Recombination Events near the Immunoglobulin C_μ Gene Join Variable and Constant Region Genes, Switch Heavy-Chain Expression, or Inactivate the Locus†

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ABSTRACT: Immunoglobulin heavy-chain expression is initiated by recombination between a variable region (V_H) gene and one of several joining region (J_H) genes located near the μ constant region (C_μ) gene, and the active V_H gene can subsequently switch to another C_H gene. That the general mechanism for C_H switching involves recombination between sites within the J_H–C_μ intervening sequence and the 5′ flanking region of another C_H gene is supported here by Southern blot hybridization analysis of eight IgG- and IgA-secreting plasmacytomas. An alternative model requiring successive V_H linkage to similar J_H clusters near each C_H gene is shown to be very unlikely since the mouse genome appears to contain only one complement of the J_H locus and no J_H gene was detectable within large cloned sequences flanking germline $C_{\gamma 3}$ and $C_{\gamma 1}$ genes. Thus, V_H–J_H joining and C_H switching are mediated by separate regions of "the joining–switch" or J–S element. In each plasmacytoma examined, the J–S element had undergone recombination within both the J_H locus and the switch region and was shown to be linked to the functional C_H gene in an IgG3, an IgG1, and three IgA secretors. Both J_H joining and C_H switching occurred by deletion of DNA. Switch recombination occurred at more than one site within the J–S element in different lines, even for recombination with the same C_H gene. Significantly, although heavy-chain expression is restricted to one allele ("allelic exclusion"), all chromosomal complements of the J_H and switch regions were rearranged in each plasmacytoma. Some rearrangements were aberrant, involving, for example, deletion of all J_H genes from the allele. Hence, an error-prone recombination machinery may account for allelic exclusion in many plasmacytomas.

More than one structural gene contribute to the expression of both the light and the heavy chain of immunoglobulins [for a review, see Adams (1980)]. Both chains contain a constant region (C)[1] and a variable region (V), which interacts with antigen, and the heavy chain (μ, α, γ_1, etc.) defines the immunoglobulin class (IgM, IgA, IgG_1, etc.). The heavy-chain gene family comprises an extensive array of V_H genes (Kemp et al., 1979) separate from a cluster of C_H genes, which appear to occur as single copies in the order 5′ μ-γ_3-γ_1-γ_{2b}-γ_{2a}-α 3′ (Honjo & Kataoka, 1978; Cory et al., 1980a; Yaoita & Honjo, 1980). As in the light chain (Bernard et al., 1978; Max et al., 1979; Sakano et al., 1979), the last portion of the V_H region is encoded not by a V_H gene but by a separate joining region (J_H) gene. Recently, four J_H genes have been identified (Early et al., 1980a; Bernard & Gough, 1980; Sakano et al., 1980; Newell et al., 1980; Gough & Bernard, 1981), located about 7.7 kb 5′ to the C_μ gene (Cory et al., 1980b). Yet another genetic element, the D_H gene, encodes a few amino acids preceding the J region (Schilling et al., 1980; Early et al., 1980a; Sakano et al., 1980). Somatic recombination joins a V_H, D_H, and J_H gene (Early et al., 1980a; Cory et al., 1980b; Maki et al., 1980), apparently by deletion of intervening DNA sequences (Cory & Adams, 1980; Cory et al., 1980a), as in V_λ–C_λ joining (Sakano et al., 1979). Since heavy-chain expression is restricted to one allele ("allelic exclusion"; Pernis et al., 1965; Cebra et al., 1966), V–(D)–J joining might be confined to one allele (Seidman & Leder, 1978; Joho & Weissman, 1980).

An intriguing feature of heavy-chain expression is that a bone marrow derived (B) lymphocyte, which initially expresses a μ chain, can later switch to another heavy chain, for example, γ or α, bearing the same V_H sequence (Kincade et al., 1970; Abney et al., 1978; Sledge et al., 1976; Wang et al., 1977). Recent work has established that this "C_H switching" occurs by successive deletion of C_H genes and the intervening DNA (Honjo & Kataoka, 1978; Cory & Adams, 1980; Cory et al., 1980b; Coleclough et al., 1980; Yaoita & Honjo, 1980; Rabbitts et al., 1980). Two models for switching are illustrated in Figure 1. Accumulating evidence favors model 1 (Davis et al., 1980a; Kataoka et al., 1980a) which postulates a single germline set of J_H genes located near the C_μ gene. Recombination between a particular V_H, D_H, and J_H gene permits μ expression, and a subsequent recombination associates this V–D–J region with another C_H gene. Model 2 postulates a similar set of germline J_H genes near each C_H gene, so that only the V_H gene moves during C_H switching. In support of model 1, Davis et al. (1980a) showed that a region 5′ to a functional C_α gene derives from the germline J_H–C_μ intervening sequence. Similar results were obtained for a rearranged $C_{\gamma 1}$ gene (Kataoka et al., 1980a) and $C_{\gamma 2b}$ gene (Takahashi et al., 1980; Maki et al., 1980; Sakano et al., 1980), but it remains conceivable that both pathways operate.

We present data here which add support for model 1 as the general mechanism of C_H activation. Contrary to model 2, the J_H locus appears to be represented only once in the mouse genome. Model 1 predicts that the DNA segment encompassing the J_H genes and much of the J_H–C_μ intervening sequence, termed here the joining–switch (J–S) element, will occur within a different DNA context in each plasmacytoma. Using the hybridization technique of Southern (1975), we verify that this is the case and present further evidence that both recombination with J_H genes and switch events occur by deletion. Our most unexpected finding is that multiple joining and switch events occurred in each plasmacytoma. Some of these events correspond to aberrant recombinations that presumably play a role in allelic exclusion.

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[1] Abbreviations used: V, variable; C, constant; J, joining; S, switch; H, heavy chain; cDNA, complementary DNA; EDTA, ethylenediaminetetraacetic acid; kb, kilobase; bp, base pairs.

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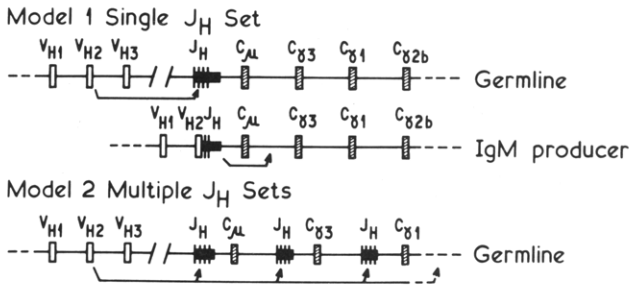

FIGURE 1: Two models for C_H switching. In model 1, a single set of J_H genes is located 5′ to the C_μ gene in the germline. IgM expression requires linkage of a V_H gene (V_{H2}), together with a D_H element, to one of these J_H genes (for simplicity, D_H elements are not shown). A subsequent switch to expression of a different immunoglobulin class (IgG3 in this example) involves association of the V_{H2}–D_H–J gene with the appropriate C_H gene ($C_{\gamma 3}$). In model 2, a set of J_H genes is located 5′ to each C_H gene, and expression of different heavy chains requires successive association of the V_{H2} gene with different J_H gene sets.

Materials and Methods

"Southern Blot" Hybridization. DNA was isolated from BALB/c embryos and plasmacytomas, digested, fractionated, transferred to filters, and hybridized as described previously (Cory & Adams, 1980). Conditions of high stringency were used for genomic blots (Cory & Adams, 1980), but to detect J_H genes within cloned sequences, hybridization and washing were carried out at low stringency (55 °C in 1.5 M NaCl, 0.15 M sodium citrate, and 5 mM EDTA).

Probes. Restriction endonuclease digests of recombinant plasmids were fractionated by polyacrylamide gel electrophoresis (Sanger & Coulson, 1978). Fragments were eluted (Maxam & Gilbert, 1977) and labeled with [^{32}P]dATP by nick translation (Rigby et al., 1977). Digests and subclones used to isolate probes a–g in Figure 2 are described elsewhere (Cory et al., 1980a). The cDNA fragments used as probes for J_1, J_2, and J_4 sequences were the following. J_1 was a 137-bp *Hinf*I fragment of pM603α.8 (Adams et al., 1980a) spanning the *Hha*I site within the J region. J_2 was a *Mbo*II fragment of pH76μ17 encoding amino acids 91–124 in the HPC 76 μ chain [fragment b in Figure 1 of Gough et al. (1980)]. J_4 was a 220-bp *Hpa*II fragment of pM173γ2a.15 (Adams et al., 1980a). The V_{HS107}-specific cDNA probe was a 390-bp *Hha*I fragment of pS107α.4 (Adams et al., 1980a) extending 22 bp into the pBR322 sequence, while the 5′ C_α-specific cDNA probe was a 400-bp *Pvu*II–*Eco*RI fragment of the same clone, the 5′ end corresponding to amino acid 191. The C_μ-specific cDNA probe was fragment c of plasmid pH76μ17 in Figure 1 of Gough et al. (1980).

Fragments of the germline sequence 5′ to $C_{\gamma 1}$ (Figure 7) were fragment h, a 10.0-kb subcloned *Hin*dIII–*Eco*RI fragment of G1.1 (Cory et al., 1980b), and fragment i, a 300-bp fragment generated by *Kpn*I digestion of fragment h. Fragments of the germline sequence 5′ to $C_{\gamma 3}$ (Figure 8) were fragment j, an 8.6-kb subcloned R1*–*Bam*HI fragment of G3.1 (Cory et al., 1980b) where R1* is an *Eco*RI site generated by the cloning technique (Kemp et al., 1979), and fragment k, an ~900-bp fragment generated by *Hin*dIII + *Xba*I digestion of fragment j. The $C_{\gamma 3}$-specific probe was a 3-kb *Hha*I fragment of the $C_{\gamma 3}$-bearing *Bam*HI–*Hin*dIII fragment subcloned from G3.1 (Adams et al., 1980b).

Results

The J_H Locus near the C_μ Gene Is Unique in the Genome. Figure 2 depicts the organization of J_H genes near the germline C_μ gene. We have access to this region through the cloned μ gene of plasmacytoma HPC 76 (Gough et al., 1980; Cory et al., 1980a). If homologous J_H loci occurred 5′ to each C_H gene (model 2 in Figure 1), a probe from the C_μ-associated locus should hybridize to several restriction fragments of embryo DNA. Instead, Southern blot analyses show that this region behaves like a unique sequence. Thus, as shown in Figure 3A, a single *Eco*RI fragment (6.4 kb) is detected by probes a, b, and c from the cloned J region in Figure 2. Similarly, in *Bam*HI digests (Figure 3B), a single fragment of 1 kb is detected by probe a and a single fragment of 9.7 kb by probe c and by probe b (not shown). A unique fragment was also detected in a *Bgl*II digest by probe c (lane c, Figure 8B), in *Pvu*II, *Bgl*I, and *Sac*I digests by probe b, and in a *Xba*I digest by a J_{H4}-specific fragment of probe c (not shown). Thus, sequences within the J_H locus appear to be represented only once per haploid mouse genome. Any closely homologous sequence larger than, say, 100 bp should have been detectable, but not a J gene (~50 bp) with a highly divergent flanking sequence.

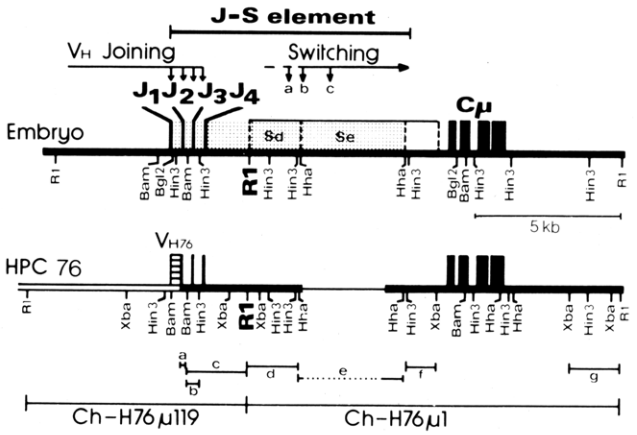

FIGURE 2: Structure of the J_H–C_μ region in the BALB/c germline and at the functional allele of the IgM-secreting plasmacytoma HPC 76. The map was derived from Southern blot analysis of embryo and HPC 76 DNAs and analysis of cloned HPC 76 fragments Ch-H76μ119 and Ch-H76μ1 (Gough et al., 1980; Bernard & Gough, 1980; Cory et al., 1980a). The HPC 76 genome has suffered a deletion of sequences represented by the thin line. The active μ gene was formed by recombination between the V_{H76} gene and the second (Bernard & Gough, 1980) of the four J_H genes (Early et al., 1980a; Bernard & Gough, 1980; Sakano et al., 1980; Newell et al., 1980; Gough & Bernard, 1981). The stippled area defines the joining–switch (J–S) element, while S_d and S_e define the regions of that element detected by cloned fragments d and e. Arrows indicate switch sites for recombination in fragment a with the $C_{\gamma 2b}$ gene in MOPC 141 (Takahashi et al., 1980; Sakano et al., 1980), fragment b, the $C_{\gamma 1}$ gene in MC101 (Kataoka et al., 1980a), and fragment c, the C_α gene in McPC 603 (Davis et al., 1980a). Cloned fragments a–g of μ119 and μ1 used as probes in the present study are described more fully elsewhere (Cory et al., 1980a).

No J_H Genes Detectable near Germline $C_{\gamma 3}$ and $C_{\gamma 1}$ Genes. To increase the sensitivity of detection, we also searched for J_H genes in large cloned fragments of mouse embryo DNA. Clone G3.1 contains 8.6 kb of DNA 5′ to the germline $C_{\gamma 3}$ gene, and G1.1 and G1.2 contain 13.2 and 9.8 kb 5′ to the $C_{\gamma 1}$ gene (Adams et al., 1980b). Digests of DNA from these clones and one digest that is known to bear J_H genes (μ119 in Figure 2) were hybridized to a J_3 + J_4 probe from μ119 (Figure 2) and to J_1-, J_2-, and J_4-specific probes from different cDNA clones (see Materials and Methods). The J_1 probe hybridized strongly to the expected μ119 fragments (Figure 3C, lanes a, b, and c), by cross-hybridization to the other J_H genes, but did not hybridize to G3.1 (lane d) or G1.1 (lane e). Similarly, no J_H sequences were detected in any C_γ clone with the J_2 or J_4 cDNA probe (not shown) or with the genomic J_3 + J_4 probe (lanes a, b, and c in Figure 3D), even though each probe gave

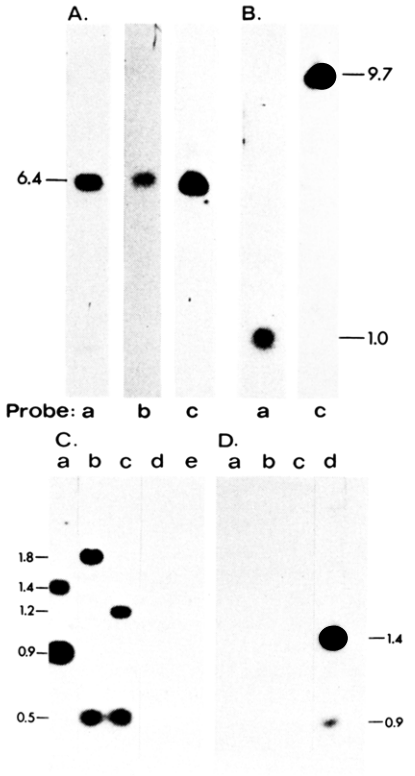

FIGURE 3: Evidence against multiple J_H sets. (A) and (B) present evidence that the J_H locus near C_μ is unique in the genome. Southern blot analysis was performed on *Eco*RI (A) and *Bam*HI (B) digests of embryo DNA with the indicated μ119 probes of Figure 2. (C) and (D) show a test for J_H genes near germline $C_{\gamma 3}$ and $C_{\gamma 1}$ genes. Southern blot analysis was performed on digests of DNA from Ch-H76μ119 (Figure 2) and from clones of embryo DNA (Adams et al., 1980b) bearing the $C_{\gamma 3}$ gene (G3.1) and the $C_{\gamma 1}$ gene (G1.1, G1.2). The probe in (C) was a J_1-bearing cloned cDNA fragment (see Materials and Methods) and in (D) was the μ119 fragment c which bears the J_3 and J_4 genes. The DNA digests in (C) were the following: (a) *Hin*dIII + *Eco*RI, μ119; (b) *Bam*HI + *Eco*RI, μ119; (c) *Bam*HI + *Xba*I, μ119; (d) *Xba*I, G3.1; (e) *Xba*I + *Hin*dIII, G1.1. Those in (D) were the following: (a) *Xba*I, G3.1; (b) *Bam*HI, G1.1; (c) *Xba*I + *Hin*dIII, G1.2; (d) *Hin*dIII + *Eco*RI, μ119. All samples in (C) were loaded on the same gel and transferred to the same filter, as were those in (D). Fragment sizes are indicated in kilobase in all the figures.

strong signals with the expected fragments of μ119, as in lane d (Figure 3D). Since all known J_H amino acid sequences can be classified as J_1, J_2, J_3, or J_4 (Sakano et al., 1980; Gough & Bernard, 1981) and we probed for all four genes, we conclude that no known J_H or closely related sequence occurs within the region 8.6 kb 5′ to the $C_{\gamma 3}$ gene or the region 13.2 kb 5′ to $C_{\gamma 1}$ represented in G1.1. A caveat regarding G1.1 is that this clone has suffered a 2.8-kb deletion within the sequence 5′ to the $C_{\gamma 1}$ gene (Adams et al., 1980b).

Multiple Rearrangements of the J_H Locus in Plasmacytomas. Since the J–S element contains an *Eco*RI site (indicated bold in Figure 2 and all subsequent maps) between the J_H locus and the switch region, *Eco*RI digests can be scored for recombination events within the J_H locus separately from those within the switch region. Figure 4 compares the hybridization obtained with probes containing the $J_3 + J_4$ gene (fragment c in Figure 2) and the J_2 gene (fragment a). The DNAs examined were from embryo (E), liver (L), and plasmacytomas secreting IgM (HPC 76), IgG3 (Y5606), IgG1 (MOPC 21), IgG2b (MPC 11), IgG2a (HOPC 1 and MOPC 173), and IgA (S107, McPC 603, MOPC 315). Significantly, no plasmacytoma retained a J_H region in germline context. Only traces of the germline 6.4-kb fragment remain, and, this almost certainly derives from nonlymphoid cells which infiltrate the subcutaneous tumors (Cory & Adams, 1980; Yaoita & Honjo, 1980). The sizes of the rearranged J_H-bearing fragments (Table I) varied, as expected for recombination with different V_H and/or D_H genes. Most plasmacytomas contained the J_H region in more than one context. Y5606, MOPC 173, S107, and MOPC 315, for example, each contain two different fragments labeled by probe c (lanes d, g, j, and l in Figure 4A), and McPC 603 (lane k) contains three. We infer that different recombination events occurred within the J_H locus on different homologous chromosomes in these hypotetraploid cells.

V_H/J_H Recombination Occurs by Deletion. Certain rearranged fragments detected with the $J_3 + J_4$ probe (Figure 4A) were not labeled by the J_2 (Figure 4B) or J_3 probe (Table I). These results enabled us to deduce the probable recombination site for each rearranged fragment (Table I, column 6) and support other evidence (Cory & Adams, 1980; Cory et al., 1980a) that DNA between the recombining V_H region gene elements is deleted. In MOPC 173, for example, the 4.2- and 1.7-kb fragments revealed by the $J_3 + J_4$ probe (lane g, Figure 4A) did not hybridize to either the J_2 probe (lane f, Figure 4B) or the J_3 probe (not shown). Since no other rearranged fragment was labeled by the J_2 or J_3 probe, we infer that recombination occurred at or near the J_4 gene and deleted the

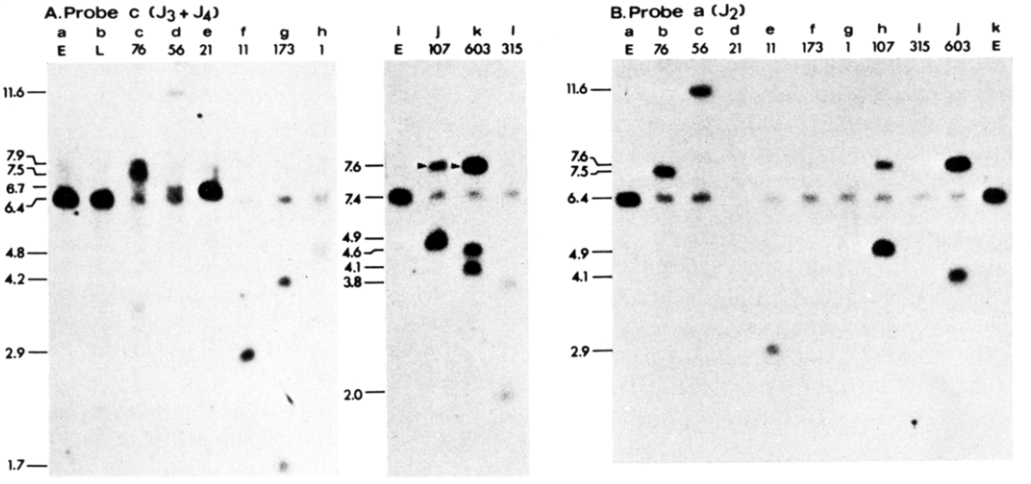

FIGURE 4: Recombination within the J_H locus in plasmacytoma DNAs. Southern blot analysis was performed on *Eco*RI digests with the indicated μ119 probes. DNAs screened were from BALB/c embryos (E) and liver (L) and from plasmacytomas HPC 76 (IgM), Y5606 (IgG_3), MOPC 21 (IgG_1), MPC 11 (IgG_{2b}), MOPC 173 and HOPC 1 (IgG_{2a}), and S107, McPC 603, and MOPC 315 (IgA). The arrows indicate those fragments which also hybridized to a V_{HS107}-specific cloned cDNA fragment (see Materials and Methods).

Table I: Rearranged *Eco*RI Fragments Detected by J_H Gene Probes[a] in Plasmacytoma DNAs

DNA	length[b] (kb)	J_2 probe (fragment a)	J_3 probe (fragment b)	$J_3 + J_4$ probe[d] (fragment c)	J gene assigned[e]	J gene expressed
embryo	6.4	+	+	1.0	–	–
HPC 76 (IgM)	7.9	–	–	<0.2		
	7.5	+	+	0.4	J_1 or J_2	J_2[f]
Y5606 (IgG3)	11.6	+	+	0.05	J_1 or J_2	
	6.7	–	–	0.12	J_4	
MOPC 21 (IgG1)	6.6[c]	–	–	0.7	J_4	J_4[g]
	6.6	–	+		J_3	
MPC 11 (IgG2b)	2.9	+	+	0.2	J_1 or J_2	
MOPC 173 (IgG2a)	4.2	–	–	0.1	J_4	J_4[g]
	1.7	–	–	0.1	J_4	
HOPC 1 (IgG2a)	4.8	–	–	0.04	J_4	J_4[g]
S107 (IgA)	7.6	+	+	0.3	J_1 or J_2	J_1[h]
	4.9	+	+	0.9	J_1 or J_2	
McPC 603 (IgA)	7.6	+	+	1.7	J_1 or J_2	J_1[h]
	4.6	–	–	0.5	J_4	
	4.1	+	+	0.5	J_1 or J_2	
MOPC 315 (IgA)	3.8	–	–	0.1	J_4	
	2.0	+	+	0.05	J_1 or J_2	J_2[h]

[a] J_H gene probes were subcloned fragments a, b, and c from phage Ch-H76μ119 (see Figure 2). [b] Underlined fragments are those bearing expressed J genes (see text). [c] Nucleotide sequencing studies have shown that J_4 is expressed in MOPC 21 (Gough & Bernard, 1981), while the labeling pattern is indicative of recombination at J_3. We surmise that there are two 6.6-kb fragments, one resulting from recombination at J_3, the other at J_4. This conclusion is consistent with the observation that the $J_3 + J_4$ probe hybridized to this fragment more strongly than the J_3 probe, as judged by comparison with the level of hybridization of each probe to embryo DNA on the same filter. [d] Each plasmacytoma DNA was compared with an equal amount of embryo DNA on the same filter. Intensities of bands on autoradiographs were measured by using a Canalco model J microdensitometer. Results are expressed as copies per haploid genome, assuming a value of 1.0 for embryo DNA. Values are the average of two experiments except for MPC 11, MOPC 173, and HOPC 1, which were only measured once. [e] Assignments were made as indicated in the text. Since μ119 did not provide a J_1 probe, we are unable to distinguish between recombination at J_1 and J_2. [f] Bernard & Gough (1980). [g] Gough & Bernard (1981). [h] Early et al. (1980a).

J_2 and J_3 genes together with their flanking sequences. The same inferences were made for certain fragments in Y5606, HOPC 1, McPC 603, and MOPC 315. Table I shows that, in seven of the plasmacytomas, one of the recombination sites assigned from our data (column 6) is consistent with the J_H gene expressed in that tumor (column 7).

As reported by Davis et al. (1980a,b) for McPC 603, we determined that the 7.6-kb fragment (arrowed in Figure 4A) of S107 and of McPC 603 bears the expressed V_H–J_1 gene by showing that this was the only J-bearing fragment labeled by a cloned S107 cDNA probe (Adams et al., 1980a) specific for the V_H sequence expressed in both these lines (not shown). Since none of the other fragments bearing rearranged J genes in these lines hybridized to the V_{HS107} probe, the other alleles may have recombined with a different V_H and/or D_H gene.

Deletion of the Entire J_H Locus from Some Chromosomes. The level of J_H sequences in certain plasmacytomas is markedly lower than in embryo. Figure 4A shows that much weaker bands were obtained with Y5606 (lane d), MPC 11 (f), MOPC 173 (g), HOPC 1 (h), and MOPC 315 (l) than with embryo (a, i) or liver (b). The quantitative data in Table I (column 5), based on densitometry of autoradiographs (Lis et al., 1978; Cory & Adams, 1980), indicate that these lines retain only 0.04–0.2 the embryo level. Deletion of part of the J_H locus as a result of the joining does not account for this: even recombination at J_4 for all copies of the J_H locus would delete only 40% of sequences complementary to probe c since this 2-kb fragment contains 1.2 kb 3′ to J_4. Thus, the marked reduction in a number of the lines suggests that several copies of the entire J_H locus have been deleted. However, since one copy per tetraploid cell would correspond to a minumum of $0.25 \times 0.6 = 0.15$, the lower values obtained for some fragments must be due in part to variation in transfer efficiency (Southern, 1975).

Switch Events Are Not Confined to One Allele. To determine whether the J–S element recombines with a C_H gene in each plasmacytoma and whether switching is restricted to one allele, we scored *Eco*RI digests with fragments d, e, and f (see Figure 2). If recombination has occurred, probes bearing sequences 5′ to the recombination site will hybridize to a new fragment. With embryo DNA, probes d and f labeled only the expected 12.4-kb fragment (lane a in Figure 5A,B), indicating that these sequences are probably unique, but probe e also weakly labeled others, which presumably contain partially homologous sequences from elsewhere in the genome. Even though switching has not occurred in the IgM-secretor HPC 76 (lane c in Figure 5A; lane b in Figure 5B,C), switch sequences hybridize to smaller fragments due to deletions within the J–S element (Cory et al., 1980a).

Probe d labeled a variety of fragments in the non-IgM plasmacytomas (Figure 5A), illustrating clearly that this switch sequence recombines with different sequences during lymphocyte maturation. Germline context was not maintained at either allele in any plasmacytoma examined—the fragment of 12–13 kb in Y5606 (lane d) is shown below to bear rearranged sequences. In several cases probe d hybridized to two or three different fragments within one cell line, for example, MOPC 21 (lane e), HOPC 1 (lane g), and the three IgA producers (lanes i, j, and k). We infer that different recombination events can occur for different chromosomal complements of the switch region. The level of switch sequences is shown in Table II (column 3). Lines low in J region sequences were also low in switch sequences (compare Tables I and II), suggesting that some deletions include much of the J–S element.

Multiple Switch Recombination Sites within the J–S Element. A comparison of the probe d pattern (Figure 5A) with those of probes e and f (Figure 5B,C) shows that recombination occurs at different sites within the J–S element. In MPC 11, for example, the 3.1-kb fragment labeled by probe d (lane f, Figure 5A) is not detected by probe e or f (lane e in Figure 5B,C); hence, recombination must have occurred 5′ to the sequence detected by probe e, that is, within region S_d in Figure 2. In contrast, the 5.8-kb MOPC 173 fragment is

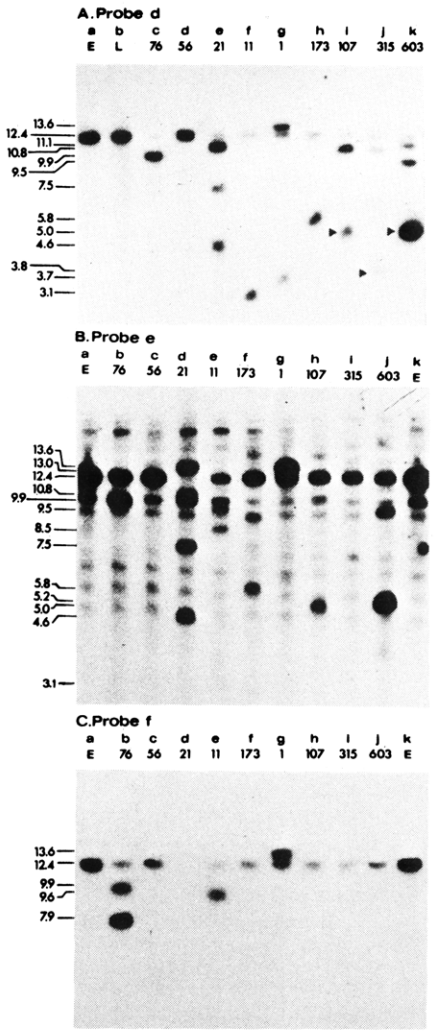

FIGURE 5: Switch recombination in plasmacytomas. Southern blot analysis was performed with the indicated μ1 probes (see Figure 2) on the *Eco*RI digests detailed in Figure 4. Although probe e is derived from a region which has suffered a deletion (Figure 2), in an *Eco*RI digest it is a valid probe for detecting recombination within the entire switch region S_e. Arrows indicate those fragments which also hybridized to a cloned 5′ C_α-specific cDNA probe (see text).

labeled by both probe d (lane h, Figure 5A) and probe e (lane f, Figure 5B) but not by probe f (lane f, Figure 5C), so the recombination site must lie within the sequence scored by probe e, i.e., within region S_e. Table II (last column) provides seven examples of recombination within region S_d and six within S_e. Within one cell line—for example, S107 or McPC 603—the recombination site apparently varied for different alleles.

Region f in Figure 2 probably does not form part of the J–S element since recombination within this region was not detected. The only rearranged fragments revealed by probe f (Figure 5C) were those bearing rearranged C_μ genes in HPC 76, MPC 11, and HOPC 1 (Cory & Adams, 1980) since they hybridized to a C_μ probe and to probe g, which bears sequences 3′ to the C_μ gene (Table II). The failure of probe f (and probe g) to hybridize to any other fragments indicates that switch recombination involved deletion of these sequences.

Curiously, two fragments (a 13 kb in MOPC 21 and a 8.5 kb in MPC 11) hybridized only with probe e. They may reflect aberrant recombination events in which joining occurred to a site within S_e rather than to a J_H gene, followed by deletion of sequences 3′ to S_e.

The C_α Gene Can Recombine with Different Switch Sequences. We attempted to demonstrate linkage between switch

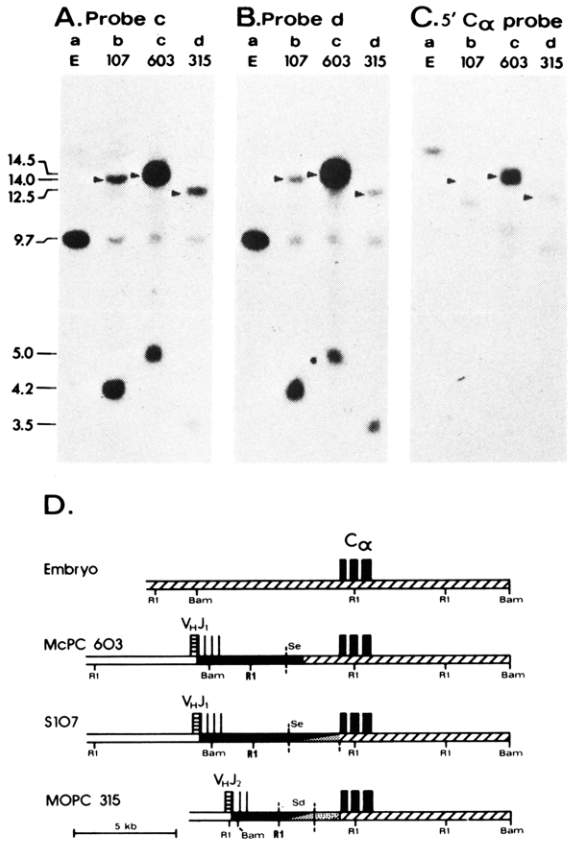

FIGURE 6: The J–S element has recombined with a C_α gene in IgA-secreting plasmacytomas. In (A–C), Southern blot analysis was performed on *Bam*HI digests of DNA from embryos (E) and the IgA-secretors S107, McPC 603, and MOPC 315 fractionated on the same gel. The probe in (A) was the $J_3 + J_4$ probe c, in (B) switch region probe d, and in (C), a cloned 5′ C_α-specific cDNA probe (see Materials and Methods). (D) shows the structures inferred. Sequences from the vicinity of the germline V_H gene are open, those from near the germline C_α gene are hatched, those from the germline J–S element are solid, and those of uncertain origin (see text) are stippled. Broken lines indicate the boundaries of the germline switch region (S_d or S_e in Figure 2). The germline (embryo) restriction map was established by Southern blot analysis of *Bam*HI, *Eco*RI, and *Bam*HI + *Eco*RI digests with cloned cDNA probes (Cory & Adams, 1980; our unpublished results). Since sequences 3′ to C_α remained in germline context (Cory & Adams, 1980; Davis et al., 1980a), sites 5′ to C_α could be positioned from the sizes of fragments hybridizing to 5′ C_α, V_{HS107}, switch, and J region probes. Since McPC 603 and S107 express J_1 and MOPC 315 expresses J_2 (Early et al., 1980a), the *Bam*HI site 5′ to C_α should correspond to the *Bam*HI site between J_2 and J_3 (Figure 2) and thus be located 2-kb 5′ to the bold *Eco*RI site; the mapping data confirmed this expectation. The V_{H315} J_2 gene is contained within an ~2-kb *Eco*RI fragment (Table I).

sequences and expressed C_H genes. The IgA-secretors S107, McPC 603, and MOPC 315 each contain more than one C_α gene rearrangement (Cory & Adams, 1980; Early et al., 1979). Figure 6 shows that one of the *Bam*H1 fragments bearing a rearranged C_α gene (indicated by an arrow) was also labeled by J region probe c and switch probe d. Similarly, one *Eco*RI fragment in each line was labeled by both switch and C_α probes, as indicated by an arrow in Figure 5A. We infer that one copy of the C_α gene has recombined with the J–S element and represents the functional C_α allele, as proven for McPC 603 (Davis et al., 1980; Early et al., 1980a). The other rearranged J–S and C_α sequences in the three IgA plasmacytomas may result from "aberrant" recombination events (see Discussion).

Surprisingly, the results with each of the three probes in Figure 6 indicate that the level of the functional C_α sequences in McPC 603 (lane c) is markedly elevated. Since this result

Table II: Rearranged *Eco*RI Fragments Detected by Switch Region Probes[a] in Plasmacytoma DNAs

DNA	length[b] (kb)	probe d[c]	probe e	probe f	C_μ	probe g	switch region
embryo	12.4	1.0	+	+	+	+	–
HPC 76 (IgM)	9.9	0.4	+	+	+	+	–
	7.9	–	–	+	+	+	–
Y5606 (IgG3)	12.8	0.4[f]	–	–	–	–	S_d
MOPC 21 (IgG1)	13.0[d]	–	+	–	–	–	–
	10.8	0.8	+	–	–	–	S_e
	7.5	0.2	+	–	–	–	S_e
	4.6	0.4	+	–	–	–	S_e
MPC 11 (IgG2b)	9.6[e]	–	+	+	+	+	–
	8.5[d]	–	+	–	–	–	–
	3.1	0.4	–	–	–	–	S_d
MOPC 173 (IgG2a)	5.8	0.4	+	–	–	–	S_e
HOPC 1 (IgG2a)	13.6[e]	0.3	+	+	+	+	–
	3.7	0.2	–	–	–	–	S_d
S107 (IgA)	10.5	0.9	–	–	–	–	S_d
	5.0	0.3	+	–	–	–	S_e
McPC 603 (IgA)	11.1	0.3	–	–	–	–	S_d
	9.5	0.4	+	–	–	–	S_e
	5.2	3.2	+	–	–	–	S_e
MOPC 315 (IgA)	10.6	0.1	–	–	–	–	S_d
	3.8	0.2	–	–	–	–	S_d

[a] Probes were fragments d, e, f, and g from phage Ch-H76μ1 (see Figure 2) and a C_μ-specific fragment of the cDNA plasmid clone pH76μ.17 (see Materials and Methods). [b] Underlined fragments probably derive from the functional allele; for MOPC 21, both the 7.5- and 4.6-kb fragments are linked to C_{γ_1}, but whether both represent expressed γ1 genes is unclear (see text). [c] Results were obtained as described in Table I and are expressed as copies per haploid genome, assuming a value of 1.0 for embryo DNA. [d] These fragments hybridized only to the probe e, so they probably reflect aberrant recombination events (see text). [e] These fragments bear C_μ genes which are believed to have been inactivated by aberrant recombination (see text). [f] This value represents hybridization of probe d to contaminating germline J–S element sequences as well as to switch Y5606 sequences.

was reproducibly obtained with more than one probe and digest, it cannot be merely an artifact of poor transfer. Comparing the labeling to that of other McPc 603 fragments (Tables I and II) raises the possibility that the functional C_α gene has been amplified severalfold by some unknown mechanism. The size of the relevant fragment in Figure 6 indicates that the region involved must be at least 14.7 kb long.

Figure 6D depicts the structures inferred for the functional C_α genes, that for McPC 603 agreeing well with the structure of the cloned gene (Davis et al., 1980a). The data in Figure 5 and Table II localize the recombination site to region S_d for MOPC 315 and S_e for McPC 603 and S107, i.e., within the regions delineated by a sloping boundary to the J–S derived sequence (solid) in Figure 6D and subsequent maps. Since the switch site differs between MOPC 315 and the other two IgA secretors, the J–S element cannot contain a single site specific for the C_α gene. For McPC 603, sequences from the region 5′ to the C_α gene (hatched) directly abut sequences from the J–S element (Davis et al., 1980b). While this may also hold for S107 and MOPC 315, if multiple switches sometimes occur, a portion of the stippled sequences might originate from elsewhere between C_μ and C_α in the germline (see Discussion).

Two Types of Switched $C_{\gamma 1}$ Genes in One Cell Line. The IgG1-producer MOPC 21 contains two types of rearranged $C_{\gamma 1}$ sequences (Cory et al., 1980b). Surprisingly, we find that both have recombined with the J–S element, as illustrated in Figure 7A. Figure 7B shows that fragment i from the region 5′ to the germline $C_{\gamma 1}$ gene (see Figure 7A) hybridizes to 7.5- and 4.6-kb *Eco*RI fragments in MOPC 21 DNA (lane b) instead of the germline 16-kb fragment (lane a). The rearrangements result from deletions which terminate, as indicated by the arrows in Figure 7A, about 2 kb 5′ to the *Eco*RI site near the $C_{\gamma 1}$ gene for the 7.5-kb fragment and about 0.5 kb 5′ to that site for the 4.6-kb fragment (Cory et al., 1980b, and unpublished observations). Since these fragments are the same size as two of the three fragments labeled by switch probes d (Figure 7B, lane d) and e (not shown), we infer that recombination between region S_e of the J–S element and the $C_{\gamma 1}$ flanking region generated the deletions. Confirmatory evidence was obtained from *Hin*dIII digests (not shown): rearranged *Hin*dIII fragments of 16.8 and 13.6 kb were labeled by $C_{\gamma 1}$ gene probes (Cory et al., 1980b), by $C_{\gamma 1}$ flanking probes i and h (see Figure 7A), and by switch probe e but not d or f.

What do the two types of $C_{\gamma 1}$ sequences represent? Since there appears to be only one $C_{\gamma 1}$ gene copy per cell (Cory et al., 1980b), the MOPC 21 (P3) line we analyzed may be heterogeneous, perhaps because of a recent deletion within the region bracketed in Figure 7A.

The 10.8-kb fragment labeled by probe d (track d) and probe e (Table II) must represent linkage of the J–S element to a region other than the $C_{\gamma 1}$ gene, possibly to another C_H gene.

Structure of a Functional $C_{\gamma 3}$ Gene. The IgG3-secretor Y5606 contains only one copy of the $C_{\gamma 3}$ gene per cell located within rearranged sequences (Cory et al., 1980b). Figure 8 presents evidence that this functional $C_{\gamma 3}$ gene is linked to the J–S element. A rearranged 16.3-kb *Bgl*II fragment (arrowed) was labeled by both a $C_{\gamma 3}$ probe (lane b, Figure 8B) and by J region probe c (lane d). Similarly, a 13-kb rearranged *Eco*RI fragment was labeled by a $C_{\gamma 3}$ probe (Cory et al., 1980b), by a small fragment (k in Figure 8A) near the germline $C_{\gamma 3}$ gene (lane b, Figure 8C), and by switch probe d (not shown). Finally, a rearranged *Bam*HI fragment of 12.5 kb (arrowed in Figure 8D) hybridized to both probe k (lane b) and the J region probe c (lane d).

Figure 8A indicates that recombination to the J–S element accounts for the deletion of most of germline region j in Y5606 (Cory et al., 1980b). Since $C_{\gamma 3}$-bearing *Hin*dIII fragments of embryo and Y5606 are the same size (not shown), recombination must have occurred 5′ to the *Hin*dIII site, 2 kb upstream from $C_{\gamma 3}$. Analysis of Y5606 *Hin*dIII digests with probes c, d, and e (not shown) indicates that the J–S switch site lies within region S_d and 1.6 kb 3′ to the *Eco*RI site, i.e., very close to the *Hin*dIII site derived from the germline se-

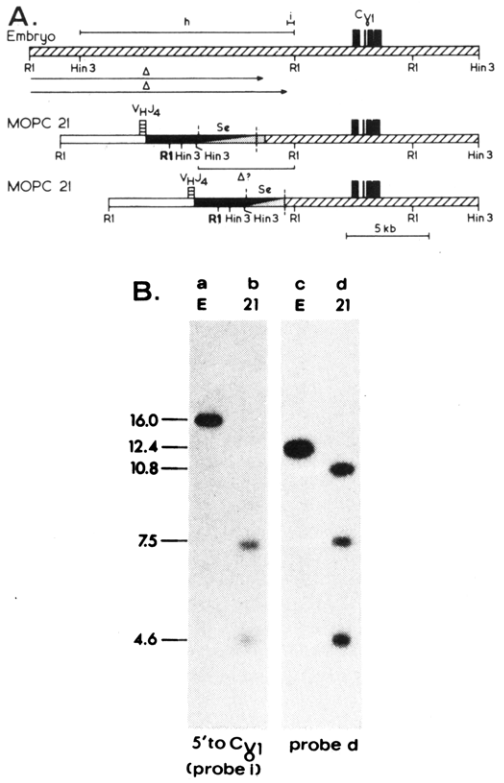

FIGURE 7: Two rearrangements of the $C_{\gamma 1}$ locus in the IgG_1-secretor MOPC 21 result from switch recombination. (A) Structure of the two genes. The J–S-derived sequence (solid) may be contiguous with the $C_{\gamma 1}$ flanking sequence (hatched), or part of the stripped region may be derived from elsewhere between C_μ and $C_{\gamma 1}$. Fragments h and i of the cloned germline sequence were used as probes. Both rearranged sequences are shown as bearing the expressed V_{H21} J_4 gene, but this is not established. (B) Southern blot analysis was performed on *Eco*RI digests of embryo (E) and MOPC 21 DNA. The probes were fragment i from the germline sequence 5′ to the $C_{\gamma 1}$ and μ_1 switch region probe d.

quence flanking $C_{\gamma 3}$. It therefore seems likely that all DNA between the expressed V–J and $C_{\gamma 3}$ genes in Y5606 derives from either the J–S element or DNA flanking the germline $C_{\gamma 3}$ gene.

The Rearranged J–S Element in γ2a and γ2b Producers. Although we have not established linkage between the J–S element and the functional C_H genes in MOPC 173, HOPC 1, or MPC 11, each of these lines does contain a switch region in altered context which is likely to be linked to the active C_γ gene. Figure 9 shows the structures of the presumptive active J–S elements inferred from Tables I and II. Significantly, it appears that switch recombination took place differently for the two γ2a secretors, occurring within region S_d of the J–S element for HOPC 1 and within S_e for MOPC 173 (see legend to Figure 9).

Inactive C_μ Genes Are Retained in Two Plasmacytomas Expressing Other C_H Genes. In contrast to a number of other plasmacytomas, only half the C_μ sequences have been deleted from the IgG2b-secretor MPC 11 and the IgG2a-secretor HOPC 1, and DNA 5′ to the residual C_μ genes differs from the germline (Cory & Adams, 1980). We show here that these genes have undergone aberrant joining events. In MPC 11, the C_μ gene occurs within a 9.6-kb *Eco*RI fragment labeled by switch probes e and f as well as by probe g from the flanking sequence 3′ to the C_μ gene, but not by switch probe d (Figure 5 and Table II). As illustrated in Figure 10, these results indicate that recombination took place to a site within region S_e of the J–S element rather than to a J_H gene. Recombination appears to have deleted the J_H genes of this allele since only

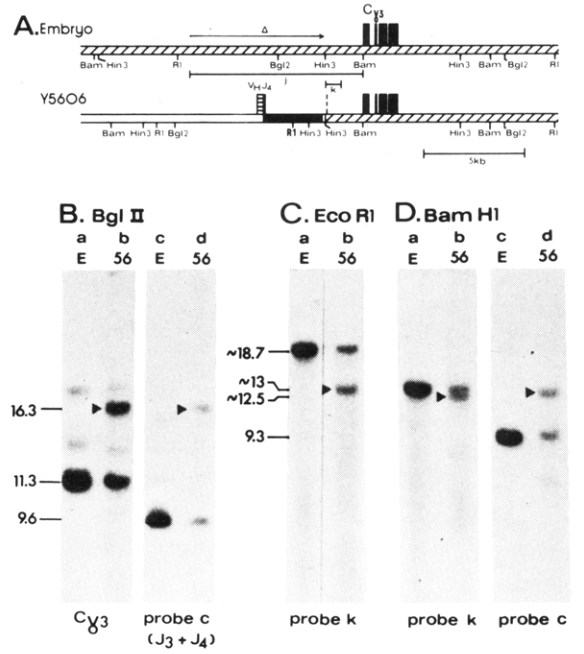

FIGURE 8: A functional $C_{\gamma 3}$ gene is formed by somatic recombination. (A) Structure of the active gene. Comparison with Figure 2 shows that the germline *Bgl*II site between J_1 and J_2, the *Bam*HI site between J_2 and J_3, and the *Hin*dIII site between J_3 and J_4 have all been eliminated from Y5606 DNA, consistent with recombination of J_4 with the V_{H56} gene and the location of the V_H J_4 within a 6.7-kb *Eco*RI fragment (see Table I). (B–D) Southern blot analyses. Digests of embryo (E) and Y5606 DNA with (B) *Bgl*2, (C) *Eco*RI, and (D) *Bam*HI with a cloned probe specific for $C_{\gamma 3}$ sequences, $J_3 + J_4$ probe c (Figure 2), and fragment k from the germline sequence 5′ to the $C_{\gamma 3}$ gene. *Eco*RI and *Bam*HI digests were fractionated on the same gel, separate from the *Bgl*II digests. Arrows indicate the rearranged fragments in Y5606 DNA.

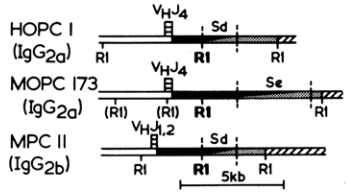

FIGURE 9: The rearranged J–S element in IgG_{2a}- and IgG_{2b}-secreting plasmacytomas. Sequences from the vicinity of the germline $C_{\gamma 2a}$ and $C_{\gamma 2b}$ genes are hatched and other conventions are as in Figure 6. The V_{H173} J_4 gene expressed in MOPC 173 must be located in either a 4.2- or 1.7-kb rearranged *Eco*RI fragment (Table I), and this fragment presumably abuts the only *Eco*RI fragment (5.8 kb) detected by switch region probes (Table II). Likewise, the V_{H1} J_4 gene must be located in a 4.8-kb *Eco*RI fragment (Table I), contiguous in HOPC 1 DNA with the 3.7-kb fragment which hybridizes to switch probe d (Table II). In both HOPC 1 and MOPC 173 DNAs, the next *Eco*RI fragment downstream probably is the ~21-kb $C_{\gamma 2a}$-bearing fragment (Cory et al., 1980b). In MPC 11, the V_{H11} $J_{1(2)}$ gene must be located in the only *Eco*RI fragment (2.9 kb) detected by J region probes (Table I) which probably abuts a 3.1-kb switch fragment (see Table II).

one *Eco*RI fragment of MPC II is labeled by J region probes (Table I) and that fragment must bear the V_{H11}–$J_{1(2)}$ gene of the active $C_{\gamma 2b}$ gene. The same arguments apply to HOPC 1 except that aberrant recombination appears to have occurred to a site within region S_d of the J–S element (Figure 10).

Discussion

The Role of Deletions in Immunoglobulin Heavy Chain Expression. The current picture of heavy-chain gene activation (Figure 11) is that expression of μ chains requires recombination (R1) between a specific V_H, D_H, and J_H gene and

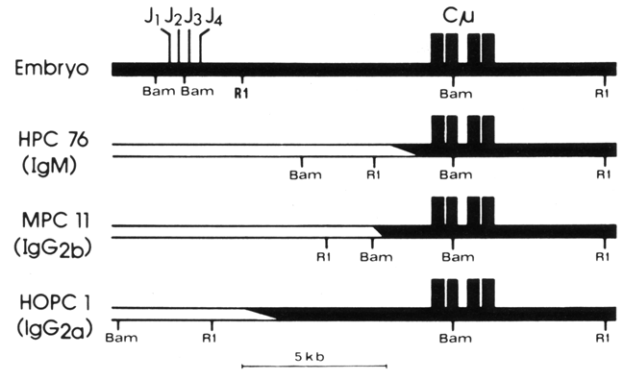

FIGURE 10: Inactivated C_μ genes in three plasmacytomas. Sequences from the vicinity of the germline C_μ gene are solid, and those represented open may be derived from the germline V_H locus. Sequences immediately 3′ to the C_μ gene are retained in germline context in all three plasmacytomas (Cory & Adams, 1980). Restriction sites 5′ to C_μ were positioned from the sizes of *Eco*RI and *Bam*HI fragments hybridizing to 5′ C_μ-specific probes. The structure of the inactive allele in HPC 76 is derived elsewhere (Cory et al., 1980a) and is shown here for comparison.

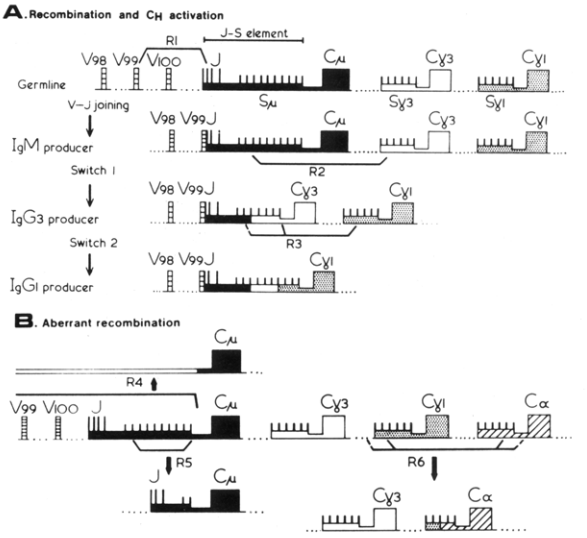

FIGURE 11: Recombination events within the heavy chain locus. S_μ, $S_{\gamma 3}$, $S_{\gamma 1}$ etc., define the switch regions associated with the C_μ, $S_{\gamma 3}$, $C_{\gamma 1}$ etc., genes, switch sequences being indicated as vertical bars. R1, R2, and R3 in (A) refer to recombination events which form functional heavy chain genes, while R4, R5, and R6 in (B) refer to "aberrant" recombination events. R1 is thought to involve two recombination events linking separate V_H, D_H, and J_H elements (Early et al., 1980a), but for simplicity D_H genes have been ignored here. R2 represents a switch recombination from C_μ to any other C_H gene (to $C_{\gamma 3}$ in the diagram), while R3 represents a second switch within a lymphocyte clone. R4 represents aberrant joining to a site 3′ to the J_H locus. R5 is a deletion within S_μ. R6 is a deletion spanning one or more C_H genes but not involving the J–S element.

subsequent expression of other heavy chains (except δ) requires recombination (R2) between a donor switch site (S_μ) within the J–S element and an acceptor site (e.g., $S_{\gamma 3}$, $S_{\gamma 1}$) located 5′ to another C_H gene. Our results add support for this as the general mechanism of C_H activation since each non-IgM plasmacytoma exhibited recombination within both the J_H locus and switch region and the J–S element formed part of a functional $C_{\gamma 1}$ gene (Figure 7), $C_{\gamma 3}$ gene (Figure 8), and three C_α genes (Figure 6). Some lymphocyte clones probably switch heavy-chain expression more than once (Gearhart et al., 1980) by a second switch recombination like R3 (Takahashi et al., 1980; Kataoka et al., 1980a,b).

Evidence is accumulating that both $V_H/D_H/J_H$ joining and C_H switching occur by deletions. Deletion of specific V_H genes has been documented (Cory & Adams, 1980), and J_H genes 5′ to the recombination site were absent in several plasmacytomas (Table I) and in three diploid B lymphoma lines (Cory et al., 1980a). With regard to C_H switching, germline sequences 3′ to the J–S element—namely, the C_μ gene and regions f and g surrounding it (Figure 2)—were absent from at least one and usually both alleles in all non-IgM plasmacytomas examined (Table II). For functional $C_{\gamma 1}$ and $C_{\gamma 3}$ genes (Figure 7 and 8), we also defined the right boundary to the deletion by showing that germline sequences 5′ to $S_{\gamma 1}$ and $S_{\gamma 3}$ sites were deleted (Cory et al., 1980b).

In apparent conflict with the deletion model for C_H switching, γ2a-secreting mutants of the IgG_1-secretor MOPC 21 (X63) reverted to γ1 secretors, seemingly expressing the original V_H region (Radbruch et al., 1980). One explanation might be that the deleted region persisted in a fraction of cells as a replicon capable of reinsertion. A second might be that the MOPC 21 parent contains two copies of the V_{H21}–$C_{\gamma 1}$ gene—not unexpected in a plasmacytoma—but only one copy is active; switching would then delete the active $C_{\gamma 1}$ gene, and reversion might represent activation of the silent V_{H21}–$C_{\gamma 1}$ gene. Our own data on MOPC 21 are somewhat ambiguous: the estimated $C_{\gamma 1}$ copy number was one per cell (Cory et al., 1980b), but these cells clearly contain two types of $C_{\gamma 1}$-bearing structures (Figure 7). A third explanation may be provided by recent evidence that certain switches involve recombination between sister chromatids (Honjo et al., 1980).

The Significance of "Aberrant" Rearrangements. We have identified three unusual types of rearrangement within the heavy-chain locus. The first is "abberant joining", R4 in Figure 11B, in which recombination occurs to a site within the J_H–C_μ intervening sequence, thereby deleting the J_H locus. Such events were documented in three lines (Figure 10), and in a number of others, quantitative data (Tables I and II) strongly suggest that the entire J–S element has been lost from some chromosomal complements. This result is not due to chromosome loss, because the C_α level is not reduced in these lines (Cory et al., 1980b). Since such deletions presumably would inactivate the entire C_H locus on those chromosomes, they may be related to allelic exclusion (see below). Aberrant joins also occur in the κ locus (Perry et al., 1980; Seidman & Leder, 1980).

The second "aberrant" event, R5 in Figure 11B, is a deletion confined to the J–S element. As shown in Figure 2, the functional μ gene in the IgM-secretor HPC 76 occurs within a smaller *Eco*RI fragment due to a 2.7-kb deletion within region S_e (Cory et al., 1980a), and similar deletions probably exist in some other IgM secretors (Yaoita & Honjo, 1980; Early et al., 1980b). Deletions within the switch element may limit the switching options within a clone, perhaps even freezing it at the stage of IgM expression (Cory et al., 1980a). The repetitive sequences identified within region S_e (Takahashi et al., 1980; Sakano et al., 1980) probably potentiate such deletions and those generated during cloning (Davis et al., 1980a; Newell et al., 1980).

Finally, some deletions, like R6, must originate within the C_H locus proper rather than within the J–S element, to account, for example, for the significantly lower levels of $C_{\gamma 3}$, $C_{\gamma 1}$, and $C_{\gamma 2b}$ sequences than of C_μ or C_α sequence in MPC 11 and HOPC 1 (Cory et al., 1980b). Such deletions, which may result from recombination between, e.g., $S_{\gamma 1}$ and S_α sequences, could also account for the rearranged C_α genes found in many IgG secretors (Cory & Adams, 1980; Coleclough et al., 1980). Events like R6 would restrict the switching possibilities for a clone.

Mechanism of Switch Recombination. Switch recombination cannot occur by homologous recombination between identical sequences 5′ to each C_H gene because both sequence data (Kataoka et al., 1980a; Takahaski et al., 1980; Sakano et al., 1980) and hybridization results indicate that neither the region 5′ to the C_μ gene (this paper) nor that 5′ to the $C_{\gamma 1}$ and $C_{\gamma 3}$ genes (Adams et al., 1980b) contains any large sequences in common with other C_H genes. The available evidence (see Davis et al., 1980b,c; Kataoka et al., 1980a,b) appears compatible with a mechanism based upon partial homology of C_H flanking sequences and/or multiple specific sites 5′ to each C_H gene. Evidence for multiple copies of partially homologous repeats 5′ to C_γ genes has led to suggestions that switch recombination involves crossing over between such units (Kataoka et al., 1980b; Tyler & Adams, 1980). This model predicts that numerous potential switch sites exist within the J–S element and 5′ to each C_H gene. Cloning studies (Davis et al., 1980a; Kataoka et al., 1980a; Takahashi et al., 1980; Sakano et al., 1980) had already established that a different S_μ site recombines with a $C_{\gamma 1}$, a $C_{\gamma 2b}$, and a C_α gene (Figure 2). Our data showed that even within one cell line different S_μ sites were used on different homologous chromosomes and that different S_μ sites recombined with the C_α genes of two IgA plasmacytomas (Figure 6), and probably with the $C_{\gamma 2a}$ genes in two IgG_{2a} secretors (Figure 9). Since submission of this manuscript, Davis et al. (1980b) have demonstrated with cloned sequences that multiple sites 5′ to the C_α gene can recombine with multiple sites 5′ to the C_μ gene. Such results, and events like R5 and R6 (Figure 11), seem to fit better with a process which is largely stochastic rather than tightly regulated.

Error-Prone Recombination Can Partly Account for Allelic Exclusion. How a lymphocyte restricts expression of each immunoglobulin chain to one allele remains a puzzle (see Adams, 1980). Can recombination events account for this? One model would be that V–J joining is permitted for only one allele in a given cell, as Joho & Weissman (1980) suggested to account for their observation that about half the C_κ sequences in a normal B lymphocyte population remained in germline context. However, no allele of the J_H locus remained in germline context in any of the nine plasmacytomas examined here or in three diploid B lymphomas (Cory et al., 1980a).

Stochastic models permit recombination to occur independently at a certain frequency for each allele. Allelic exclusion would occur, at least in the majority of cells, if (a) recombination is a low-frequency event for either allele and/or (b) the process is very error prone. For the κ locus, Perry et al. (1980) found that 15 out of 30 κ-expressing plasmacytomas retained one C_κ allele in germline context and that at least 13 of the others had undergone aberrant recombination. Thus a combination of models a and b could account for events at the κ locus. For the heavy-chain locus, our results argue against model a since germline context was not retained for either J_H allele in any of these terminally differentiated plasmacytomas or in either of two lines representative of early B cells (Cory et al., 1980a). Consistent with model b, aberrant joins had deleted the J_H locus from one allele in three lines (Figure 10), and some chromosomal complements of the entire J–S element were deleted from several others. Other types of errors may include out-of-phase joining (Altenburger et al., 1980), D_H–J_H joining without a V_H gene, and joining to a pseudo-V gene. Thus functional inactivation of one allele of the J_H locus due to an error-prone recombination machinery probably accounts for allelic exclusion in a significant fraction of cells.

Acknowledgments

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Mitochondrial Adenosinetriphosphatase Inhibitor Protein: Reversible Interaction with Complex V (ATP Synthetase Complex)†

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ABSTRACT: Mitochondrial ATPase inhibitor protein (IF_1) reacts reversibly with complex V and inhibits up to 90% of its ATPase activity. Both the rate and extent of inhibition are pH and temperature dependent and increase as the pH is lowered from pH 8 to 6.7 (the lowest pH examined) or as the temperature is increased from 4 to 36 °C. Nucleotide triphosphates plus Mg^{2+} ions are required for inhibition of complex V ATPase activity by IF_1. In the presence of Mg^{2+} ions, the effectiveness order of nucleotides is ATP > ITP > GTP > UTP. Highly purified complex V, which requires added phospholipids for expressing ATPase and ATP–P_i exchange activities, cannot be inhibited by IF_1 plus ATP–Mg^{2+} unless phospholipids are also added. This indicates that the active state of the enzyme is necessary for the IF_1 effect to be manifested, because F_1–ATPase, which does not contain nor require phospholipids for catalyzing ATP hydrolysis, can be inhibited by IF_1 plus ATP–Mg^{2+} in the absence of added phospholipids. The IF_1-inhibited complex V, but not IF_1-inhibited F_1–ATPase, can be reactivated by incubation at pH >7.0 in the absence of ATP–Mg^{2+}. The reactivation rate is pH dependent and is influenced by temperature and enzyme concentration. Complex V preparations contain small and variable amounts of IF_1. This endogenous IF_1 behaves the same as added IF_1 with respect to conditions described above for inhibition and reactivation and can result in 25–50% inhibition in different complex V preparations. However, complex V lacking endogenous IF_1 can be reconstituted from F_0, F_1, oligomycin sensitivity conferring protein, and phospholipids. Inhibition of this reconstituted preparation in the presence of ATP–Mg^{2+} depends entirely on addition of IF_1. In general, the ATP–P_i exchange activity of complex V is more sensitive to the chemical inhibitors of F_1–ATPase than its ATPase activity. This is not so, however, for IF_1. Under conditions that IF_1 caused ~75% inhibition of ATPase activity of complex V, no more than 10% of the ATP–P_i exchange activity was inhibited.

The mitochondrial ATPase inhibitor protein (IF_1) is a low molecular weight, water-soluble, and heat-stable protein first discovered and isolated from bovine heart mitochondria by Pullman & Monroy (1963). Similar inhibitor proteins have also been isolated from rat liver (Chan & Barbour, 1976a; Cintron & Pedersen, 1979), *Candida utilis* (Satre et al., 1975), *Saccharomyces cerevisiae* (Ebner & Maier, 1977), chloroplasts (Nelson et al., 1972), and *Escherichia coli* (Nienvenhuis et al., 1974; Smith & Sternweis, 1977; Laget & Smith, 1979). The mitochondrial IF_1 has been extensively studied by various investigators (Horstman & Racker, 1970; Brooks & Senior, 1971; Ernster et al., 1973; Van de Stadt et al., 1974; Gómez-Puyou et al., 1977; Ferguson et al., 1977; Harris et al., 1979; for review, see Ernster et al., 1979). In chloroplasts and *E. coli*, the ATPase inhibitor protein constitutes the ϵ subunit of F_1–ATPase with a molecular weight of 15 000–16 000. However, in bovine heart mitochondria, IF_1 and the ϵ subunit of F_1 are distinct entities with molecular weights, respectively, of 10 500 and 5700–7500. It is generally believed

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