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PHYSICAL LINKAGE OF A VARIABLE REGION SEGMENT AND THE JOINING REGION SEGMENT OF THE HUMAN IMMUNOGLOBULIN HEAVY CHAIN LOCUS

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SUMMARY: We have isolated overlapping cosmid clones containing both human V_{H-VI} and D gene segments from genomic libraries of a human lymphocyte line which has the germline context of the immunoglobulin loci. Characterization of the cosmid clones revealed that the V_{H-VI} gene was located about 20 kb upstream to the D_4 segment with the same transcriptional orientation relative to the D and J_H segments. No other V_H genes were found downstream to the V_{H-VI} gene using V_H probes hybridizing all the six human V_H families so far identified. The results indicate that the V_{H-VI} gene is the most proximal V_H segments among the known V_H family members and that the distance between the V_H and J_H segments is not longer than 70 kb in the human genome.

A number of important unresolved questions must be answered to elucidate the molecular events underlying the generation of the immunoglobulin repertoire and the regulatory expression of immunoglobulins with particular specificities (1). Among these are the precise number of functional V_H genes as opposed to pseudogenes, the relative orientation of the different V_H genes, and the overall organization of the immunoglobulin locus.

In order to provide conclusive answers concerning these problems, we have initiated a project whose aim is to characterize structurally the entire human V_H locus. By isolating 18 independent cosmid clones containing 55 V_H segments, we have already shown that human V_H segments were classified into three

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families and that members of different V_H families are interspersed among themselves (2). Further isolation and characterization of germline V_H segments revealed the presence of three additional V_H families which had not been identified as proteins (3-5). Isolation of overlapping cosmid clones, which cover about 120-kb region, allowed us to estimate the distance between the D and J_H segments to be 22 kb (6). We have also isolated a novel D (D_5) segment located among V_H segments (6, 7).

Another approach using pulse field gel electrophoresis (PFG) has provided several useful informations on the overall organization of the V_H locus: a) the total size of the human V_H locus is approximately 2500-3000 kb (4, 6); b) some of the D segments are interspersed among the V_H genes (6); c) the distance between the J_H and the closest V_H segment to be less than 300 kb (6). In fact, the V_{H-VI} probe hybridized to a 100-kb SpeI fragment which also hybridized to the C_δ gene (4). The conclusions based on the PFG studies, however, are limited because of the possible overlap of the separate DNA fragments with the similar size.

During the course of our studies to link physically all the V_H and D segments, we identified the V_{H-VI} segment about 20 kb upstream to the D_4 segment in a previously isolated D-bearing cosmid clone. We have also isolated a new cosmid clone which extends about 30 kb upstream of the V_{H-VI} gene. No other V_H families were found within the 30-kb regions 5' and 3' to the V_{H-VI} segment.

MATERIALS AND METHODS

Materials. α [^{32}P]dCTP was purchased from New England Nuclear Inc. (Boston, MA.), enzymes were obtained from Takara Shuzo Co. Ltd. (Kyoto), Toyobo (Osaka), or New England Biolabs (Beverly, MA). The cell line FLEB 14-14 was established before (8). Cosmid vector used for BamHI libraries was kindly provided by Drs. P. Little and S. Cross (London) (9). V_{266BL} (V_{H-I} family),

V_{CE-I} (V_{H-II} family), V_{HBV} (V_{H-III} family) and V_{71-2} (V_{H-IV} family) probes were as described (2, 3). Genomic V_{H-V} and V_{H-VI} family probes were generous gifts from Dr. F. Alt (Columbia Univ.) (4). The 0.38-kb HincII-PstI fragment of 5-1R1 clone and the 0.3-kb EcoRI-StuI fragment of 6-1R1 clone were used as V_{H-V} and V_{H-VI} probes, respectively (4).

Methods. Southern blot hybridization was carried out as described (10). Filters were washed at 65°C in 0.1 X SSC (SSC is 0.15 M NaCl and 15 mM sodium citrate)-0.1% (w/v) sodium dodecyl sulfate. Cosmid libraries using Lorist 2 as a vector were constructed as described (9). Nucleotide sequencing of the DNA was carried out by the chain termination method (11, 12).

RESULTS AND DISCUSSION

We have isolated and characterized overlapping cosmid clones which link the D_4, D_1, D_2, D_3 and J_H segments in this order within a 70-kb region (6, 7). One of these clones, D_{34} contains the D_4 segment and extends further 25 kb upstream of the D_4 segment. Subsequently, we have screened, with the V_{H-VI} probe, a cosmid library containing partial BamHI digests of DNA from FLEB 14-14 cell. Five cosmid clones obtained all contained a 0.75-kb EcoRI fragment that hybridized with the V_{H-VI} probe. No other V_H probes hybridized with any of these clones. One of these clone V65 was digested with EcoRI, and the digests were compared with those of the D34 clone (Fig. 1A). The V65 clone contained four EcoRI fragments of 0.3, 0.75, 1.6 and 2.3 kb which are identical with that of D34 clone located at the 5' side (7). Among these the 0.75-kb fragment hybridized with the V_{H-VI} probe (Fig. 1B). When the EcoRI digests of the D34 clone were hybridized with various V_H probes, only the V_{H-VI} probe hybridized to a 0.75-kb fragment as shown in Fig. 1B. The results clearly indicate that the D34 and V65 clones overlap with each other and share the 0.75-kb EcoRI fragment which seems to contain the V_{H-VI} segment. The V65 clone is likely to extend to the 5' most BamHI site of the D34 clone, located about 10.5 kb 3' from the 5' end of the

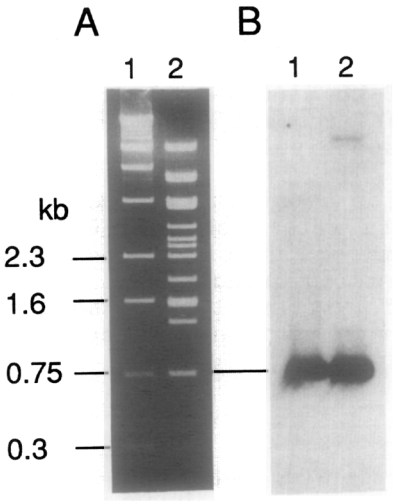

Fig. 1. EcoRI digests of D34 and V65 clones and their Southern blot hybridization using V_{H-VI} probe. DNA of cosmid clones D34 (lane 1) and V65 (lane 2) were digested with EcoRI. (A) Ethidium bromide staining. (B) Autoradiogram of Southern blot hybridization. Numbers indicate sizes of fragments in kb.

D34 clone (Fig. 2). V65 clone seems to extend 30 kb further upstream of the D34 clone as well.

We subcloned the 5' most 3.75-kb fragment of the D34 clone (Fig. 2) and determined the nucleotide sequence of its 3' 400-bp region, which was identical to the published sequence of the V_{H-VI} gene segment (4) except for few bases in the untranslated region. The 3' end of the coding region of the V_{H-VI} segment was located 200 bp 5' to the 3' end of the subcloned fragment. The sequence read indicates that the transcriptional orientation of the V_{H-VI} segment is identical to those of the D and J_H segments. We, therefore, conclude that the V_{H-VI} segment is located about 70 kb upstream of the J_{H1} segment and the most proximal to the J_H segment among known V_H family members.

We then constructed physical maps of the 155-kb region from 30 kb 5' to the V_{H-VI} segment to 20 kb 3' to the C_δ gene using five restriction endonucleases (BssHII, ClaI, SalI, SfiI and SpeI) which have relatively rare cutting sites in mammalian genomes (Fig. 2B). The V_{H-VI}, D_{1-4}, J_H and C_μ genes were shown

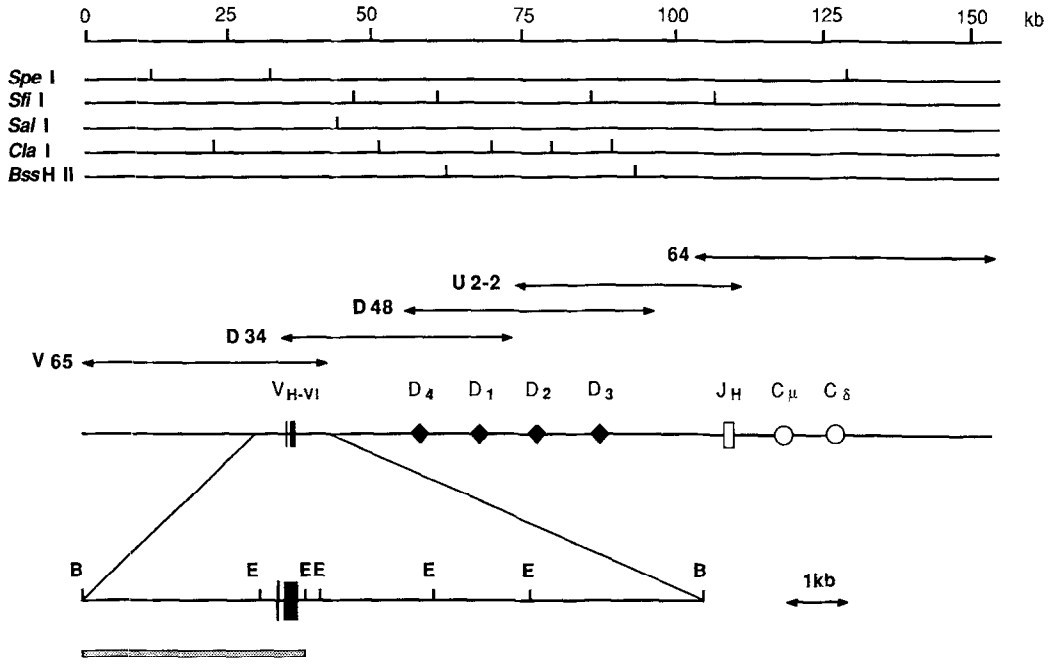

Fig. 2. Organization of the 155-kb region containing human V_{H-VI}, D, J_H, C_μ and C_δ genes. Restriction sites are shown by vertical lines at the top. Inserts of cosmid clones are shown below by horizontal arrows. Locations of gene segments are indicated at the bottom. The transcriptional orientations of all the segments are from left to right. The symbols (■, ◆, □ and o) indicate the V_{H-VI}, D, J_H and C segments, respectively. The subcloned segment is shown by a shadowed bar.

to be in a single 85-kb SpeI fragment, which is in general agreement with the PFG data by Berman et al. (4). The presence of four ClaI sites between the V_{H-VI} segment and the J_H cluster does not agree with the observation that the 158-kb ClaI fragment hybridized with both the V_{H-V} and J_H probes using PFG (13). This discrepancy might be explained by methylation, polymorphism due to the difference of the cell lines used for PFG, or fortuitous coincidence of sizes of two ClaI fragments, each containing the V_{H-V} or J_H segment.

Accumulating evidence indicates that the relative frequencies of DNA rearrangements of the various V_H (4, 5, 13-15) and J_K (16) segments are not equal. The murine J_{K1} and J_{K2} segments were two to five times more frequently rearranged than the J_{K4}

and J_{K5} segments, the J_{K3} being a pseudogene (16). Alt and his associates (4) proposed that V_H segments most proximal to the J_H cluster preferentially rearrange in fetal tissues where clonal selection is less evident. In fact they have shown that the V_{H-VI} segment is more frequently used in fetal tissues than in the adult tissues (4). Our conclusion that the V_{H-VI} segment is most proximal to the J_H segments among known V_H families is consistent with their hypothesis. However, the elucidation of the overall V_H organization and more extensive studies on the relative V_H usage are necessary to directly test the possibility that such biased usage of V_H segments is ascribed to the distance from the J_H segments (or the enhancer sequence).

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