

Rearranged sequences of a human *Kpn* I element

(transposable elements/genome organization/moderately repetitive DNA)

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ABSTRACT The complete nucleotide sequence of a human *Kpn* I element inserted into α -satellite DNA is presented. This sequence reveals several features of interest. First, a large block of DNA normally associated with *Kpn* I elements has been deleted. Second, the order of the remaining sequences is permuted in a manner that cannot be accounted for by simple inversion. Third, a significant open reading frame of 675 bases is detected.

The organization of the human genome follows the short-period interspersion pattern with moderately repetitive sequences usually about 300 base pairs (bp) long scattered among single-copy DNA (1-3). There are, however, some longer interspersed repeats, with the *Kpn* I family the best-studied (4). *Kpn* I elements from African green monkeys (AGM) and humans have been independently discovered and defined by a number of groups (5-10). They have been found near structural genes (5) and inserted into satellite DNA (10). Electron microscope heteroduplex studies comparing randomly cloned *Kpn* I elements suggest that their sequences are generally colinear and at least somewhat well-conserved, within the detectable limits of this technique (5). But whole-genome Southern blots and restriction analyses of individual elements have shown extensive restriction site polymorphism and significant size differences among elements (5, 8, 11, 12).

This laboratory has isolated and characterized human DNA segments carrying unusual monomeric domains of α -satellite sequence (13). Of particular interest are the nonsatellite sequences that have been found occasionally interrupting the long tandem arrays of satellites (10, 13, 14). Because satellite sequences undergo continual rectification by unequal crossover (15) and gene conversion, it seems possible that the interrupting sequences represent mobile elements that have inserted into satellite relatively recently in evolutionary time. In this study I focused on a part of the pa7 clone, which has been shown to carry a nonsatellite sequence inserted into α -satellite DNA (13). The invading sequence is shown here to be an unusual *Kpn* I element.

The *Kpn* I elements are abundant (about 30,000 copies per haploid genome) and particularly interesting structurally. No terminal repeats have yet been found, making them unlike most transposable elements (16, 17), and their variable constructions include deletions and inversions (11). In this paper the complete nucleotide sequence of an element inserted into human α -satellite DNA is presented. The sequence reveals the presence of a large sequence permutation, thereby describing yet another type of polymorphism found in the *Kpn* I family of primate dispersed repetitive elements. Moreover, a large open reading frame is found, suggesting a coding function.

MATERIALS AND METHODS

Enzymes and polynucleotide linkers were purchased from New England BioLabs, and isotopes were from New England Nuclear.

DNA Sequence Determination. DNA sequence was determined by using the partial chemical cleavage method of Maxam and Gilbert (18). DNA was labeled at the 3' end with the Klenow fragment of DNA polymerase I and at the 5' end by polynucleotide kinase.

Subcloning. The 4.0-kilobase (kb) *Sal* I-*Eco*RI DNA segment carrying a *Kpn* I element with flanking α -satellite DNA was purified from the previously described pa7 clone (13) and ligated into pBR322 DNA cut with *Sal* I and *Eco*RI.

In order to facilitate the sequence determination, two experiments designed to introduce restriction sites at useful locations were performed. In the first experiment the subclone was opened at the *Bgl* II site and then treated for various periods with the exonuclease BAL-31. Progress of the digestion was followed by gel electrophoresis. *Cla* I linkers were blunt-end-ligated to the ends and cleaved by *Cla* I, and the molecules were recircularized by ligation. After transformation of *Escherichia coli* HB101 and selection on ampicillin plates, random colonies were used for "miniprep" (19) and restriction analyses to determine the exact locations of the introduced *Cla* I sites. A second experiment used the same overall plan, only the initial cleavage was with *Sal* I, and then *Xho* I linkers were added.

Other. DNA purification (19), Southern blot (20), nick-translation (21), and hybridization (22) have been described.

RESULTS

This study is based on the premise that sequences found inserted into satellite DNA represent putative transposable elements. This laboratory previously has identified such nonsatellite sequences inserted into human monomeric α -satellite DNA, and one cloned segment in particular, named pa7, was shown to carry two related nonsatellite sequences arranged in inverted orientation (13). A 4.0-kb *Eco*RI-*Sal* I restriction segment of pa7 with one of these regions of interest was subcloned into the plasmid pBR322 in order to facilitate a more detailed analysis of its structure.

Homology to the *Kpn* I Family of Elements. To determine if the inserted nonsatellite sequence belongs to the *Kpn* I family, the following experiments were performed. The subcloned restriction segment of pa7 was cleaved with various restriction enzymes, blotted by the Southern technique, and hybridized to "consensus" subclones of the human *Kpn* I family that were generously provided by J. Maio and co-workers (23). A "normal" *Kpn* I element may contain up to five internal *Kpn* I restriction sites, giving four conserved segments after *Kpn* I cleavage. Such internal *Kpn* I segments have been cloned from total human DNA, and their sequences have been aligned with respect to the element designated *Kpn* β as shown in Fig. 1A (23), indicating that *Kpn* β , which is found about 3 kb beyond the human β -globin gene (5), is rather normal in sequence arrangement. A detailed description of these probes can be found in Shafit-Zagardo *et al.* (23). Southern blots indicated that the inserted element in the pa7 clone is homologous to three of the "consensus" *Kpn* I subclones and, therefore, is a member of the *Kpn* I family. Furthermore the results suggested that this element, desig-

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Abbreviations: kb, kilobase(s); bp, base pair(s); AGM, African green monkey.

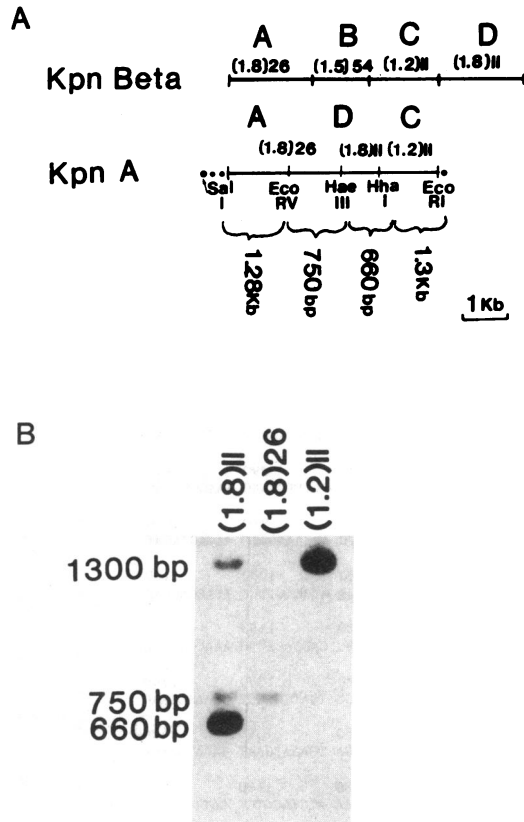


FIG. 1. Relationship between *Kpn* A and the *Kpn* β element found near a human β -globin gene. (A) Comparison of *Kpn* A and *Kpn* β sequences. The short vertical lines in *Kpn* β demarcate the position of sequences present in various *Kpn* I "subclones," which are aligned and numbered as described by Shafit-Zagardo *et al.* (23). For *Kpn* A, the solid line represents the *Kpn* A sequence, and the surrounding dots are α -satellite DNA; the positions of a few reference restriction sites are shown, and the results of cross-hybridization Southern blots are summarized. Note that the (1.5)54 sequences are absent in *Kpn* A, and the remaining sequences are present in a different order from that in *Kpn* β . The letters A, B, C, and D are included to aid comparison and do not relate to any previous nomenclature of *Kpn* I elements. (B) Southern blots showing homology of *Kpn* A to the *Kpn* I family. Three identical Southern blots of the purified 2.7-kb *Eco*RI-*Eco*RV DNA segment subsequently digested with *Hae* III/*Hha* I and, after the gel electrophoresis and blot, hybridized with the various *Kpn* I subclones as shown. Note that the (1.8)11 probe hybridizes predominantly with the 660-bp *Hae* III-*Hha* I segment, but it also hybridizes weakly with both of the other segments, showing that the (1.8)11 sequence extends somewhat beyond the *Hae* III and *Hha* I sites.

nated *Kpn* A [A for alphoid (α) satellite], carries an interesting arrangement of sequences.

First, the (1.5)54 *Kpn* I subclone probe failed to hybridize, showing that these sequences are absent from *Kpn* A; this result is consistent with prior reports demonstrating that internal deletions can occur in *Kpn* I elements (12). Second, and perhaps more interesting, the sequences that are present

appear to be permuted or scrambled relative to the "normal" consensus arrangement exemplified by *Kpn* β . For example, when the purified 2.7-kb *Eco*RI-*Eco*RV segment of *Kpn* A is digested with *Hae* III and *Hha* I, three DNA fragments are generated. Fig. 1B shows the results when these DNAs are separated on gels, Southern blotted, and hybridized to the subcloned *Kpn* I probes; in Fig. 1A the results of these and other cross-hybridization experiments are summarized. The most striking observation is that the *Kpn* A element contains a rearrangement of sequence, with the right-hand end, as normally written [probe (1.8)11], located within the central portion of the element.

DNA Sequence. The complete nucleotide sequence of *Kpn* A, along with flanking α -satellite DNA, was determined by the procedure of Maxam and Gilbert (18). The sequence strategy and a partial restriction map are shown in Fig. 2. In Fig. 3 the sequence of the element and some flanking satellite DNA is presented.

The junctions between the *Kpn* A and the flanking DNA are of particular interest because mobile elements are usually terminated by direct or inverted repeats, and they usually generate short target-site duplications upon insertion (17). An advantage to working with elements inserted into satellite DNA is that it becomes easier to define the boundaries because the appearance of satellite DNA sequence marks the end of the element. Therefore, I compared the sequence in Fig. 3 with known monomeric α -satellite DNA sequence (13) by using the Stanford Seq program. The result was clear. The first 114 bp of sequence in Fig. 3 are homologous to bases 1-114 of the monomeric α -satellite DNA sequence determined by Potter and Jones (13). Moreover, at the end of the *Kpn* I element, this homology resumes at base 3567 of Fig. 3, with the next expected base of the α -satellite DNA. That is, it appears that the satellite DNA has been precisely interrupted by this block of *Kpn* A DNA, with homology breaking at one base in the satellite sequence and then continuing at the other boundary with the next base.

With the boundaries thus defined, I was surprised to observe the absence of any apparent target site duplications at these junctions. However, there is no visible change in the satellite DNA sequence aside from the interruption.

In performing the computer search for homology to α -satellite DNA, I unexpectedly found an internal *Kpn* A region extending from base 2123 to 2171 with 75% homology to bases 51-94 of the monomeric α -satellite DNA (13). I then searched the published 1.9-kb sequence of Manuelidis (9), which represents a portion of the *Kpn* I element deleted in *Kpn* A. Here there was yet another region of highly significant homology, including bases 1841-1877 of the Manuelidis sequence and bases 249-287 of the monomeric α -satellite DNA, with a 75.6% match. It should be emphasized that these homologies between *Kpn* I and α -satellite DNAs have *E* values of <0.01 , where *E* is the expected number of matches of this significance or greater that one would find in comparing random sequences of the same length.

Permuted Sequence. To understand better the arrangement of the sequences in *Kpn* A, I compared them to known *Kpn* I sequences. In particular I found it useful to compare the sequences of *Kpn* A to the sequence of the AGM *Kpn* I-LS1

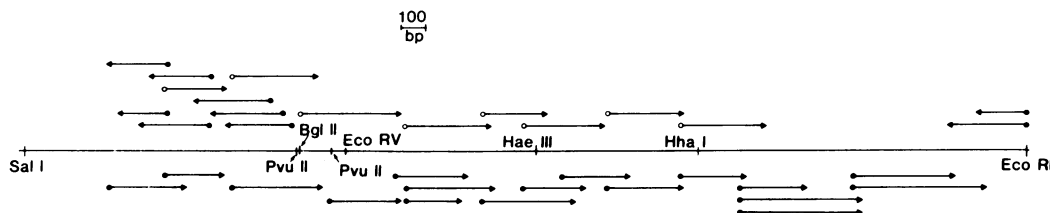


FIG. 2. *Kpn* A restriction endonuclease map and sequence strategy. Each arrow represents the length of sequence determined in one independent *Kpn* A experiment. ●, 3' end-labeled; ○, 5' end-labeled.


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3210 3220 3230 3240 3250 3260 3270 3280 3290 3300
AAGAGCCAC ATTGCCAAGT CAACCCTAAG TCAAAAAGAAC AAAGCTGGAG GCATCACGGT ACCGGACTTC AAATTATACT ACAAGGCTAC AGTAACCAAA
.....TCT G.A.G...A .T.....G. CT.....T.....T.....C.....T.....G.....
3310 3320 3330 3340 3350 3360 3370 3380 3390 3400
ACAGGATGGT ACTGGTACCA AACAGCGGAT ATAGATCCAT GGAACAGAAG AAAGCCCTCA GAAATAACGC TGCATATCTA CAATATCTGT ATCTTTGACA
...C.....A.....AT...-...C.A... .A.....C.....G.....A.....CAT.C.....A.....A.....
3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
AAACTGAGAA AAACAAGCAA GGAGGAAAGC ATTCCCTATT TAATAAATGG TGCTGGGAAA ACTGGCTAGC CATATGTAGA AAGCTGAAAC TGGAAACCTT
...C.....C.....T.G.....G.....G.....T.....TC.....A.....C.....
3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
CCATACACCT TATACAAAAA GTAATTC AAG ATTGATTAAA CACTTAAATG TTAAACCTAA AACCATAGAA ACTGCTTTT GATGTGTCCA TACAATTCAC
T.TC.....G.....G.....G.....A.....C.....G.....A.....C.....
T.....G.....G.....A.....C.....
LS1 645
AATGTTGAAC CTTTCTTTG ATTGAGCAGT TTTGAAACAG TCTTTTGTGA GAATCTGCAA GTGGGTATT GGAGCG
atagctaac ctttcttttc attgagcagt tttgaaacgc tctttttaga gaatctcaa gtggatatt ggagca

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FIG. 3. Complete base sequence of *Kpn* A, with flanking α -satellite DNA sequence. The vertical lines (at bases 114 and 3566) mark the boundaries between *Kpn* A and α -satellite DNA. The sequence in small letters represents a monomeric α -satellite DNA sequence determined by Potter and Jones (13) and used here for comparison to help determine the *Kpn* A boundaries. Bases that do not match are underlined, deletions are marked with a dash, and insertions are indicated with an inverted V. The overlined sequence within *Kpn* A, extending from base 1551 to base 2225, is the large open reading frame. Two large blocks of homology with the sequence of Lerman *et al.* designated *Kpn* I-LS1 (11) are also shown, with dots indicating where the bases are the same in the two sequences. Only two *Kpn* I restriction recognition sites are present in *Kpn* A, beginning at bases 3314 and 3258.

element determined by Lerman *et al.* (11) and to the sequence determined by Potter and Jones (13), which is essentially a human subset of the AGM *Kpn* I-LS1 sequence. The results are summarized in Fig. 4. The right-hand end of *Kpn* A was found at an internal position in both of the elements it was compared to, as expected from the Southern blot cross-hybridization data with the "consensus" *Kpn* I subclone probes. Conversely I also found that internal sequences of *Kpn* A are at the right hand termini of the other two elements. This rearrangement of the *Kpn* A sequence did not involve a simple inversion and, therefore, requires a more interesting explanation.

Lerman *et al.* (11) have compared LS1 with the apparently "normal" $\alpha 7$ *Kpn* I element by Southern blot hybridizations and some sequence analysis. They concluded that the LS1 element carries a "normal" arrangement for the sequences present, which in turn suggests that it is *Kpn* A that is rearranged and not vice versa. This is further indicated by the comparison of *Kpn* A with the human *Kpn* I 3' sequence (element *Kpn* 3') (summarized in Fig. 4), where again the same type of rearrangement is suggested for *Kpn* A.

In contrast with the permuted configuration at the right end of *Kpn* A, our sequence comparisons suggest that the left end of *Kpn* A carries the "consensus" sequence normally found at this position. Two sequences at the left ends of *Kpn* I elements (J. W. Adams, A. Cline, and A. Nienhuis, personal communication) are homologous to the left end of *Kpn* A for 233 bp, demonstrating that at least this arrangement is fairly common.

It is important to note that most of the *Kpn* A sequence as

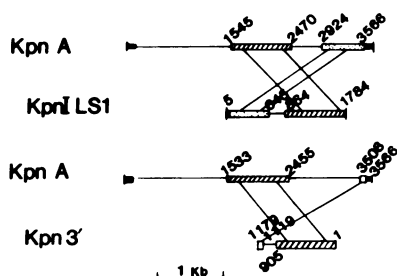


FIG. 4. Summary of sequence comparisons. The *Kpn* A (Fig. 3) sequences are compared to the *Kpn* I-LS1 sequence of Lerman *et al.* (11), and to the sequence of the *Kpn* 3' element determined by Potter and Jones (13). The numbers mark the boundaries between the blocks of homology. The short thick lines at the ends of *Kpn* A represent flanking α -satellite DNA sequence. The sequences determined for *Kpn* I-LS1 and *Kpn* 3' only include the right-hand portion of a full element. In both cases, their right-hand termini are found at an internal position in *Kpn* A. Note that the numbering of *Kpn* 3' increases from right to left because the published sequence (13) is the inverse complement of those reported for *Kpn* A and *Kpn* I-LS1.

shown in Fig. 3 represents previously unreported *Kpn* I element sequence. This includes over 1000 bases near the left end and the 453 bases (2471–2923) between the two blocks with homology to *Kpn* I-LS1 sequence.

Although some of the *Kpn* A sequences are permuted relative to the other elements, the blocks of similar sequence shown in Fig. 4 retain excellent homology (>90% for human-human and $\approx 85\%$ for AGM-human). However, the computer failed to find any regions within the *Kpn* A sequence showing extensive self-homology or symmetry. That is, there were no regions of inverted or direct repeat sequence showing 75% or greater homology.

All three registers of both strands of the sequence were searched for open reading frames, and the longest found was 675 bp beginning with base 1551 and ending with base 2225, as shown in Fig. 3. This demonstrates a real potential to encode protein.

DISCUSSION

Transposable elements are multifunctional mobile DNA sequences that have been isolated and studied in a variety of bacteria and eukaryotes (17). In recent years our progress in understanding these sequences has been rapid in those systems that can be genetically manipulated. In humans, however, a system of inherent interest, their study is difficult. In order to partially circumvent this problem, my strategy has been to isolate human transposable elements by using satellite DNAs as traps.

Satellite DNA represents a fairly homogeneous medium that continually corrects itself (and removes invading sequences) by the rectification processes of unequal crossover (15) and gene conversion. Therefore any sequences interrupting satellite DNA might well represent mobile elements that were inserted in recent evolutionary time. I and others have previously reported the isolation of satellite DNA segments carrying such nonsatellite sequences (10, 12–14).

In this report I present the complete nucleotide sequence of an invading element, *Kpn* A. Because of its small size and the lack of apparent restriction site similarity, I was somewhat surprised when sequence comparisons and Southern blot hybridizations with known *Kpn* I element subclones as probes demonstrated that I was dealing with a *Kpn* I element. A typical full-length *Kpn* I element is likely to be over 6 kb compared to the 3452 bp of *Kpn* A.

The sequence of *Kpn* A reveals several points of interest. First, the satellite DNA is cleanly interrupted, with no apparent deletion or duplication of target sequence. This contrasts with most transposable elements (17) and is unlike another case (12), where the insertion of an element designated *Kpn* I-RET generated a 14-bp target-site duplication. The absence of target-site duplication is not, however, entirely without precedent. For example, some *Alu* elements are not

flanked by them, whereas the others are flanked by duplications of variable size (24).

Second, a large block of sequence found within the consensus *Kpn* I element has been deleted from *Kpn* A. This was shown by Southern blot hybridizations in which the (1.5)₅₄ subclone probe failed to hybridize to *Kpn* A and by sequence comparisons in which there was no homology with the 1.9-kb sequence of Manuelidis (9). This extends the work of Thayer and Singer (12), where deletions within *Kpn* I elements were first demonstrated at the sequence level.

Finally, the base sequence reveals an interesting permutation. In *Kpn* A I find DNA that is normally terminal is now central and vice versa. This rearrangement is of particular interest because it is not generated by a simple inversion. That is, the direction of the sequences in *Kpn* A remains normal, and only the order of the sequences is changed.

It is interesting to note that one of the breakpoints involved in the formation of *Kpn* A is extremely close to a breakpoint found in *Kpn* I-RET. The right hand end of *Kpn* A, base 3566 in Fig. 3, is not a normal *Kpn* I family terminus and, therefore, presumably acquired this position by a DNA break. As shown in Fig. 3, this corresponds to base 645 of LS1. Thayer and Singer have shown that *Kpn* I-RET carries a deletion of LS1 sequences extending from base 651 to base 1381 (12). The close proximity of the *Kpn* A breakpoint (LS1, base 645) and the *Kpn* I-RET breakpoint (LS1, base 651) suggests that this region might be particularly active in producing altered *Kpn* I elements.

A careful comparison of *Kpn* I-LS1 and *Kpn* A sequences suggests possible mechanisms for generating some of the observed sequence differences. In the block of sequence extending from LS1 864 to 1784, corresponding to *Kpn* A 1545–2470, there are three significant insertions/deletions of 21, 31, and 17 bp. In two of these cases, I find short sequence homologies at the boundaries that could generate deletions by undergoing recombination. For example, after *Kpn* A base 1619 (LS1 931), I find LS1 has an extra 21 bp not present in *Kpn* A. At the boundaries, the sequence G-G-C-A is repeated, and a recombination between these repeats would produce the observed deletion, leaving behind one copy of the repeat as seen in *Kpn* A. The situation is similar at *Kpn* A base 1876, where 31 bp appear that are absent in LS1. At the left boundary of LS1 is the sequence T-A-G-C and at the right boundary of the deleted sequence we find T-A-C-C. Again, recombination between these imperfect repeats could generate the observed difference. Alternatively these deletions could be the result of "slipped mispairing" during DNA replication (25). In any case, the presence of such short sequence homologies flanking deletion hot spots in *E. coli* has been well-documented (26), and the sequence comparisons of *Kpn* I elements suggest that similar molecular mechanisms are at work here.

The mechanism by which *Kpn* I elements disperse to new sites is unknown, but as Lerman *et al.* (11) have pointed out, structurally they resemble the various mobile DNA sequences that are terminated by an oligo (dA) region of nucleotides. The processed genes with their introns cleanly removed and with their ends variably truncated, perhaps by incomplete reverse transcription, constitute one such category of element (27–29). The *Alu* sequences, with an internal promoter and efficient self-priming system, appear to be especially well adapted to movement in this manner (30, 31), and the F family of mobile elements in *Drosophila* provides yet another example of oligo (dA)-terminated mobile sequences (32). Because long transcripts of *Kpn* I elements have indeed been detected (8, 11, 33), this mode of transposition would seem plausible, although it remains to be demonstrated.

In conclusion, the sequence presented in this paper further describes additional polymorphisms in the *Kpn* I element structures. If the *Kpn* I elements are eventually found to be

"scrambled clusters" as described in *Drosophila* (34) and chicken (35), then this sequence data will help to define the subunits. In any event, the rearrangements present in *Kpn* A indicate a remarkable structural plasticity in this family of sequences.

Joseph Maio generously gave purified insert DNA from his *Kpn* clones. A. Nienhuis provided unpublished data. This work was supported by Grants GM30816 and CA00852 from the National Institutes of Health.

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