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The human kappa deleting element and the mouse recombining segment share DNA sequence homology

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ABSTRACT

We have used cloned mouse and human DNA probes to identify regions of conserved homology between the human and murine DNA segments, (termed kappa deleting element (kde) and recombining segment (RS) respectively) which are frequently recombined in lambda-producing B cells. Heteroduplex analysis indicated extensive homology in the region immediately downstream of the recombination site of both segments. This was confirmed by Southern and direct nucleotide sequence analyses. Fifty percent homology was detected within the 500 nucleotides that neighbour the recombination points in the kde and RS segments. These results indicate that the kde and RS sequences are evolutionarily conserved and may be functionally relevant to normal B cell development.

INTRODUCTION

Recombination of the immunoglobulin light chain gene segments during B cell differentiation occurs in an ordered fashion, such that recombination of the kappa genes initiates prior to lambda gene rearrangement (1-4). Thus, kappa-producing B cells usually retain germline lambda genes, whereas the kappa genes are either rearranged or deleted in most lambda producing B cells (1-4). We have previously reported that the progression from kappa to lambda gene rearrangement in both murine and human B cells is frequently associated with a specific deletional recombination which uniformly eliminates the constant kappa (C_k) and a portion of the joining kappa (J_k)-C_k intron, including the kappa enhancer sequence (5-7). The kappa gene deletion is mediated by the site-specific rearrangement of a DNA segment or element into a palindromic heptameric sequence located either in the J_k-C_k intron or within the variable kappa (V_k) gene region. This recombinatorial element, termed kappa deleting element (kde) in man and recombining sequence (RS) in mouse, is rearranged in all kappa-deleted lambda producing B cells, including those instances in which J_k genes are eliminated and recombination occurs directly to a V_k gene.

The similarity of the deletional recombination to normal V-J joining as

well as its mediation by specific recombinatorial sequences in two different species, strongly suggests that deletional recombination has functional significance and that the kde and RS may be important in the regulation of ordered light chain gene recombination. Detection of homology between these two elements would lend further support to this hypothesis.

In order to establish whether the human kde and murine RS share significant homology, we have compared their structure by heteroduplex mapping, Southern blot analysis and nucleotide sequence comparison. In this article we report the detection of a region of conserved homology between the mouse RS and human kde. The conserved segment is situated in the most 5' ends of the two recombined elements, thus encompassing the region of the recombinatorial breakpoint.

MATERIAL AND METHODS

Heteroduplex analysis

Recombinant phage clones containing the rearranged kde within a cloned 10kb Eco RI fragment and the rearranged RS within a cloned 8.4 kb Bam HI fragment were subjected to heat denaturation followed by overnight renaturation in 50% formamide, 12.5 mM EDTA, 50 mM NaCl and 100 mM Tris pH 8.5 at room temperature. Electron microscopic analysis was performed by M. Sullivan (Genentech).

Southern blot analyses

DNA from the plasmid subclone of the germline human kde was restriction enzyme digested, electrophoresed on a 1% agarose gel, transferred to nitrocellulose by the method of Southern, and hybridized to randomly-primed fragments from the murine RS plasmid subclone (8,9). The RS probes included 1.4 and 2.4 kb SacI fragments and a 1.8 kb SacI-BamHI fragment isolated from the rearranged RS subclone (5). Hybridization filters were washed at 55°C in 0.1X SSC.

Isolation and sequencing of the human germline kde

A 2.5 kb unique HindIII-Bam HI fragment from the rearranged human kde was used to screen a Charon 4A human liver genomic library (provided by E. Fritsch) and to isolate the germline kde. DNA sequencing of the germline kde was carried out by the dideoxynucleotide chain-termination method using M13 sequencing vectors (10,11).

RESULTS

Heteroduplex comparison of the rearranged human kde and murine RS

Our first attempt at examining homology involved heteroduplex mapping. This was possible because both human and mouse elements were originally cloned

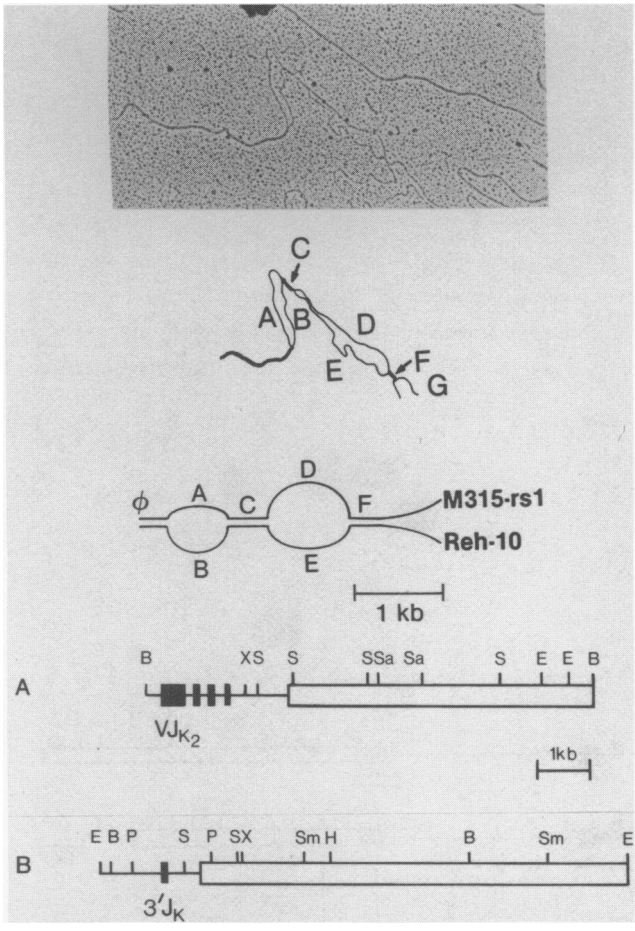

Figure 1: Heteroduplex comparison of the mouse recombining segment (RS) and human kappa deleting element (kde). The top panel shows the actual electron micrograph of the heteroduplex formed between the RS-containing phage clone (M315-rs1) and the kde-containing phage clone (Reh10). Arrows in the line drawing indicate the regions of homology which correspond to joining kappa regions (C) within the clones and the 5' ends of the RS and kde (F). Measurements (in kb) corresponding to the regions demarcated in the schematic drawing were based on analysis of eight molecules. A = 0.8 ± .08, B = 1.1 ± 0.1, C = 0.3 ± 0.08 (heteroduplexed J_k region), D = 1.9 ± 0.4, E = 1.0 ± 0.4, F = 0.3 ± 0.1 (heteroduplexed RS and kde). The bottom panel shows restriction maps of the rearranged murine RS (A) and human kde (B). Restriction enzymes are: EcoRI (E), HindIII(H), PstI (P), BamHI(B), SacI(S), SmaI(Sm), Sau 3A(Sa) and XhoI (X).

in phage vectors and their orientation previously determined by restriction map analyses (5,6). As shown in Figure 1, the heteroduplex formed between the 10 kb human segment and the 8.4 kb mouse segment shows two distinct regions of homology. One is a 300 base pair segment which corresponds to J_k sequences

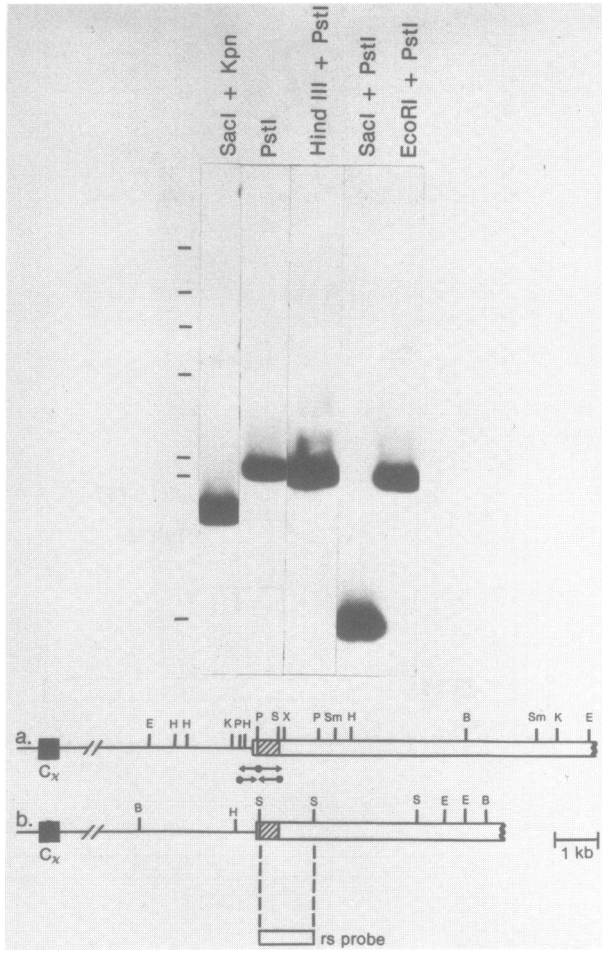

Figure 2: Comparative hybrdization analysis of the cloned human kde and murine RS. The probe used was a 1.4 Kb SacI-SacI fragment mapping to the 5' end of RS. DNA from a plasmid subclone of the human germline kde was digested with the enzymes indicated. The filters were washed at a final stringency of 0.1 x SSC at 55°C. The markers are HindIII digested lambda DNA. a) Restriction map of the germline kde. Arrows below indicate strategy for chain termination DNA sequence analyses. The region of homology is indicated by cross-hatching. b) Restriction map of the germline RS. Restriction enzymes are: Eco RI(E), HindIII(H), Kpn I(K), PstI(P), SacI(S), XhoI(X) and SmaI(Sm).

present in both clones. The second homology region is a 300 base pair segment, beginning about 1.9 kb (mouse) and 1.0 kb (human) 3' to the J_k region homology segment and corresponding to the most 5' ends of the recombined kde and RS. The clones diverge at this point and no further homologous regions

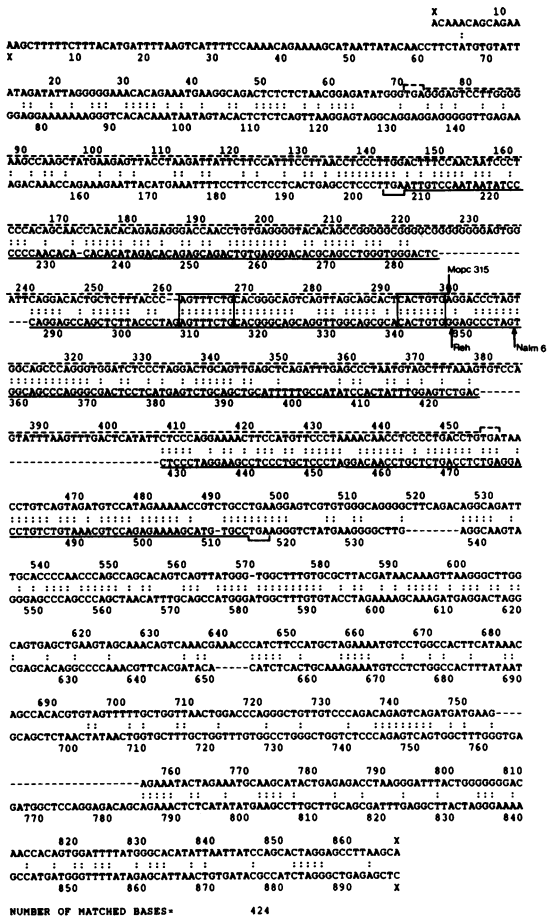

Figure 3: Sequence of the germline RS and kde DNA. The murine sequence (7) is shown in the top line, and the kde sequence is aligned below. The upstream palindromic nonamer and heptamer signal sequences are shown in the open boxes. Arrows indicate the recombination breakpoints for RS (M315) and for two human kde alleles (Reh and Nalm 6). The dashed line above the RS sequence and the solid line below the kde sequence indicate long protein open reading frames. Nucleotide identity is shown by the dotted lines between the two sequences.

have been detected. Thus heteroduplex analysis indicates conservation between mouse and man recombining segments and maps the conserved homology to the region of both segments which encompasses the recombination breakpoint.

Comparison of kde and RS by Southern analysis

To confirm the localization of shared sequences within the elements, Southern blots of restricted DNA from the human kde plasmid subclone, were

probed with sequences from different regions of the murine RS subclone. As shown in figure 2, a 1.4 kb base pair probe derived from the 5' region of the RS segment crosshybridized to the analagous region within the human kde. Cross-hybridization was not detected when more 3' RS sequences were used to probe the kde (data not shown). These results confirm the sharing of homologous sequences between the two elements and the localization of the conserved region to the portions of each segment mapping immediately downstream of the recombination site.

Nucleotide sequence comparison

A 9 kb clone containing the germline kde was isolated from a human liver library in Charon 4A. As the previous analyses mapped the conserved region to the most 5' region of the kde, a 0.9 Hind III-SacI fragment spanning this region was subcloned into M13 and sequenced. In figure 3, the germline kde sequence (which differed from that previously reported (12) at a few nucleotide bases, possibly due to polymorphism), is aligned with the germline RS sequence. 50% homology is apparent within the 500 base pairs immediately downstream to the recombination site and decreases thereafter. Identical palindromic recombinatorial sequences are located upstream of both elements. The longest open reading frames within the homology regions of the kde and RS segments localize slightly differently, but, in each instance, span the recombination point, potentially encoding 127 amino acids in the mouse and 102 amino acids in man. No significant homology has been found between protein sequences in the Gene Bank Data Base and the translated sequence of these long open reading frames.

DISCUSSION

We have previously reported that kappa gene deletion occurs frequently in lambda-producing murine and human B lymphocytes and that, in both species, the deletion appears to be mediated by a specific, recombining DNA element. In this study, we demonstrate that this DNA segment is evolutionarily conserved between man and mouse and that the germline sequences are flanked by identical recognition nonamer and heptamer sequences, suggesting that the recombination resembles normal V-J joining. The murine RS and human kde nucleotide sequences are 50% homologous within the first five hundred bases downstream of the recombination site and thereafter the homology diminishes. Heteroduplex and Southern analyses confirmed this localization of the conserved region. The inter-species homology demonstrated here strongly suggests that the kde and RS have a functional role. This role is not likely to be merely the mediation of deletion recombination, as very little, if any, DNA sequence from the regions

flanking the recognition segments appears to be required for normal V-J joining (13,14).

Deletion of kappa genes, particularly the kappa enhancer sequences, may allow progression from kappa to lambda gene rearrangement, either by elimination of inhibitory sequences or by directly encoding a transacting factor that can effect lambda gene recombination. In this regard, we have recently detected kde transcription in a lambda-producing myeloma cell line using a probe from the highly conserved region of the kde. Similarly, a murine probe from this region has revealed transcription of rearranged RS segments in some Abelson murine leukemia virus transformed cell lines (M. Moore, J. Durdik, E. Selsing, unpublished). These data, although preliminary, provide further indication that the murine and human deletional elements may be biologically significant. Further studies are underway to elucidate the relationship between these elements and the ordering of light chain gene rearrangement.

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