Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3280–3284, May 1982
Immunology

Polymorphisms in immunoglobulin heavy chains suggesting gene conversion

(amino acid sequence/genetic mechanisms/antibody diversity)

STEPHEN H. CLARKE*, J. LATHAM CLAFLIN†, AND STUART RUDIKOFF*‡

*Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; and †Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109

Communicated by Michael Potter, February 22, 1982

ABSTRACT **Complete heavy (H) chain variable region (V region; amino acids 1–118) sequences have been determined for three phosphocholine (*P*Cho)-binding monoclonal antibodies of CBA mouse strain origin. Two of these were found to differ from the sequence of the BALB/c T15 germline V_H segment (segment of the V region that includes amino acids 1–95) at four positions but were identical to the allelic form of T15 (C3) found in C57BL. The third V_H segment, HP101.6G6 (6G6), was clearly the product of a second, related V_H gene, probably the allele of the BALB/c *V11* gene, a second member of the *P*-Cho V_H gene family. Thus, more than one V_H gene is capable of encoding heavy chains of *P*Cho-binding antibodies. The 6G6 V_H segment differs from V11 at seven positions; four of these distinguishing amino acids are encoded in other members of the *P*Cho V_H gene family. We postulate that the origin of the 6G6 V_H sequence can most easily be explained by a process of gene conversion occurring between at least three members of the *P*Cho V_H family.**

The question of the mechanisms involved in the generation of the vast array of diversity shown by immunoglobulins has long been one of the intriguing problems in immunology. Recent studies at both the protein and nucleic acid levels have demonstrated that immunoglobulin light (L) (1–5) and heavy (H) (6–9) chains are encoded in multiple genetic elements and that the assortment of these various elements produces much of the diversity shown by immunoglobulins at the protein level. Further structural diversity arises as a result of alterations in the recombination sites of these gene segments, generating both amino acid-sequence and -length differences in the third hypervariable regions (3–5, 8–11). Finally, the potential random association of L and H chains and the occurrence of somatic point mutations (12–16) may further amplify the diversity generated at the somatic level.

In addition to these somatic mechanisms, the unique nature of the immunoglobulin genes provides the opportunity for additional mechanisms to operate both somatically and in the germ line to potentially generate additional diversity. Immunoglobulin L and H chain variable (V) region segments (amino acids 1–95; V_L and V_H segments, respectively) are encoded in multigene families, which each consist of >100 genes (17–19). These large families can be divided further into subsets whose members share a high degree of homology as identified by protein (17, 18) or nucleic acid (13, 14) sequence determination or by the ability of a given L or H chain DNA probe to hybridize with multiple, apparently closely related genomic bands (19, 20). The existence of such subsets of homologous gene structures provides an opportunity for gene interaction and, consequently, the generation of antibody diversity. In the present communication, we present evidence for such gene interaction in the form of gene conversion and discuss the potential role of this mechanism in antibody V region diversity.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Hybridoma Proteins. CBA/J hybridomas were prepared as described (21) and injected into (BALB/c × CBA/J)F_1 mice. The resulting ascites fluid containing hybridoma proteins was then affinity-purified on a phosphocholine (*P*Cho)-Sepharose column (22). H and L chains were partially reduced and alkylated, dialyzed into 6 M urea/1 M acetic acid overnight, and separated by gel filtration on Sephadex G-100 columns equilibrated in 6 M urea/1 M acetic acid.

Cyanogen Bromide (CNBr) Cleavage. H and L chains were cleaved with CNBr at a CNBr/protein ratio of 4:1 (wt/wt) in 70% formic acid (23), and the fragments were separated on a Sephadex G-100 column equilibrated in 5 M guanidine/0.2 M NH_4HCO_3. Pooled fractions were dialyzed extensively against 0.2 M NH_4HCO_3 and lyophilized. To separate fragments linked by intrachain disulfide bonds, a complete reduction and alkylation was then carried out. Resulting peptides were separated by gel filtration on a Bio-Gel A-0.5m agarose (Bio-Rad) column equilibrated in 6 M guanidinium Tris buffer, pH 8.0. Pooled fractions were dialyzed extensively against 0.2 M NH_4HCO_3 and lyophilized.

Sequence Determinations. Automated Edman degradations were performed on intact chains and CNBr fragments with a modified Beckman 890 C sequencer (24, 25) and a 0.25 M Quadrol buffer program. Identification of phenylthiohydantoin amino acids was by high-pressure liquid chromatography (26).

RESULTS

Complete H chain V region sequences (amino acids 1–118) were determined for three *P*Cho-binding monoclonal antibodies (HP100.6F9, HP140.7C6, and HP101.6G6) of CBA/J origin by automated degradation of intact H chains and appropriate CNBr fragments. These proteins subsequently will be designated 6F9, 7C6, and 6G6. CNBr-cleaved H chains were chromatographed on a Sephadex G-100 column equilibrated in 5 M guanidine/0.2 M NH_4HCO_3 (Fig. 1*A*). Peaks P3 and P4, which were found to contain V region fragments, were pooled, reduced, [^{14}C]alkylated, and applied to a Bio-Gel A-0.5m agarose column equilibrated in 6 M guanidinium Tris, pH 8.0 (Fig. 1*B*). Similar cleavage patterns were obtained for all three H chains. V region fragments used for sequence studies were obtained as follows:

Abbreviations: L, light; H, heavy; V region, variable region of the H chain (amino acids 1–118); V_H and V_L segments, H and L chain V region segments including amino acids 1–95; V_H, gene segment encoding amino acids 1–95; D_H, H chain diversity segment; J_H, H chain joining segment; *P*Cho, phosphocholine; CNBr, cyanogen bromide.

‡ To whom reprint requests should be addressed.

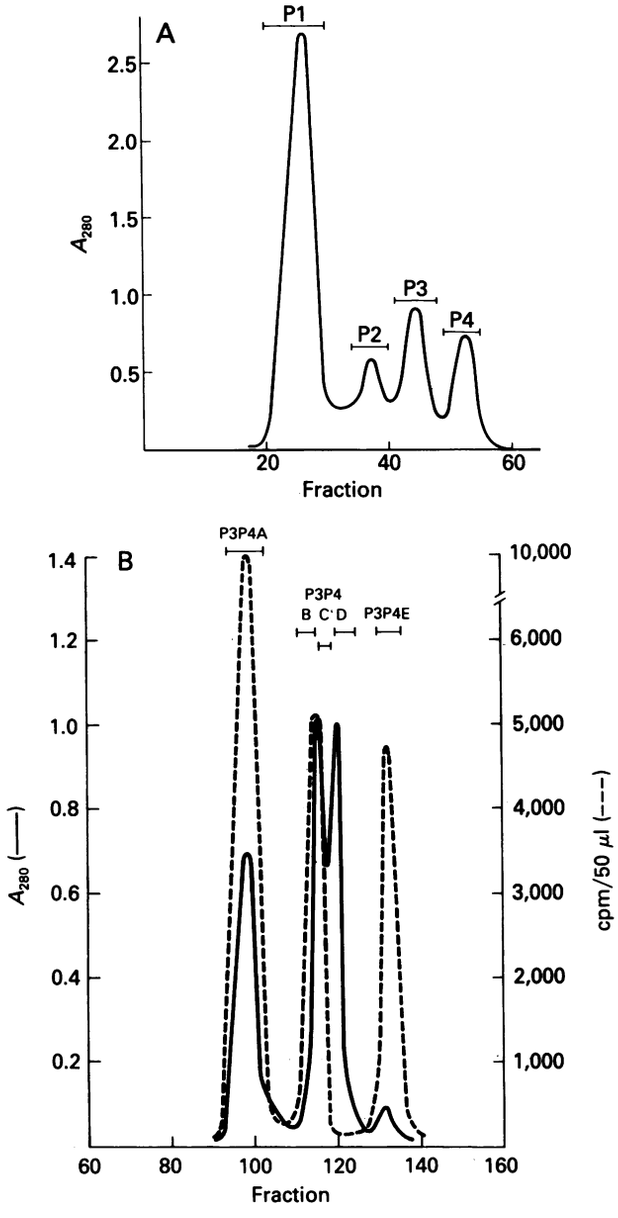

FIG. 1. (*A*) Sephadex G-100 chromatography of CNBr-cleaved H chains. Columns were equilibrated in 5 M guanidine/0.2 M NH_4HCO_3. P, peak. (*B*) Bio-Gel A-0.5m agarose chromatography of completely reduced and [^{14}C]alkylated pooled fragments from P3 and P4 in *A*. Columns were equilibrated in 6 M guanidine (pH 8.0).

residues 1–34, P3P4E; residues 35–82, P3P4D; and residues 83–140, P3P4B. Sequences determined are presented in Fig. 2. The 6F9 and 7C6 sequences were identical to the sequence of the V_H segment of C3 to position 94 and expressed the four characteristic allelic residues at positions 14, 16, 40, and 44 found in C57BL anti-*P*Cho antibodies (28, 29).

DISCUSSION

The murine immune response to *P*Cho has been well characterized in a number of laboratories. Three of the originally described BALB/c *P*Cho-binding myeloma proteins T15, M603, and M167 (30) subsequently have been shown to possess similar H chains (23, 31), although their L chains are quite different and belong to three separate V_κ subgroups (18). These three myeloma proteins have proven to be prototypes for the murine response to *P*Cho in that all inbred strains of mice use the same or similar L chains in response to *P*Cho immunization (32).

Although the H chains from T15, M603, and M167 are similar, a number of amino acid substitutions exist among these molecules. Recent studies comparing the protein sequences of these H chains and those from a number of BALB/c anti-*P*Cho hybridomas (12) to the DNA sequences of genes from the *P*Cho gene family (13) have concluded that the entire V_H repertoire seen in this response is generated from a single V_H gene and that variations such as those seen among the T15, M167, and M603 H chains are the result of somatic mutation. This particular V_H gene directly encodes the T15 V_H segment, defining T15 V_H as a BALB/c germ line gene product. In the present study, we have determined the complete V region sequences from three *P*Cho-binding monoclonal antibodies of CBA origin. The three CBA proteins analyzed were selected because of the observation that each expressed a different L chain, which in turn corresponded to one of the BALB/c myeloma L chains. 6F9 expresses an M603-like L chain; 7C6, a T15-like L chain; and 6G6, an M167-like L chain. Amino-terminal sequences of these L chains (not shown) demonstrated that each was identical to the BALB/c prototype germ line sequence for the first 50 positions, with the exception of a single framework substitution in the 6G6 L chain.

When an assessment is made of the CBA H chain sequences (Fig. 2) it can be seen that 6F9 and 7C6 differ only at positions 95 and 100a. These two residues are at the junctions of V_H–diversity (D_H) and D_H–joining (J_H) segments, and the differences are likely due to alterations in the recombination sites between the appropriate segments. With the exception of position 95, these two sequences differ from the BALB/c T15 germ line V_H segment sequence at four positions (14, 16, 40, and 44) and are identical to that of C3. C3[§] is a *P*Cho-binding myeloma protein of C57BL allotype that appears to represent an allelic form of the T15 V_H segment (29). The identification of the C57BL type sequence in CBA is in agreement with previous structural (28) and serologic (33) analyses describing the segregation of these polymorphic forms. The two CBA proteins have identical D_H segments, which differ from those found in T15 and C3, and also use the J_H1 segment found in all other *P*Cho-binding H chains previously reported. Therefore, CBA and C57BL express the same apparently allelic form of the BALB/c T15 V_H gene and demonstrate the conservation of this V_H gene in inbred mice.

The third CBA H chain, 6G6, differs from the T15 V_H and C3 V_H segments at 17 and 20 positions, respectively (Fig. 2), and is apparently not derived from either the T15 V_H or C3 V_H genes. A comparison of the 6G6 V_H sequence with the deduced amino acid sequences of the three functional genes belonging to the T15 V_H family of BALB/c mice (13) indicates that the 6G6 V_H segment is not identical to any of these (Fig. 3*A*). The T15 V_H family in BALB/c mice is comprised of four genes designated *V1* (*VT15*), *V3*, *V11*, and *V13*. *VT15* translates exactly into the T15 V_H segment and *V3* is a pseudogene which, by definition, is not expressed. *V11* and *V13* are ≈85% homologous to the *VT15* gene. The 6G6 V_H segment differs from the *V11*- and *V13*-encoded amino acid sequences at 7 and 14 positions, respectively, and more closely resembles the V11 amino acid sequence than that of VT15, indicating that a second V_H gene can encode the H chains of anti-*P*Cho antibodies. In fact,

[§] The C3 myeloma protein originated in CBB22, a congenic strain derived by introgressively backcrossing the immunoglobulin H chain constant region, *C* (color), and *B* (black) loci of C57 onto the BALB/c background.

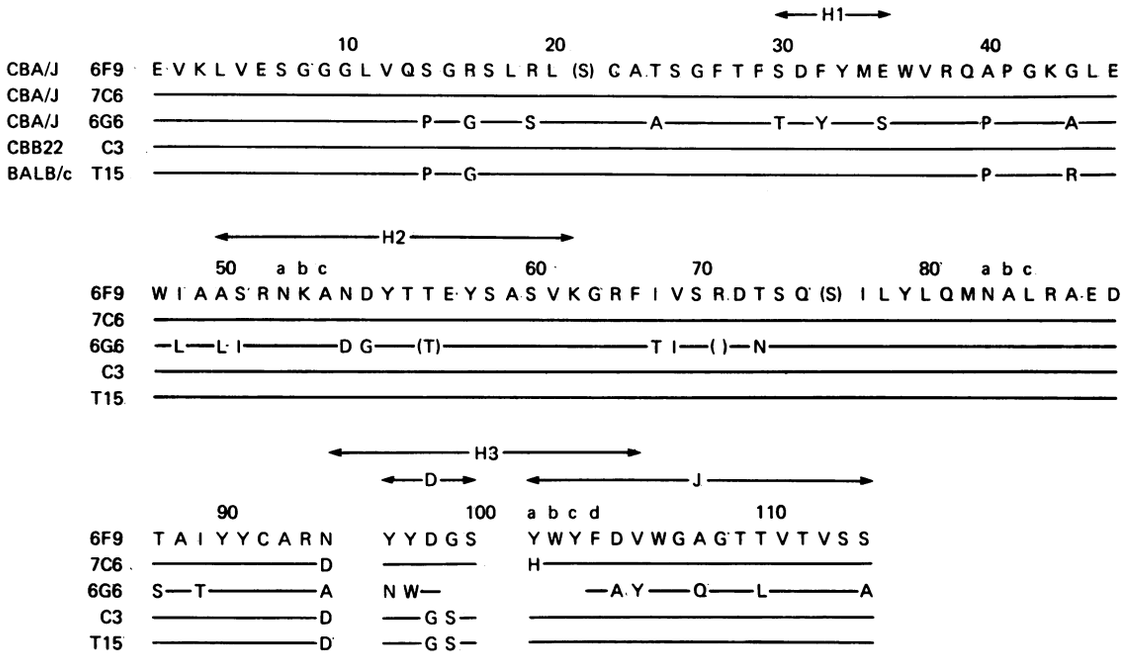

FIG. 2. Complete V region sequences of CBA H chains compared to C3 (C57BL origin) and T15 (BALB/c origin) H chains. Residues in parentheses were tentatively identified. Open parentheses indicate an unidentified amino acid. Numbering is from ref. 27.

although it has been concluded that essentially the entire BALB/c anti-*P*Cho response is generated from the *VT15* gene (13), the H chain of one *P*Cho-binding hybridoma protein of BALB/c origin, HPCG15 (12), expresses three linked amino acids characteristic of the *V11* and *V13* genes and also appears to be the product of a second V_H gene. Thus, at least two V_H genes in both strains can encode anti-*P*Cho V_H segments. The 6G6 protein has been shown to bind *P*Cho and several *P*Cho analogues (34), indicating that H chains encoded by this V_H gene exhibit similar specificity to those encoded by the *VT15* gene. The 6G6 V_H segment may be the product of a gene allelic to the *V11* gene of BALB/c mice or, alternatively, it may be the product of an additional gene belonging to this V_H family.

For the purposes of the following discussion, we have assumed that the V_H gene families encoding the response to *P*Cho are similar in BALB/c and CBA/J mice. This assumption is supported by (*i*) the essentially identical amino acid sequences of the L chains from these two strains and (*ii*) the close homology between the T15 V_H (BALB/c) and the 6F9 and 7C6 V_H (CBA/J) segments, as well as the V11 (BALB/c) and 6G6 (CBA/J) V_H segments of their respective strains. Based on the above observations, we have concluded that 6G6 is likely to be coded for by the BALB/c *V11* homologue in CBA/J. When 6G6 is compared to the V11-encoded sequence (Fig. 3*A*), seven substitutions are observed. Three of these, occurring at positions 19, 53, and 95 of the 6G6 V_H segment are not found in any member of this V_H family. Because these substitutions require only single base-pair changes at the DNA level, they are possibly the result of point mutations in the germ line, as it has been shown that IgM proteins rarely demonstrate somatic point mutations (12–14). Therefore, the substitutions observed in 6G6 are likely either to be encoded in the germ line or to arise by somatic events other than point mutation. The alanine for aspartic acid substitution at position 95 of the 6G6 V_H segment alternately may be generated by variation in the V_H–D_H recombination site. The four remaining substitutions in the 6G6 V_H segment at positions 24, 49, 50, and 82b are also encoded at these positions in other members of this V_H family. Several possibilities exist to explain this observation. First, the four shared substitutions may have arisen by point mutations. Because the probability is extremely low that four independent point mutations would occur in the 6G6 V_H gene to generate the same amino acids present in other members of the T15 V_H gene family, we consider this to be an unlikely explanation. Therefore, alternative mechanisms, such as gene recombination or gene conversion, may be responsible for these substitutions.

Generation of the 6G6 V_H segment from other members of this family by gene recombination requires a complex series of events such as that illustrated in Fig. 3*A*. Beginning at the amino-terminal end and not including the three unique substitutions, the 6G6 sequence is identical to the V11 amino acid sequence, up to position 24, at which point an alanine is substituted for the threonine encoded by the *V11* gene. Because alanine is encoded by the *V13* gene, a crossover to the *V13* gene at this position is required to generate the 6G6 sequence. The 6G6 amino acid sequence is then identical to the *V13*-encoded sequence to position 40, at which the proline found in the 6G6 sequence is not encoded by the *V13* gene. Proline is found at position 40 in the V11 amino acid sequence, and a second crossover back to the *V11* gene would be required to generate the observed 6G6 amino acid sequence. Four additional crossover events as depicted in Fig. 3*A* would be necessary to produce the "shared" amino acids found in the 6G6 sequence. Thus, to generate the 6G6 V_H amino acid sequence by gene recombination, at least six crossovers between three or more genes would be required without altering the reading frame. Because this mechanism necessitates such a complex series of recombinations within a single V_H gene, we feel it is also an unlikely explanation.

A third explanation for the generation of the 6G6 sequence is the occurrence of gene conversion. Gene conversion is a mechanism whereby two closely related genes interact in such a way that all or part of the DNA sequence of one becomes identical to that of the other. Conversion can occur between genes on the same chromosome, homologous chromosomes, or nonhomologous chromosomes (reviewed in ref. 35). Conversion has been described in yeast (36–39) and recently has been suggested to occur among mammalian genes to account for the

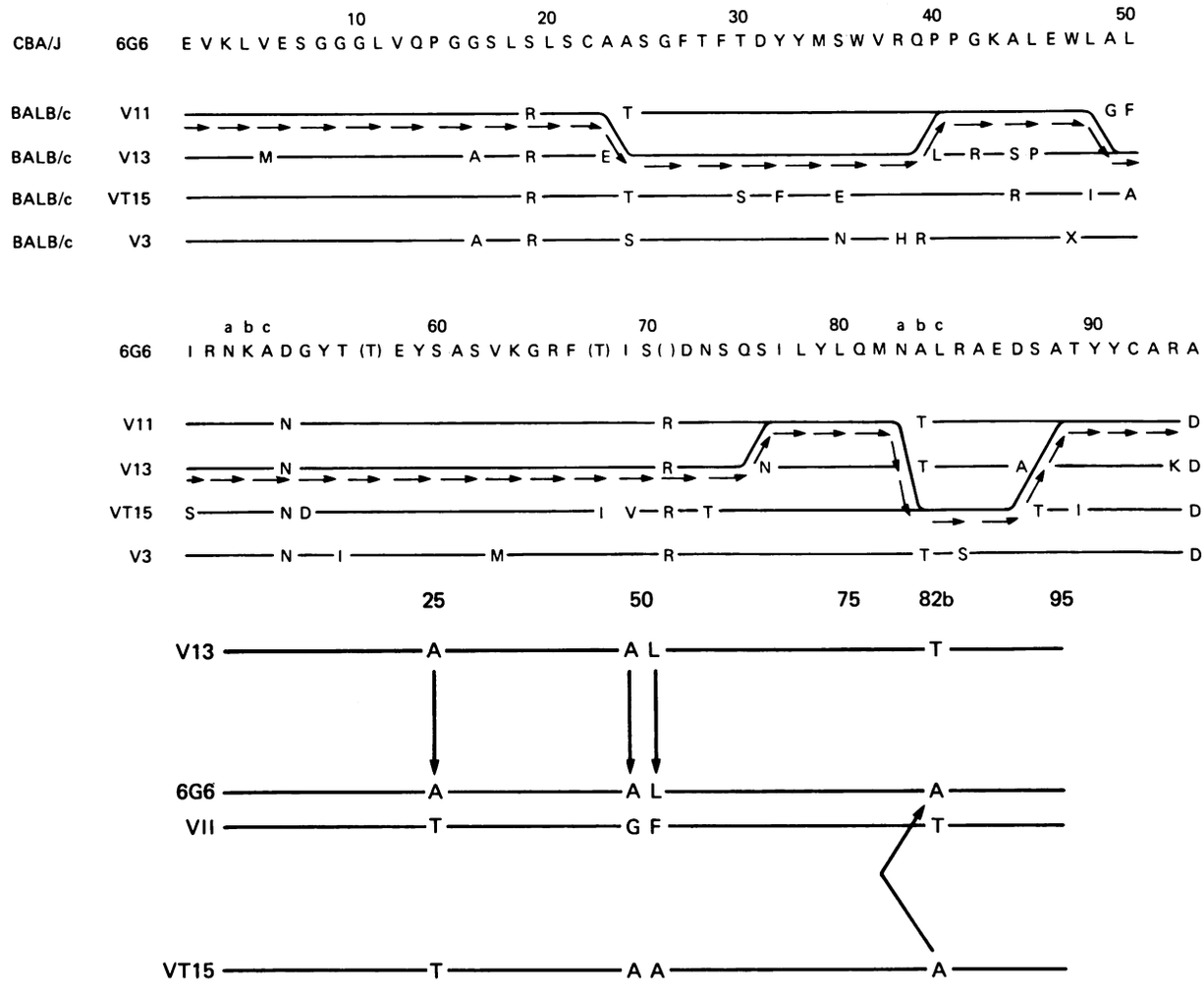

FIG. 3. (*A*) Comparison of 6G6 V_H sequence to the BALB/c anti-*P*Cho V_H family. Arrows represent one of several possible pathways required for assembly of 6G6 from the *P*Cho-V_H family by multiple somatic recombination events. X at position 47 in the V3 sequence represents a stop codon. (*B*) Schematic representation of gene conversion events occurring during the generation of 6G6. 6G6 is most homologous to V11. At four of the positions (24, 49, 50, 82b) where differences were observed between 6G6 and V11, the substituted amino acid was found to be encoded by other members of the *P*Cho-V_H family, as indicated by vertical arrows representing postulated conversion events.

maintenance of homology between members of a multigene family (40). In relation to immunoglobulins, aspects of gene conversion have been included in recombination models addressing diversity in κ L chains (20). Schrier *et al.* (41) proposed gene conversion to have occurred between the immunoglobulin constant region *γ2a* and *γ2b* genes of BALB/c mice and also have interpreted the homology between the *γ1* and *γ2b* genes of BALB/c mice observed by Miyata *et al.* (42) to be the result of gene conversion. The four amino acid substitutions in the 6G6 V_H segment at positions 24, 49, 50, and 82b, where the substituted amino acids are found to be encoded by other members of the T15 V_H gene family (Fig. 3*A*), can be explained readily by gene conversion (Fig. 3*B*). In this instance, conversion appears to have occurred between three members of the T15 V_H gene family to create the 6G6 V_H gene sequence. Thus, the 6G6 sequence provides evidence for possible conversion among V_H gene families. From the present data, it cannot be determined whether the presumed conversion has occurred somatically or among germ line genes because this process can occur at either meiosis or mitosis. This question is being approached by isolation and sequence determination of the germ line *V11* gene of CBA/J origin. It should be noted that gene conversion also may be responsible for generating the observed amino-terminal H-chain sequence of the BALB/c hybridoma protein HPCG15 (12). The V_H segment of this H chain does not appear to be the product of the *VT15* gene and more closely resembles *V11* and *V13* gene products. HPCG15 is not identical to the amino acid sequence encoded by either but does contain amino acids characteristic of both, suggesting, as in the case of 6G6, the occurrence of gene conversion. It should be emphasized that, in both examples (6G6 and HPCG15), it is possible that the observed sequences were derived from an as yet unidentified germ line gene(s) that contains elements of the various members of this V_H gene family. Even if this were the case, the "unidentified" gene(s) expressing these "mixed" sequences would have had to be generated by conversion or complex recombination processes such as those described above.

Gene conversion has recently been suggested (35) to account for the conservation of immunoglobulin framework regions described by Kabat *et al.* (43). These conserved stretches of sequence were originally proposed to be encoded by a series of minigenes that are assembled somatically. Although this hypothesis proved to be correct for the D and J regions, there is no evidence that the remainder of the variable region is assembled from minigenes. Baltimore (35) has postulated that the conservation of framework segments observed in the remainder of the V region may have arisen by gene conversion and furthermore that families of closely related *V* genes can be main-

tained over long periods of time by this process. The potential conversion described for the 6G6 V_H segment differs from that suggested above in that it does not involve entire framework regions. For example, conversion appears to have occurred between the *V11* and *V13* genes within the first framework region to generate the alanine at position 24 of the 6G6 V_H segment (Fig. 3*B*). There are other amino acids characteristic of the *V13*-encoded amino acid sequence at positions 5, 16, and 23 (Fig. 3*A*), also in the first framework region, that are not present in the 6G6 V_H segment. Similar examples are found in the second and third framework regions as well. Therefore, the conversion described here for the 6G6 V_H segment is limited to short stretches of sequence and does not extend to entire framework segments as would be necessary to explain the assortment described by Kabat *et al.* (43).

Gene conversion potentially adds interesting facets to the diversity exhibited by the multigene immunoglobulin family. If the process occurs during differentiation, structural diversity can be generated by the production of "hybrid" molecules expressing segments encoded by several related genes, as may be the case for 6G6. It should be cautioned that the amount of functional diversity, in terms of antibodies with altered binding specificity, is likely to be considerably less than the structural diversity generated by conversion, in that substitutions such as those in 6G6 which occur in framework regions are not likely to affect antigen binding. However, we previously have shown that in certain instances substitutions in framework regions may alter antigen binding (15). If gene conversion occurs in the germ line, at least two quite different end results may be obtained. First, as at the somatic level, new structures may be generated that will, in turn, be maintained in the germ line. Second, gene conversion in effect may compensate for mutations occurring during evolution. Because mutation appears to be the major force in evolution, the accumulation of such events "drives" a given gene sequence to continual change. Gene conversion in multigene families such as the immunoglobulins may provide a mechanism for "correcting" such mutations by restoring sequences strongly selected for, and presumably important in, survival. The observation of gene conversion in immunoglobulin V regions thus provides an additional mechanism contributing to antibody diversity and also potentially serving to conserve sequences in related families during evolution.

1. Weigert, M., Gatmaitan, L., Loh, E., Schilling, J. & Hood, L. (1978) *Nature (London)* **276,** 785–790.
2. Brack, C., Hirowa, A., Lenhard-Schueller, R. & Tonegawa, S. (1978) *Cell* **15,** 1–14.
3. Max, E., Seidman, J. G. & Leder, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3450–3454.
4. Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. (1979) *Nature (London)* **280,** 288–294.
5. Rudikoff, S., Rao, D. N., Glaudemans, C. P. J. & Potter, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4270–4274.
6. Rao, D. N., Rudikoff, S., Krutzsch, H. & Potter, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2890–2894.
7. Schilling, J., Clevinger, B., Davie, J. M. & Hood, L. (1980) *Nature (London)* **283,** 35–40.
8. Early, P. W., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19,** 981–992.
9. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286,** 676–683.
10. Weigert, M., Perry, R., Kelley, D., Hunkapiller, T., Schilling, J. & Hood, L. (1980) *Nature (London)* **283,** 497–499.
11. Gough, N. M. & Bernard, O. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 509–513.
12. Gearhart, P., Johnson, N. D., Douglas, R. & Hood, L. (1981) *Nature (London)* **291,** 29–34.
13. Crews, S., Griffin, J., Huang, H., Calame, K. & Hood, L. (1981) *Cell* **25,** 59–66.
14. Bothwell, A. L. M., Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. & Baltimore, D. (1981) *Cell* **24,** 625–637.
15. Cook, W. D., Rudikoff, S., Giusti, A. & Scharff, M. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1240–1244.
16. Rudikoff, S., Giusti, A. M., Cook, W. D. & Scharff, M. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1979–1983.
17. Hood, L., Loh, E., Hubert, J., Barstad, P., Eaton, B., Early, P., Fuhrman, J., Johnson, N., Kronenberg, M. & Schilling, J. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 817–836.
18. Potter, M. (1977) *Adv. Immunol.* **25,** 14–211.
19. Cory, S., Tyler, B. M. & Adams, J. M. (1981) *Mol. Appl. Genet.* **1,** 103–116.
20. Seidman, J. G., Leder, A., Nau, M., Norman, B. & Leder, P. (1978) *Science* **202,** 11–17.
21. Claflin, J. L., Hudak, S. & Maddalena, A. (1981) *J. Exp. Med.* **153,** 352–364.
22. Chesebro, B. & Metzger, H. (1972) *Biochemistry* **11,** 766–771.
23. Rudikoff, S. & Potter, M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2109–2112.
24. Wittman-Liebold, B., Graffunder, H. & Kohls, H. (1976) *Anal. Biochem.* **75,** 621–633.
25. Hunkapiller, M. W. & Hood, L. (1978) *Biochemistry* **17,** 2124–2133.
26. Zimmerman, C. L., Appella, E. & Pisano, J. J. (1977) *Anal. Biochem.* **77,** 569–573.
27. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1976) *Variable Regions of Immunoglobulin Chains* (Bolt, Bernak, & Newman, Cambridge, MA).
28. Claflin, J. L. & Rudikoff, S. (1979) *J. Immunol.* **122,** 1402–1406.
29. Rudikoff, S. & Potter, M. (1980) *J. Immunol.* **124,** 2089–2092.
30. Potter, M. & Lieberman, R. (1970) *J. Exp. Med.* **132,** 737–751.
31. Rudikoff, S. & Potter, M. (1974) *Biochemistry* **13,** 4033–4037.
32. Claflin, J. L. (1976) *Eur. J. Immunol.* **6,** 669–674.
33. Lieberman, R., Rudikoff, S., Humphrey, W., Jr. & Potter, M. (1981) *J. Immunol.* **126,** 172–176.
34. Andres, C. M., Maddalena, A., Hudak, S., Young, N. M. & Claflin, J. L. (1981) *J. Exp. Med.* **154,** 1584–1598.
35. Baltimore, D. (1981) *Cell* **24,** 592–594.
36. Radding, C. M. (1978) *Annu. Rev. Biochem.* **47,** 847–880.
37. Scherer, S. & Davis, R. W. (1980) *Science* **209,** 1380–1382.
38. Jackson, J. A. & Fink, G. R. (1981) *Nature (London)* **292,** 306–311.
39. Klein, H. L. & Petes, T. D. (1981) *Nature (London)* **289,** 144–148.
40. Slightom, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21,** 627–638.
41. Schrier, P. H., Bothwell, A. L. M., Mueller-Hill, B. & Baltimore, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4495–4499.
42. Miyata, T., Yasunaga, T., Yamawaki-Kataoka, Y., Obata, M. & Honjo, T. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2143–2147.
43. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2429–2433.