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Cloning of human immunoglobulin μ gene and comparison with mouse μ gene

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ABSTRACT

We have cloned a 12 kb DNA segment containing human μ gene and its flanking sequence from human fetal liver DNA library using mouse μ gene as a probe. Partial nucleotide sequence determination shows that the cloned DNA contains the sequence encoding human μ chain. This is the first constant region gene of the human heavy chain that is cloned. We have compared human and mouse μ genes by heteroduplex analysis and Southern blot hybridization. The results clearly show that not only the sequence encoding the CH_4 domain but also the 5'-flanking (S_μ) sequence is conserved between human and mouse μ genes, suggesting that the nucleotide sequence in the S_μ region has an important biological function, presumably a recognition signal for the class switch recombination as proposed previously.

INTRODUCTION

Immunoglobulins comprise two heavy (H) and two light (L) chains, each consisting of a variable (V) region and a constant (C) region. The H chains are classified into five major classes μ, δ, γ, α and ε.

During the differentiation of antibody-producing cells, two types of DNA recombination occur in the H chain genes. First, the V_H, D and J_H gene segments are joined to generate a contiguous coding sequence for the entire V region (1,2). The second type of DNA recombination, which is called S-S recombination, occurs between a pair of S (switch) regions, one located in the 5'-flanking sequence of the C_μ gene and the other in the 5'-flanking sequence of the C_γ or C_α gene, resulting in the replacement of the C_μ gene with the C_γ or C_α gene (2-5). The sequences surrounding the V-D-J recombination and the S-S recombination sites are quite different from each other. The inverted-repeats sequences are found around the V-D-J recombination sites (1,2) whereas tandem direct repeat sequences are present in the S regions which seem to play some role in the S-S recombination (2-6).

In this report we compare the structures of human and mouse μ genes using heteroduplex analysis and Southern blot hybridization. The S_μ regions

of the two μ genes are more homologous to each other than a part of the coding regions. The results suggest that the nucleotide sequence in the S_μ region plays an essential biological function and thereby it has been conserved in these organism for decades of million years.

MATERIALS AND METHODS

The human DNA library (7), which was kindly provided by Dr. T. Maniatis of California Inst. of Tech., was constructed by Hae III/Alu I digestion of human fetal liver DNA followed by the addition of Eco RI linkers and insertion into λ Charon 4A phage (8). Plaque hybridization (9) of the library was carried out after plating on Nunc bioassay plates (23cm x 23cm) using the nick-translated mouse μ gene fragment (3,10). Nucleotide sequence was determined by the method of Maxam and Gilbert (11) with slight modifications (12). Restriction DNA fragments were electrophored in 0.7% agarose gels (type I, Sigma) and transfered to nitrocellulose filters according to Southern (13). The filter was hybridized to an appropriate probe as described (14) with slight modifications (12). The heteroduplexes were analyzed by the formamide technique as described by Davis et al. (15) with slight modifications (16). Sources of restriction enzymes were described (12). Cloning experiments were carried out under P3-EK2 conditions.

RESULTS AND DISCUSSION

Isolation and Characterization of Human μ Gene Clone

We screened a Charon 4A library containing partial Hae III/Alu I digests of human fetal liver DNA with the 1.2 kb Hind III fragment (Hind III D in Figure 1) of mouse μ gene clone λgtWES·IgH701 (3,10), the insert of which is called M701. Five positive clones were obtained after screening 5.0 x 10^5 hybrid phages. One clone was designated as Ch4A·H·Igμ-24 and its insert is called H24. The other four clones were indistinguishable from Ch4A·H·Igμ-24 by restriction site analysis.

The restriction maps of M701 and H24 were compared as shown in Figure 1. The region of H24 that is homologous to the Hind III D fragment of M701 was determined by Southern hybridization. When digested with Eco RI, DNA of H24 produced a 0.9 kb fragment (Eco RI D) which hybridized with ^{32}P-labeled Hind III D fragment of M701 as shown in Figure 2A. A 1.2 kb fragment which is located 5' to the Eco RI D fragment hybridized weakly with the same probe.

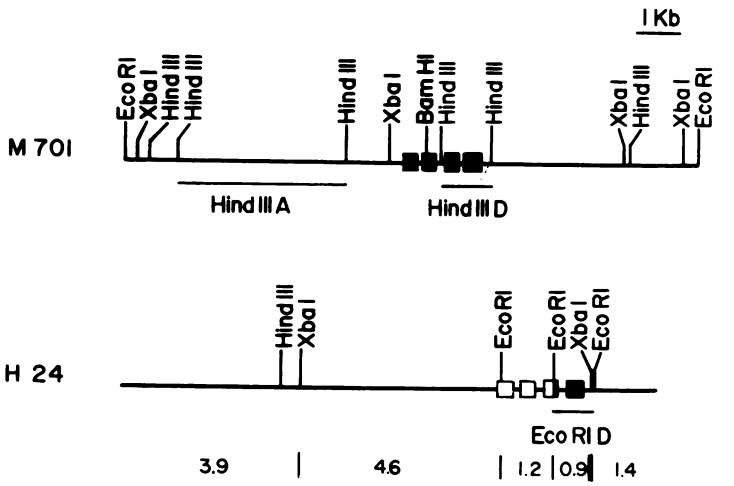

Fig. 1. Comparison of restriction site maps of the mouse (M701) and human (H24) μ genes.

Human and mouse μ gene fragments are displayed with the direction of transtciption from left to right. Coding regions are indicated by closed squares. Open squares show putative locations of the CH_1, CH_2 and CH_3 domains of the human μ gene. The Hind III A and D fragments of M701 and the Eco RI D fragment of H24 which were used for hybridization probes, were shown by horizontal bars below the restriction maps of each gene. Sizes of the Eco RI-Xba I fragment of H24 were indicated in kilobase pairs below the restriction map of H24.

Nucleotide Sequence of Human μ Gene

To confirm that H24 contains the human μ gene, we isolated the 0.9 kb Eco RI fragment (Eco RI D) of H24, digested it with Hinf I or Hap II, and determined the nucleotide sequences as shown in Figure 3. The nucleotide sequences match the amino acid sequences of the human μ chain (17). The sequences a, b and c shown in Figure 3 correspond to the end of the CH_3 domain, the middle of the CH_4 domain and the end of the CH_4 domain including the COOH-terminal segment, respectively. The nucleotide sequences unambiguously demonstrate not only that H24 contains the μ gene, but also that the human μ gene is interrupted by an intervening sequence at the junction of the CH_3 and CH_4 domains. The CH_4 domain and the COOH-terminal segment are contiguous as shown in the mouse μ gene (10,18).

When we compare the nucleotide sequences of the homologous domains of the human and mouse μ genes, the homology in the CH_4 domains (80%) and the COOH-terminal segments (86%) is higher than that in the CH_3 domain (68%).

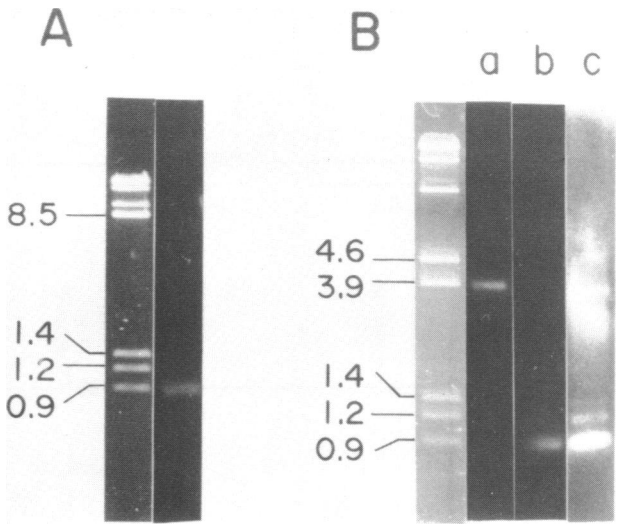

Fig. 2. Southern blot hybridization of restriction DNA fragments of the human μ gene with the ^{32}P-labeled mouse μ gene fragments.
A. H24 DNA was cleaved with Eco RI and electrophoresed in a 0.7% agarose gel. The Southern blot of the gel was hybridized with ^{32}P-labeled M701 Hind III D fragment (Figure 1) of M701. B. H24 DNA was cleaved with Eco RI and Xba I and electrophoresed in a 0.7% agarose gel. The Southern blot of the gel was hybridized with either ^{32}P-labeled Hind III A fragment of M701 (a), Hind III D fragment of M701 (b) or pBR322·IgH714 (c). pBR322·IgH714 contains the insert identical to M701 except that IgH714 has a deletion (2 kb) in the Hind III A fragment.

This result is consistent with the amino acid sequence deta (19).

We have also compared the nucleotide sequences of the intervening sequences and the 3'-untranslated regions of the two μ genes. The intervening sequences located between the CH_3 and CH_4 domains are less homologous (60% or less) than coding sequences. The 3'-untranslated region is more homologous (64%) than the intervening sequence, suggesting that the former is important for recognition of poly(A) addition, termination of transcription and/or RNA splicing for membrane-bound μ chain synthesis (20).

Homology between Human and Mouse μ Genes

Homology between human and mouse μ genes was further tested by Southern hybridization and heteroduplex analysis. When digested with Eco RI and Xba I, H24 DNA produced 4.6 and 0.9 kb fragments which hybridized with ^{32}P-labeled mouse μ gene (Figure 2B, lane c). The 4.6 kb Eco RI-Xba I fragment of H24 hybridized with ^{32}P-labeled Hind III A fragment of M701 and

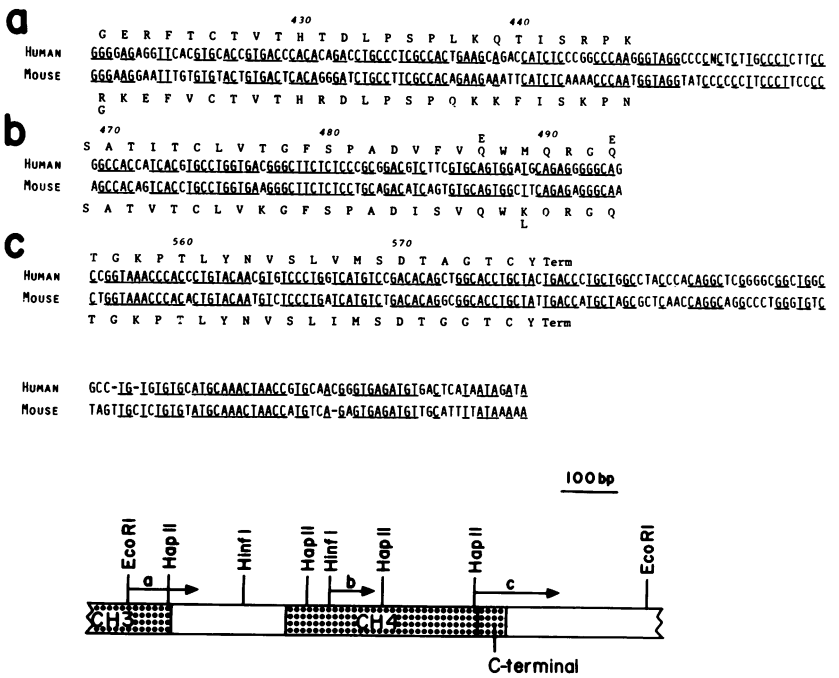

Fig. 3. Partial nucleotide sequence of human μ gene.

The nucleotide sequence of the strand corresponding to the mRNA was displayed from left to right with the direction of transcription. The amino acid predicted by the nucleotide sequence was shown above the coding sequence in italic letters. The numbers of the amino acid residues indicate those of human Ou μ chain protein (17) which is shown on the top line. Only the amino acids (residues 487 and 493) which disagree with the predicted residues were shown. Amino acids were expressed by one letter code as follws: (A) alanine; (C) cystein; (D) aspartic acid; (E) glutamic acid; (F) phenylalanine; (G) glycine; (H) histidine; (I) isoleucine; (K) lysine; (L) leucine; (M) methionine; (N) asparagine; (P) proline; (Q) glutamine; (R) arginine; (S) serine; (T) threonine; (V) valine; (W) tryptophan ; (Y) tyrosine. The nucleotide sequence of the mouse μ gene and the predicted amino acid sequence, which were taken from the previous data (18), are shown below the human sequences. Homologous nucleotides are underlined. Strategy for nucleotide sequence determination was shown below the sequence. Arrows indicate the ranges and directions of the sequences read.

the 0.9 kb fragment of H24 (Eco RI D) hybridized with ^{32}P-labeled Hind III D fragment of M701 (Figure 2B, lanes a and b).

For heteroduplex analysis experiments, we recloned the insert of mouse μ chain gene (M701) into a Charon 4A phage. During recloning of M701, we obtained a shorter clone containing 11.5 kb insert, which was called

Ch·M·Igμ-702, the insert of which is abbreviated as M702. This deletion (1.5 kb) occured in the middle portion of the 3.7 kb Hind III fragment (Hind III A). An electron microscopic picture of the heteroduplex formed between human and mouse μ gene DNAs is shown in Figure 4A. From the restriction map analysis we know that the 5' ends of the μ gene inserts are ligated with the shorter arm of Charon 4A phage DNA. Our interpretation of the heteroduplex is shown in Figure 4B and C. There are two homologous regions; one is located 4.5-5.7 kb and 2.6-3.8 kb 3' to the 5' end of H24 DNA and M702 DNA, respectively, and the other 9.5-9.8 kb and 6.8-7.1 kb 3' to the 5' end of H24 DNA and M702 DNA, respectively.

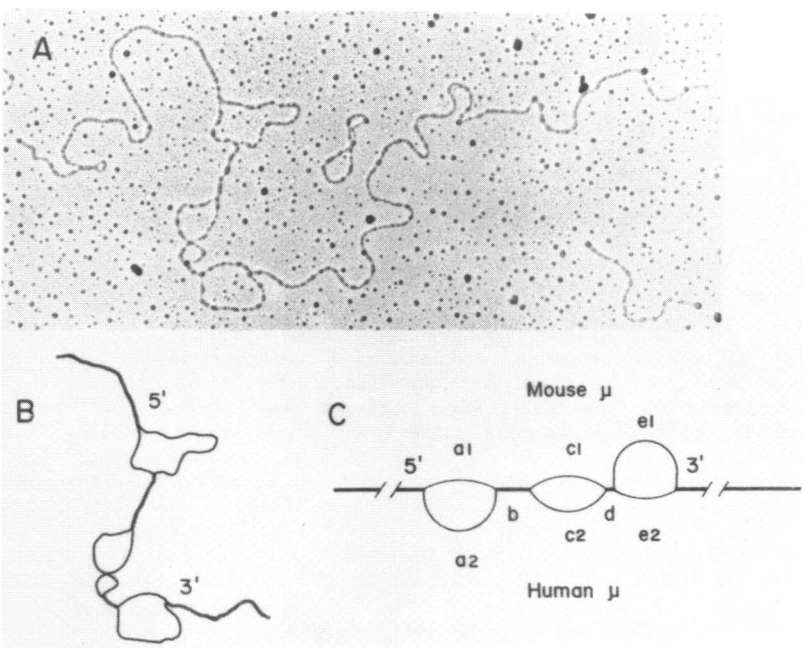

Fig. 4. Heteroduplex formed between the human (H24) and mouse (M702) μ genes.

A. Heteroduplex was formed between H24 and M702 DNAs. Electron micrographs were taken in a Hitachi-HU12A at an instrumental magnification of 10,000 fold. Heteroduplex molecules were traced on a 10-fold enlarged image with a Leiz projector, measured and calculated with a Mutoh-digigramer (Model G). For measurement of DNA length, pA043 DNA (25) was added as internal standards. short arms (10.9 kb) of Charon 4A DNA were also used as internal standards. B. Interpretation. C. Schematic representation with measured length (kb) of each segment. a1; 4.48±0.55; a2; 2.64±0.48; b; 1.16±0.30; c1; 3.86±0.83; c2; 3.00±0.51; d; 0.34±0.09; e1; 4.42±0.79; e2; 2.13±0.36.

Such interpretation is consistent with Southern blot hybridization data. The 3' homologous region (0.3 kb) corresponds to the homology observed between the Hind III D fragment of M701 and the Eco RI D fragment of H24 (Figure 2B, lane b). Since M702 has 1.5 kb deletion in Hind III A fragment, the 3' homologous region (0.3 kb) is located 8.3-8.6 kb 3' to the 5' end of M701. Considering the standard deviation of length measurement and the homology in the amino acid sequence (78-89%) and nucleotide sequence (80-86%), we have assigned the 3' homologous region as the CH_4 domain and COOH-terminal segments. The 5' homologous region lies 2.3 kb and 1.5 kb upstream from the coding region of human and mouse μ genes, respectively. The result is in agreement with the cross-hybridization of the 4.6 kb Eco RI-Xba I fragment of H24 with the Hind III A fragment of M701 (Figure 2B, lane a). This homologous region in the mouse μ gene corresponds to the S_μ region that participates in the class switch recombination (3,5). The homologous regions in M702 and H24 are schematically shown in Figure 5. Since the deleted segment (1.5 kb) in M702 lies in the middle portion of the Hind III A fragment, the homologous region might be larger in M701. The S_μ region is more homologous than the CH_1, CH_2 and CH_3 domain. Since the S_μ region does not code for any protein, the S_μ region is not under the influence of selective constraints at the protein level. Nonetheless, the S_μ region are more conserved than some of the coding domains between the human and mouse μ genes, supporting the idea that the S_μ region plays an essential biological function for immunoglobulin gene expression.

S Regions

Several rearranged γ and α genes were isolated from mouse myeloma DNAs (2-6,21). Comparison of these rearranged genes and germ line genes revealed

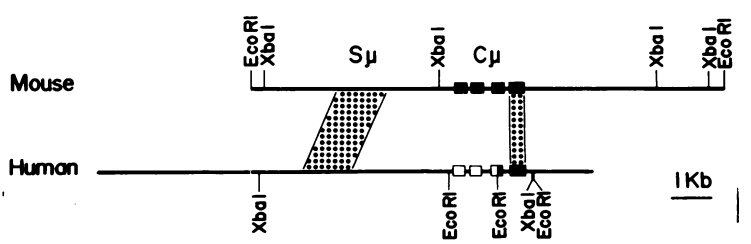

Fig. 5. Homology between the human (24) and mouse (702) μ genes. Homologous regions between M702 and H24 are schematically shown by dotted areas. Wider squares indicate coding domains. Relative locations of the CH_1 and CH_2 domains of the human μ gene are set to the same as those of the mouse μ gene.

that the sequence around the class switch recombination site is quite different from the sequence around the V-D-J recombination site. The nucleotide sequences around the S-S recombination sites have neither inverted-repeat nor common sequences. A partial nucleotide sequence determination of the mouse S_μ region (2,5; T. Nikaido et al., unpublished data) indicates that a pentameric sequence, GAGCT, GGGGT and their variants are repeated in tandem. Partial nucleotide determination of the S_γ and S_α region indicates that S_γ and S_α regions comprise tandem repetition of 49 and 80 base-pair units, respcetively (4,6,23,24). The nucleotide sequence of the S_μ region shares GAGCT and GGGGT in common with those of other S regions. The results support our previous proposal (5) that combination of tandem repetitive sequences and relatively short common sequences may serve as a recognition signal for S-S recombination.

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REFERENCES

1. Early,P., Huang,H., Davis,M., Calame, K. and Hood,L. (1980) Cell 19, 981-992.
2. Sakano,H., Maki,R., Kurosawa,Y., Roeder,W. and Tonegawa,S. (1980) Nature 286, 676-683.
3. Kataoka,T., Kawakami,T., Takahashi,N. and Honjo,T. (1980) Proc. Natl. Acad. Sci. USA 77, 919-923.
4. Kataoka,T., Miyata,T. and Honjo,T. (1980) Cell in press.
5. Takahashi,N., Kataoka,T. and Honjo,T. (1980) Gene 11, 117-127.
6. Davis,M. Kim,S. and Hood,L. (1980) Science 209, 1360-1365.
7. Maniatis,T., Hardison,R.C. Lacy,E., Lauer,J., O'Connel,C. Quan,D., Sim, G.K., and Efstratiadis,A. (1978) Cell 15, 687-701.
8. Lawn,R.M., Fritsch,E.F., Parker,R.C., Blake,G. and Maniatis,T. (1978) Cell 15, 1157-1174.
9. Benton,W.D. and Davis,R.W. (1977) Science 196, 180-182.
10. Kawakami,T., Takahashi,N. and Honjo,T. (1980) Nucleic Acids Res. 8, 3933-3946.
11. Maxam,A.M. and Gilbert,W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
12. Honjo,T., Obata,M., Yamawaki-Kataoka,Y., Kataoka,T., Kawakami,T., Takahashi,N. and Mano,Y. (1979) Cell 18, 559-568.
13. Southern,E.M. (1975) J. Mol. Biol. 98, 503-517.
14. Jeffreys,A.J. and Flavell,R.A. (1977) Cell 12, 429-439.

15. Davis,R.W., Simon,M. and Davidson,N. (1971) in Methods in Enzymology Vol 21D (S.P.Colowick and N.O.Kaplan eds.) pp413-429, New York: Academic Press.
16. Yamagishi,H., Inokuchi,H. and Ozeki,H. (1976) J. Virology 18, 1016-1023.
17. Putnam,F.W., Florent,G., Paul,C., Shinoda,T. and Shimizu,A. (1973) Science 182, 287-291.
18. Calame,K. Rogers,J., Early,P., Davis,M., Livant,D., Wall,R. and Hood,L. (1980) Nature 284, 452-455.
19. Kehry,M., Sibley,C., Fuhrman,J., Schilling,J. and Hood,L.E. (1979) Proc. Natl. Acad. Sci. USA 76, 2932-2936.
20. Early,P., Rogers,J., Davis,M., Calame,K., Bond,M., Wall,R. and Hood,L. (1980) Cell 20, 313-319.
21. Davis,M., Calame,K., Early,P.W., Livant,D.L., Joho,R., Weissman,J.L. and Hood,L. (1980) Nature 283, 733-739.
22. Maki,R., Traunecker,A., Sakano,H., Roeder,W. and Tonegawa,S. (1980) Proc. Natl. Acad. Sci. USA 77, 2138-2142.
23. Honjo,T., Kataoka,T., Yaoita,Y., Shimizu,A., Takahashi,N., Yamawaki-Kataoka,Y., Nikaido,T., Nakai,S., Obata,M., Kawakami,T. and Nishida,Y. (1980) Cold Spring Harbor Symp. in press.
24. Obata,M., Kataoka,T., Nakai,S., Yamagishi,H., Takahashi,N., Yamawaki-Kataoka,Y., Nikaido,T., Shimizu,A. and Honjo,T. (submitted).
25. Oka,A., Nomura,N., Sugimoto,K., Sugisaki,H. and Takanami,M. (1978) Nature 276, 845-847.