.26Bu4 DNA in lanes 3 and 9 to germ line DNA in lanes 4 and 8). However, both probes hybridized to a 12.5-kb *Bam*HI fragment in P3.26Bu4 (lanes 5 and 6) in addition to the germ line 6.5-kb fragment (compare lane 7). The restriction enzyme cleavage site maps at the bottom of Fig. 1A show the relationship between the germ line Sγ2a allele and the allele which has undergone this insertion.

A probe specific for Sγ1 hybridized to three rearranged fragments of 11.1, 8.5, and 2.7 kb in *Eco*RI-digested P3.26Bu4 DNA (Fig. 1B, lane 2). At least one and perhaps two Sγ1 rearrangements are expected in an IgG1 producer; the appearance of a third band was unexpected since neither Sγ1 nor Sμ contains an *Eco*RI site. The relationship between these P3.26Bu4 fragments will be discussed further below. In addition, we suggest that some of these insertions occurred after the derivation of P3 (21, 34), in that some subclones of P3 do not include the Sγ1 insertion.

Molecular clones of rearranged Sγ2a and Sγ1 fragments from P3.26Bu4 contain closely related DNA segments. Sγ2a- and Sγ1-hybridizing clones were isolated from a recombinant DNA library made by ligating *Eco*RI-digested and size-fractionated P3.26Bu4 DNA into Charon 4a. The molecular clone designated γM72 has Sγ2a sequences in its 5′ half (Fig. 2A). The 5′ portion of the molecular clone called γM8.5 contained recombined Sμ and Sγ1 sequences in the expected switched configuration. The 3′ portions of the two clones included fragments that cross-hybridized (indicated by stars and filled circles). The two clones, when used as hybridization probes against *Eco*RI-digested BALB/c DNA, hybridized to similar, complex patterns of bands (Fig. 2B), as did subclones from the 3′ portions of the parent clones (data not shown). No portion of the *Igh* locus has a restriction map and reiteration frequency similar to that of the 3′ portion of either of these clones (40). Together, these results suggest that in P3.26Bu4 one allele each of Sγ2a and Sγ1 have been interrupted by members of the same reiterated sequence family.

Sequences interrupting Sγ2a and Sγ1 in P3.26Bu4 homologous to sequences from the retroviruslike element ETn. All of the Sγ1-hybridizing sequences of γM8.5 were contained within a single 1.8-kb *Hin*dIII fragment. This fragment corresponded exactly in size to an Sγ1-hybridizing fragment present in an *Hin*dIII-*Bam*HI digest of P3.26Bu4 genomic DNA and therefore suffered no detectable deletion during clonal propagation (Fig. 3A). We determined, by dideoxy DNA sequencing, that this fragment contained an Sμ/Sγ1 recombination site, 840 base pairs (bp) of tandemly repeated Sγ1 sequences, and, at the 3′ end, nonimmunoglobulin sequences (Fig. 2A, inset). Shown in Fig. 3B are 375 bp of DNA sequence from this fragment; the first 60 bp were tandemly repeated Sγ1 sequences and the remaining 315 bp were from the unknown portion of γM8.5. These sequences are compared with the long terminal repeat (LTR) sequence of a member of the ETn family described by Brulet et al. (5, 23). The Sγ1 sequences ended and ETn sequences began precisely at the 5′ limit of the LTR. Comparison of Sγ1 sequences from γM8.5 with germ line Sγ1 sequences (28) demonstrated that ETn interrupted the expressed gene 1.5 kb from the *Eco*RI site at the end of Sγ1.

In γM72 the recombination point between Sγ2a and inserted sequences lay in a 2.4-kb *Hin*dIII fragment, which was about 0.3 kb smaller than, and therefore deleted relative to, the corresponding P3.26Bu4 genomic fragment (Fig. 3C).

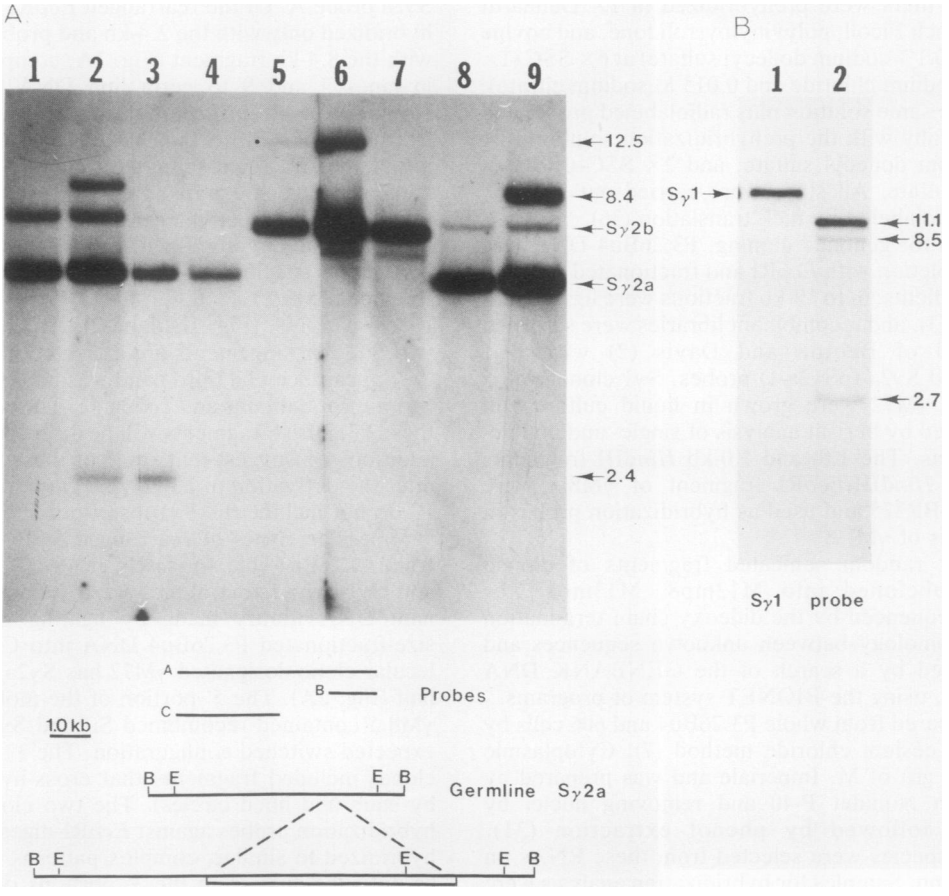


FIG. 1. Genomic Southern blots with switch region probes. (A) BALB/c kidney and liver DNAs (lanes 1, 4, 7, and 8) and plasmacytoma P3.26Bu4 DNA (lanes 2, 3, 5, 6, and 9) were digested with *Eco*RI (lanes 1 to 4, 8, and 9) or *Bam*HI (lanes 5 to 7). Lanes 1 and 2 were hybridized with pSγ2a-1, indicated on the map as probe A. Lanes 3 to 5 were hybridized with probe B, a pBR325 subclone of the 3′ *Bgl*II-*Eco*RI subfragment of pSγ2a-1. Lanes 6 to 9 were hybridized with probe C, a pBR325 subclone of the 5′ *Eco*RI-*Hin*dIII fragment of pSγ2a-1. The 12.5-kb *Bam*HI fragment in P3.26Bu4 DNA hybridizing to both probes B and C is noted by a dot to the left of lane 5. The restriction map shows the relationship of the probes to each other and to the *Bam*HI fragment carrying Sγ2a in the BALB/c germ line. The proposed structure of the rearranged Sγ2a fragment is also shown, with the inserted segment indicated by an open bar. E, *Eco*RI; B, *Bam*HI. (B) BALB/c kidney and liver (lane 1) and P3.26Bu4 (lane 2) DNAs digested with *Eco*RI. The probe was pγ1/EH10.0, which includes all of Sγ1.

The recombination site was between Sγ2a sequences and sequences normally found about 200 bp downstream from the 5′ LTR of ETn (data not shown). It is likely that an artifactual deletion suffered during the propagation of γM72 removed the original recombination site and 0.3 kb of the Sγ2a and ETn sequences (including the LTR) flanking it. Nevertheless, the presence in γM72 of sequences homologous to sequences found within an ETn element confirms that γM72 represents an ETn insertion into the Sγ2a region.

γM8.5 represents the expressed γ1 gene in P3.26Bu4, into which ETn has been inserted. IF2 is a variant subline of P3 that expresses a γ1 heavy chain with the same variable region as that expressed by P3 cells (1). However, the IF2 heavy chain lacks the first constant-region domain (1). The gene from which this protein is expressed has suffered a deletion that begins in Sγ1 sequences and extends downstream, removing much of the J-C intron, all of the first Cγ1 exon, and some of the Cγ1-hinge intron (12). Thus, the expressed γ1 gene of IF2 is rearranged relative to the expressed gene of P3. Of the three Sγ1-hybridizing *Eco*RI fragments of P3.26Bu4, only the 11.1-kb fragment remained unrearranged in IF2 (Fig. 4). Therefore, the 8.5- and 2.7-kb *Eco*RI fragments must exist together as part of the expressed γ1 gene in P3.26Bu4 DNA.

The arrangement of the 8.5- and 2.7-kb *Eco*RI fragments in the expressed γ1 gene of P3.26Bu4 is illustrated in Fig. 5. This arrangement is consistent with the restriction map of γM8.5, the site of insertion described above, and the fact that most ETn elements are 6.0 kb in length. The sequences at the Sμ/Sγ1 recombination site of γM8.5 (not shown) were identical to those in the IF2 expressed gene (13) and to those in pB.12, a *Bam*HI clone we describe elsewhere (Petrini et al., J. Immunol., in press) that overlaps the 11.1-kb *Eco*RI fragment. In Fig. 5 we show and describe the relationships between the Sγ1-hybridizing fragments of P3.26Bu4 and IF2 implied by this sequence identity and by comparisons of γM8.5 and germ line sequences.

ETn is transcribed in a number of B-lymphoid cell lines. ETn was originally discovered as a poly(A)-containing transcript in embryonal carcinoma F9 cells. ETn mRNA was not present in F9-derived differentiated cell lines or in other differentiated cell lines (5). Polyadenylated RNA hybridizing to γM8.5 subclone pH.A (Fig. 2A) was found in P3.26Bu4 and not in NIH 3T3 cells; it was also found in hybridomas in

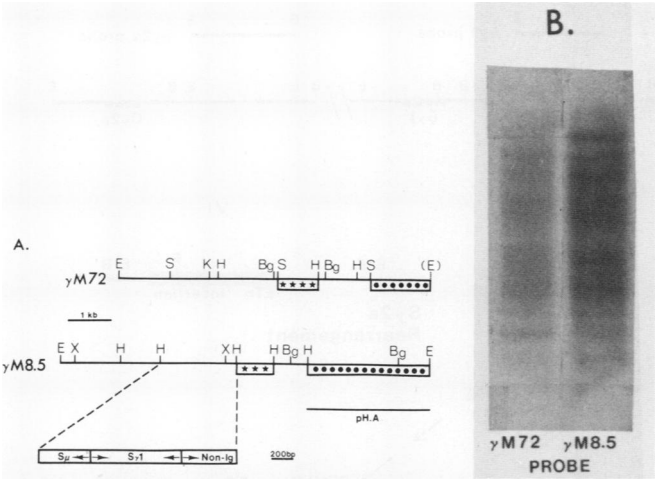
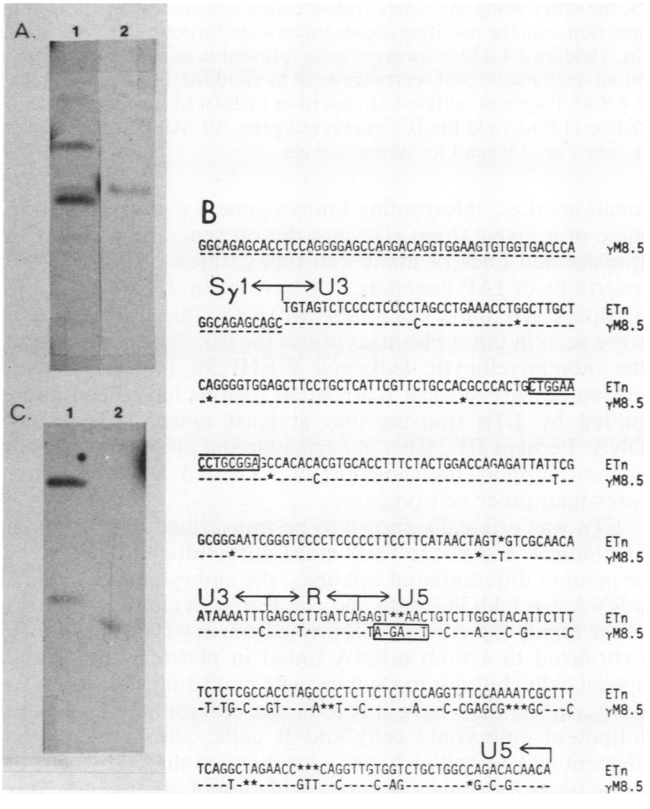


FIG. 2. Charon 4A clones γM72 and γM8.5 contain related sequences. (A) Restriction maps of the two clones. pBR325 subclones of γM8.5, p8.5/H.A, and p8.5/H.D, containing the fragments indicated by solid circles and stars, respectively, were used to probe Southern blots of restricted γM72 DNA. The fragment in γM8.5 hybridizing to p8.5/H.A is indicated by solid circles, and the fragment hybridizing to p8.5/H.D is indicated by stars. The fragment shown enlarged was subcloned and named p8.5/H.B. Random sonication was used to generate M13 subclones that were sequenced to locate Sμ, Sγ1, and nonimmunoglobulin (Non-Ig) segments within this fragment. Abbreviations: E, *Eco*RI; S, *Sac*I; K, *Kpn*I; H, *Hin*dIII; Bg, *Bgl*II; X, *Xba*I; (E), presumed *Eco*RI site joining the insert to vector sequences (this site is not present in γM72, perhaps because of a deletion suffered during clonal propagation). (B) Identical Southern blots of *Eco*RI-digested BALB/c kidney and liver DNA were blotted and probed with γM72 and γM8.5 as indicated.



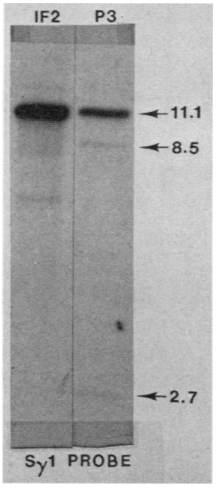


FIG. 4. γ1 switch region content of P3 and IF2 DNA. P3.26Bu4 and IF2 DNAs were digested with *Eco*RI, Southern blotted, and probed with pγ1/EH10.0, the Sγ1 probe. The intensity of a given band reflects the amount of repetitive Sγ1 sequences on the corresponding fragment. For example, the 11.1-kb fragment includes about 3 kb of tandemly repeated Sγ1 sequences, whereas the 8.5-kb fragment includes 840 bp of the same sequences.

which the fusion partner was P3X63Ag8.653 (Fig. 6, inset; unpublished data). As shown in Fig. 6, the same probe hybridized to a 6 kb species in polyadenylated RNA from plasmacytomas and lymphosarcomas. The ETn mRNA was present, in varying amounts, in all such lines examined. We have not evaluated the significance of the small quantitative differences in mRNA hybridization among different lymphoid tumors. However, the lack of signal in the thymic RNA sample probably indicates a dramatically lower level of ETn mRNA in this tissue.

DISCUSSION

To date, the rearrangements of heavy-chain switch regions that have been characterized have been in two types, S-S recombination (reviewed in reference 26) and c-*myc* translocation (reviewed in reference 25). The data presented here indicate that two unexpected rearrangements of immunoglobulin heavy-chain switch regions in the IgG1-producing plasmacytoma P3 result from insertions of 6 kb of non-IgH sequences. The insert in each case is a member of the ETn family of transposonlike elements. At least one of these

FIG. 3. Comparison of nonimmunoglobulin sequences from γM8.5 with ETn LTR sequences. (A) *Bam*HI- and *Hin*dIII-digested P3.26Bu4 (lane 1) and γM8.5 (lane 2) DNAs were electrophoresed on the same agarose gel. The resulting Southern blot was hybridized with pγ1/EH10.0, the Sγ1 probe. (B) Sequences near the Sγ1/nonimmunoglobulin recombination point of γM8.5 are compared with the sequence of an LTR of ETn reported by Kaghad et al. (23). The U3, R, and U5 designations are those reported by Kaghad et al. ETn sequences are directly above sequences from γM8.5; dashes in the γM8.5 sequence indicate identity with the ETn bases above. Bases that differ are shown as letters, and asterisks indicate spaces placed in one or the other sequence to maximize homology. Tandemly repeated Sγ1 sequences are underlined. The boxes in rows 3 and 6 indicate approximate homologies to the heat shock transcriptional control element CTNGAANNTTCNAG (33) and immunoglobulin gene promoter octamer ATGCAAAT (32), respectively (where N is A, T, G, or C). (C) *Hin*dIII-digested P3.26BU4 (lane 1) and γM72 (lane 2) DNAs, electrophoresed on the same gel, were blotted and probed with pSγ2a-1, the Sγ2a probe.

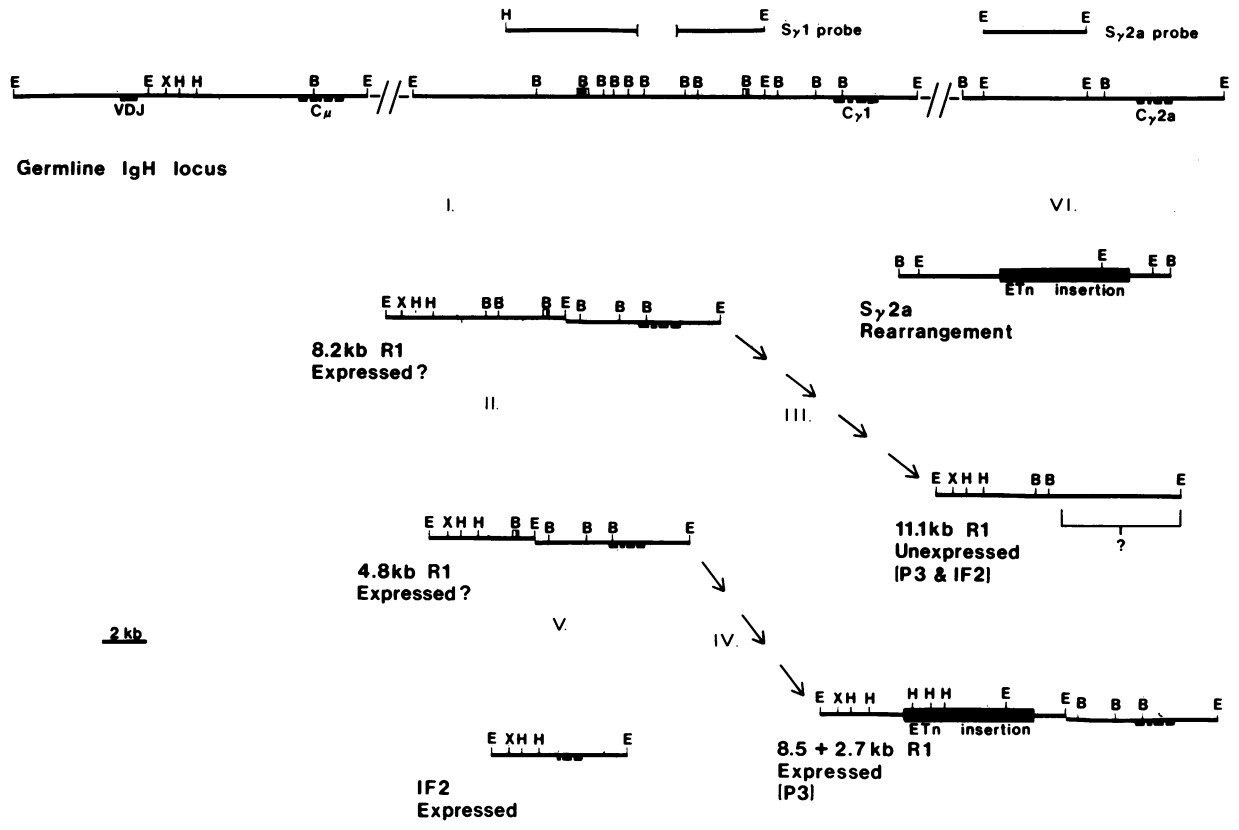


FIG. 5. Restriction map of the germ line *Igh* locus and deduced maps of Sγ1-hybridizing fragment in P3.26Bu4 and IF2 and their progenitors. Only the relevant portions of the germ line map are shown. Deletions or insertions that relate the fragments to one another, deduced by comparing γM8.5 sequences to Sγ1 germline sequences (28), are indicated by Roman numerals. I. The original Sμ/Sγ1 recombination took place 5.3 kb upstream from the *Eco*RI site at the 3′ end of Sγ1, yielding a single 8.2-kb Sγ1-hybridizing *Eco*RI fragment. This fragment represents one of the two IgH alleles in the progenitor of P3, the other being the c-*myc* translocation described elsewhere (13, 30). This expressed gene was duplicated, perhaps by chromosomal nondisjunction, and the resulting copies underwent further rearrangements as follows. II. One copy suffered deletions of 2.8 kb of Sγ1 and 0.6 kb of Sμ, yielding a 4.8-kb expressed gene (present in some P3 subclones; unpublished results). III. The other copy of the 8.2-kb fragment underwent an uncharacterized rearrangement to yield the 11.1-kb fragment. IV. During culture of the sublone P3.26Bu4, the gene represented by the 4.8-kb fragment suffered an insertion of ETn to give the 8.5- and 2.7-kb fragments. V. In another subline the same gene suffered a large deletion (12) to yield the IF2 expressed gene. VI. All P3-related lines examined to date have the ETn insertion into one of the two Sγ2a alleles. See Fig. 2 legend for abbreviations.

insertions begins exactly at the 5′ boundary of previously described ETn LTR sequences; the insertions may represent transpositions of the element. A hallmark of transpositions is the presence of a short direct repeat (6 bp in the case of ETn) of target sequences flanking the inserted element. Cloning and sequencing of both ends of each insertion will be necessary to demonstrate ETn transposition. One of these insertions of ETn is in the major intron of the expressed γ1 heavy-chain gene of P3.26Bu4. These cells continued to produce IgG1, indicating that a significant portion of the transcripts originating from the variable-region promoter of this gene avoid polyadenylation at the ETn polyadenylation sites.

Brulet et al. described ETn as a family of genetic elements having a retroviruslike structure, including LTRs and priming signals like those found in many mammalian retroviruses and in retrotransposons of lower eucaryotes (23). The structure of the element and its presence in 1,000 copies in the mouse genome led them to believe the element was a transposon (5). Ours is the first reported evidence for ETn transposition. Five other elements having similar structures have been found in the mouse (9, 22, 24, 37, 46); of these, only the IAP genome has been shown to appear in new locations (i.e., interrupting known genes) within cells from mice of a given strain. Despite the presence of particles in transformed lines of many cell types, three of the six new insertions of IAP genomes that have been described in the literature are found in P3 derivatives (8, 18), and two more were seen in other plasmacytomas (6, 16). (The sixth was in the monomyelocytic leukemia WEHI 3B [49].) The data presented here suggest that switch regions have been interrupted by ETn transposition at least twice in P3.26Bu4 DNA. Perhaps P3.26Bu4 in particular and plasmacytomas in general support transposition of ETn and IAP at greater rates than other cell types.

ETn was originally shown to be transcribed in embryonal carcinomas and not in their more differentiated derivatives or in other differentiated cell lines; the embryonal carcinoma mRNA was 6 kb in length and colinear with cloned members of the family (5). A non-LTR probe from our transposed ETn hybridized to a 6-kb mRNA found in plasmacytomas and spleen cells, but not in thymus cells or 3T3 fibroblasts. ETn transcription may have a role in the differentiation of both totipotent embryonic cells and B cells; alternatively, the element may fortuitously use a mechanism shared by cells of these types for transcriptional regulation of other genes. It is

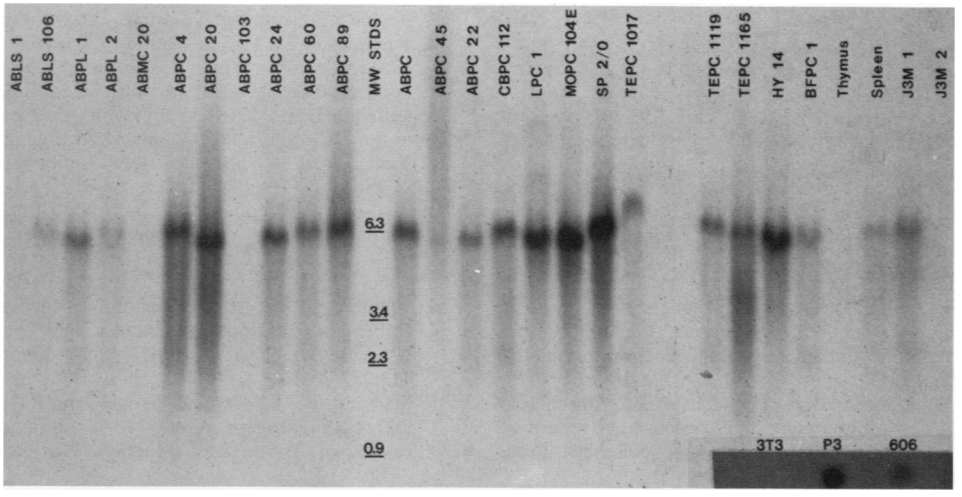


FIG. 6. ETn RNAs in P3 and other B-lineage cells. (Inset) Polydenylated RNA (1 μg) from NIH 3T3, P3.26Bu4, or hybridoma 606 cells was spotted and hybridized with probe pH.A (see Fig. 2). In the Northern blot, 5 μg of polyadenylated RNA from two lymphosarcomas (ABLS1 and ABLS106), two hybridomas (SP2/O and HY14), thymus, spleen, and a number of plasmacytomas (all other lanes) were hybridized with the same probe. The two hybridomas, SP2/O and HY14, were derived by using a P3 derivative as the myeloma fusion partner. Probing this same filter with a murine c-*myc* probe yielded a hybridization signal in all lanes, demonstrating that all RNA samples (including thymus and J3M2) were of roughly equal quality (F. Mushinski, unpublished results). In addition, the c-*myc* hybridization suggested that, for most samples, the apparent differences in the size of hybridizing RNAs were due to artifactual migration differences in some areas of the gel. MW STDS, size standards (in kilobases).

entirely possible as well that the two types of cells share no mode of transcriptional regulation and that ETn is transcribed by a different mechanism in each. For example, sequences related to two types of transcriptional control elements, the heat shock transcriptional control element (33) and an immunoglobulin transcriptional control signal (32), are found in the LTR of the ETn present in γM8.5 (denoted by boxes in Fig. 3B).

Rearrangements of switch regions by insertion or transposition have not previously been observed, although in one mutant plasmacytoma subline an unusual element containing reiterated sequences interrupts the 3′ end of an alpha constant-region gene (17). The finding of transposed ETn elements in two switch regions in P3.26Bu4 is unexpected if one assumes that transposition targets are selected at random; no P3.26Bu4-specific rearrangements were seen in hybridization patterns detected by probes from two other large gene families, rDNA and amylase (data not shown). The possibility exists that switch regions are preferred targets for ETn transposition, perhaps because it is their function to participate in somatic recombinations.

ACKNOWLEDGMENTS

We thank M. Scharff and J. Glorioso for providing cell lines, T. Rabbitts for providing IF2 DNA, M. Imperiale for providing 3T3 RNA, and J. Berry for assistance in running oligo(dT) columns. We also thank K. Marcu and M. Meisler for hybridization probes, and we are deeply indebted to J. F. Mushinski for the use of Northern blot filters. J. L. Claflin, D. Ginsberg, and M. Imperiale provided helpful discussion, and Angela Gross assisted in preparation of the manuscript; we thank them as well.

This investigation was supported by Public Health Service grant CA-39068 awarded by the National Cancer Institute. B.S. was a predoctoral fellow of the National Science Foundation during a portion of this investigation.

LITERATURE CITED

1. **Adetugbo K., C. Milstein, and D. S. Secher.** 1977. Molecular analysis of spontaneous somatic mutants. Nature (London) **265**:299–304.
2. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques *in situ*. Science **196**:180–182.
3. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L.-A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **196**:161–169.
4. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
5. **Brulet, P., M. Kaghad, Y. S. Xu, O. Croissant, and F. Jacob.** 1983. Early differential tissue expression of transposon-like repetitive DNA sequences of the mouse. Proc. Natl. Acad. Sci. USA **80**:5641–5645.
6. **Canaani, E., O. Dreazen, A. Klar, G. Rechavi, D. Ram, J. B. Cohen, and D. Givol.** 1983. Activation of the c-*mos* oncogene in a mouse plasmacytoma by insertion of an intracisternal A-particle genome. Proc. Natl. Acad. Sci. USA **80**:7118–7122.
7. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
8. **Cohen, J. B., T. Unger, G. Rechavi, E. Canaani, and D. Givol.** 1983. Rearrangement of the oncogene c-*mos* in mouse myeloma NS1 and hybridomas. Nature (London) **306**:797–799.
9. **Cole, M. D., M. Ono, and R. C. C. Huang.** 1981. Terminally redundant sequences in cellular intracisternal A-particle genes. J. Virol. **38**:680–687.
10. **Coleclough, C., D. Cooper, and R. P. Perry.** 1980. Rearrangement of immunoglobulin heavy chain genes during B-lymphocyte development as revealed by studies of mouse plasmacytoma cells. Proc. Natl. Acad. Sci. USA **77**:1422–1426.
11. **Cory, S., J. Jackson, and J. M. Adams.** 1980. Deletions in the constant region locus can account for switches in immunoglobulin heavy chain expression. Nature (London) **285**:450–456.
12. **Dunnick, W., T. H. Rabbitts, and C. Milstein.** 1980. An immunoglobulin deletion mutant with implications for the heavy-chain switch and RNA splicing. Nature (London) **286**:669–675.
13. **Dunnick, W., B. E. Shell, and C. Dery.** 1983. DNA sequences near the site of reciprocal recombination between a c-*myc*

oncogene and an immunoglobulin switch region. Proc. Natl. Acad. Sci. USA **80**:7269–7273.
14. **Finnegan, D. J.** 1985. Transposable elements in eukaryotes. Int. Rev. Cytol. **93**:281–326.
15. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42**:507–517.
16. **Greenberg, R., R. Hawley, and K. B. Marcu.** 1985. Acquisition of an intracisternal A-particle element by a translocated c-*myc* gene in a murine plasma cell tumor. Mol. Cell. Biol. **5**:3625–3628.
17. **Gregor, P. D., and S. L. Morrison.** 1986. Myeloma mutant with a novel 3′-flanking region: loss of normal sequence and insertion of repetitive elements leads to decreased transcription but normal processing of the alpha heavy-chain gene products. Mol. Cell. Biol. **6**:1903–1916.
18. **Hawley, R. G., M. J. Shulman, H. Murialdo, D. M. Gibson, and N. Hozumi.** 1982. Mutant immunoglobulin genes have repetitive DNA elements inserted into their intervening sequences. Proc. Natl. Acad. Sci. USA **79**:7425–7429.
19. **Holland, T. C., S. D. Marlin, M. Levine, and J. Glorioso.** 1983. Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. **45**:672–682.
20. **Honjo, T., and T. Kataoka.** 1978. Organization of immunoglobulin heavy chain genes and allelic deletion model. Proc. Natl. Acad. Sci. USA **75**:2140–2144.
21. **Horibata, K., and A. W. Harris.** 1970. Mouse myelomas and lymphomas in culture. Exp. Cell Res. **60**:61–77.
22. **Itin, A., and E. Keshet.** 1986. A novel retrovirus-like family in mouse DNA. J. Virol. **59**:301–307.
23. **Kaghad, M., L. Maillet, and P. Brulet.** 1985. Retroviral characteristics of the long terminal repeat of murine E.Tn sequences. EMBO J. **4**:2911–2915.
24. **Keshet, E., and Y. Shaul.** 1981. Terminal direct repeats in a retrovirus-like repeated mouse gene family. Nature (London) **289**:83–85.
25. **Klein, G.** 1983. Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. Cell **32**:311–315.
26. **Marcu, K. B.** 1982. Immunoglobulin heavy chain genes. Cell **29**:719–721.
27. **Margulies, D. H., W. M. Kuehl, and M. D. Scharff.** 1976. Somatic cell hybridization of mouse myeloma cells. Cell **8**:405–415.
28. **Mowatt, M. R., and W. A. Dunnick.** 1986. DNA sequence of the murine γ1 switch segment reveals novel structural elements. J. Immunol. **136**:2674–2683.
29. **Mushinski, J. F., S. R. Bauer, M. Potter, and E. P. Reddy.** 1983. Increased expression of *myc*-related oncogene mRNA characterizes most BALB/c plasmacytomas induced by pristane or Abelson murine leukemia virus. Proc. Natl. Acad. Sci. USA **80**:1073–1077.
30. **Neuberger, M. S., and F. Calabi.** 1983. Reciprocal chromosome translocation between c-*myc* and immunoglobulin G2b genes. Nature (London) **305**:240–243.
31. **Nevins, J. R.** 1980. Definition and mapping of adenovirus 2 nuclear transcription. Methods Enzymol. **65**:768–784.
32. **Parslow, T. G., D. L. Blair, W. J. Murphy, and D. K. Granner.** 1984. Structure of the 5′ ends of immunoglobulin genes: a novel conserved sequence. Proc. Natl. Acad. Sci. USA **81**:2650–2654.
33. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the *Drosophila* HSP 70 heat shock gene. Cell **30**:517–528.
34. **Potter, M.** 1967. The plasma cell tumors and myeloma proteins of mice, p. 105–167. In H. Busch (ed.), Methods in cancer research. Academic Press, Inc., New York.
35. **Rabbitts, T. H., A. Forster, W. Dunnick, and D. L. Bentley.** 1980. The role of gene deletion in the immunoglobulin heavy chain switch. Nature (London) **283**:351–356.
36. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
37. **Schmidt, M., T. Wirth, B. Kroger, and I. Horak.** 1985. Structure and genomic organization of a new family of murine retrovirus-related DNA sequences (MuRRS). Nucleic Acids Res. **13**:3461–3470.
38. **Shapiro, J. A.** 1983. Mobile genetic elements. Academic Press, Inc., New York.
39. **Shiba, T., and K. Saigo.** 1983. Retrovirus-like particles containing RNA homologous to the transposable element *copia* in *Drosophila melanogaster*. Nature (London) **302**:119–124.
40. **Shimizu, A., N. Takahashi, Y. Yaoita, and T. Honjo.** 1982. Organization of the constant region gene family of the mouse immunoglobulin heavy chain. Cell **28**:499–506.
41. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
42. **Stanton, L. W., and K. B. Marcu.** 1982. Nucleotide sequence and properties of the murine γ3 immunoglobulin heavy chain switch region: implications for successive Cγ gene switching. Nucleic Acids Res. **10**:5593–5604.
43. **Steffen, D., and R. A. Weinberg.** 1978. The integrated genome of murine leukemia virus. Cell **15**:1003–1010.
44. **Szurek, P., J. Petrini, and W. Dunnick.** 1985. Complete nucleotide sequence of the murine γ3 switch region and analysis of switch recombination sites in two γ3-expressing hybridomas. J. Immunol. **135**:620–626.
45. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
46. **Wichman, H. A., S. S. Potter, and D. S. Pine.** 1985. *Mys*, a family of mammalian transposable elements isolated by phylogenetic screening. Nature (London) **317**:77–81.
47. **Wivel, N. A., and G. H. Smith.** 1971. Distribution of intracisternal A-particles in a variety of normal and neoplastic mouse tissues. Int. J. Cancer **7**:167–175.
48. **Yang, S. S., and N. A. Wivel.** 1974. Characterization of an endogenous RNA-dependent DNA polymerase associated with murine intracisternal A particles. J. Virol. **13**:712–720.
49. **Ymer, S., W. Q. J. Tucker, C. J. Sanderson, A. J. Hapel, H. D. Campbell and I. G. Young.** 1985. Constitutive synthesis of interleukin-3 by leukaemia cell line WEHI-3B is due to retroviral insertion near the gene. Nature (London) **317**:255–258.