Restriction Fragment Length Polymorphism Caused by a Deletion Involving *Alu* Sequences within the Human α_2-Plasmin Inhibitor Gene†

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ABSTRACT: A restriction fragment length polymorphism within the human α_2-plasmin inhibitor gene has been detected by Southern blot hybridization using an α_2-plasmin inhibitor cDNA probe. This restriction fragment length polymorphism can be attributed to the presence of two alleles, *A* and *B*, that are distributed in Hardy–Weinberg equilibrium with frequencies of 73.5% and 2.65%, respectively, in 66 unrelated Caucasian individuals or with frequencies of 51.0% and 49.0%, respectively, in 50 unrelated Japanese individuals. The minor allele, *B*, is due to a deletion of about 720 base pairs in intron 8 of the α_2-plasmin inhibitor gene. Sequence analysis of the deletion junction in allele *B* and the corresponding regions of allele *A* demonstrated the presence of oppositely oriented *Alu* sequences at the 5′ and 3′ deletion boundaries. These data suggest that this restriction fragment length polymorphism was caused by intrastrand recombination between *Alu* sequences.

Restriction fragment length polymorphism (RFLP)[1] is an inherited variation in the length of a DNA fragment yielded by restriction endonuclease digestion [reviewed in Botstein et al. (1980) and Gusella (1986)]. RFLPs are very valuable for investigating various human genetic diseases and for mapping of genes to certain regions of the genome. Most RFLPs are caused by point mutations that directly affect the recognition sites of restriction endonucleases, either creating new sites or abolishing preexisting ones. Alternatively, RFLPs are produced by a variation in the number of tandemly repeated short DNA sequences that leads to restriction fragments of highly variable lengths. Besides these RFLPs, rare cases of RFLPs caused by deletions have been reported (Raines et al., 1985; Xu et al., 1985; Xu & Galibert, 1986), for which the mechanisms for the deletions are still unknown.

Alu sequences are interspersed, repetitive elements of about 300 base pairs (bp); there are 300 000–500 000 copies of them in the human genome, and they constitute 5–6% of the total genome [reviewed in Weiner et al. (1986)]. *Alu* elements consist of imperfect dimers formed from two directly repeated monomer units, which are now known to be derived from 7SL RNA (Ullu & Tschudi, 1984), a component of the signal recognition particle. Most copies of these sequences are flanked by short direct repeats of non-*Alu* sequences and contain polymerase III promoters that are active in vitro. These features led to the hypothesis that the *Alu* sequences are transposable DNA elements with transposition mediated by their RNA transcript. Recently, *Alu* repetitive sequences have also been strongly implicated as the mediator of unequal, homologous and nonhomologous exchange generating deletions or a duplication in the β-globin gene cluster (Jagadeeswaran et al., 1982; Ottolenghi & Giglioni, 1982; Vanin et al., 1983; Henthorn et al., 1986), the low-density lipoprotein (LDL) receptor gene (Lehrman et al., 1985, 1986, 1987a,b), the β-hexosaminidase α-chain gene (Myerowitz & Hogikyan, 1987), and the adenosine deaminase gene (Markert et al., 1988). *Alu* sequences have been also implicated in sex chromosome rearrangements (Rouyer et al., 1987). These properties of the *Alu* family suggest the existence of many polymorphisms caused by insertions of these elements or by recombinations involving these sequences. However, to our knowledge, only two cases of polymorphisms involving the *Alu* family have been reported so far: one was caused by insertion of a rodent *Alu*-like element (Shuler et al., 1983) and the other by point mutations within a human *Alu* sequence (Hobbs et al., 1985).

α_2-Plasmin inhibitor (α_2PI), also called α_2-antiplasmin, is a plasma glycoprotein characterized as the most important physiological inhibitor of the fibrinolytic system [reviewed in Aoki and Harpel (1984) and Aoki (1986)]. In the course of cloning the human α_2PI gene (Hirosawa et al., 1988) and elucidating the molecular basis for the hereditary deficiency of the α_2PI in a family affected with this decrease, we found an RFLP in the α_2PI gene, which can be detected with several restriction enzymes and provided information about the state of the deficiency in individual members of the pedigree studied (Miura et al., 1989). In this paper, we report the molecular basis of this RFLP and its distribution among unrelated normal individuals. The RFLP was found to be generated by a deletion of about 720 bp between two inversely oriented *Alu* sequences in intron 8 of the α_2PI gene, and nucleotide sequence analysis indicated that this deletion was caused by intrastrand recombination between the two *Alu* sequences.

Materials and Methods

Southern Blot Hybridization. Genomic DNA was prepared from the peripheral leukocytes obtained from 50 unrelated normal Japanese individuals, according to an established method (Maniatis et al., 1982). Lymphocyte DNA samples from normal Caucasian individuals were provided by the Centre d'Étude du Polymorphisme Humain in Paris. DNA samples (5 μg) were digested with restriction enzymes, and agarose gel electrophoresis, transfer to nylon membranes, hybridization, washing of filters, and autoradiography were carried out by standard procedures (Maniatis et al., 1982). A 750-bp *Eco*RI/*Hin*dIII fragment of a human α_2PI cDNA

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[1] Abbreviations: RFLP, restriction fragment length polymorphism; LDL, low-density lipoprotein; α_2PI, α_2-plasmin inhibitor; bp, base pair(s).

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Table I: χ^2 Test of the Hardy–Weinberg Law[a]

	Caucasians[b]				Japanese[c]			
genotype	*A/A*	*A/B*	*B/B*	total	*A/A*	*A/B*	*B/B*	total
observed (*O*)	35	27	4	66	17	17	16	50
expected (*E*)	35.7	25.7	4.63	66	13.0	25.0	12.0	50
$(O-E)^2/E$	0.0119	0.0647	0.0857		1.23	2.56	1.33	

[a] Allele frequencies: $A = 0.735$ and $B = 0.265$ in Caucasians; $A = 0.510$ and $B = 0.490$ in Japanese. [b] $\chi^2 = 0.162$; $P > 0.90$. [c] $\chi^2 = 5.12$; $0.05 < P < 0.10$.

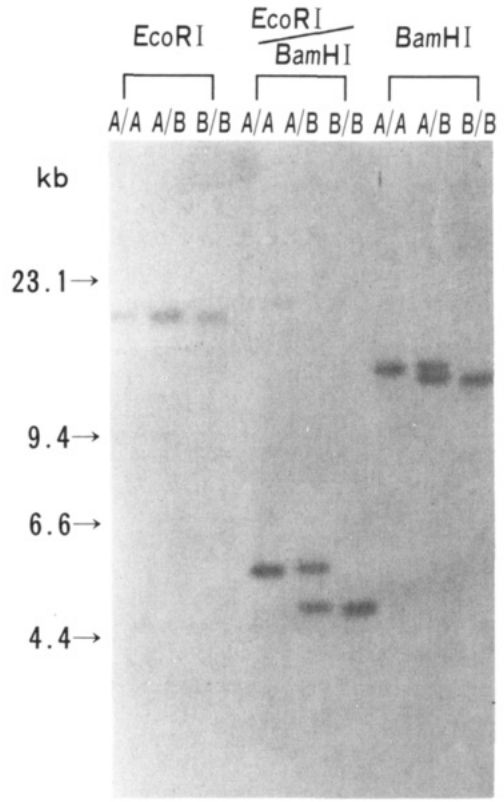

FIGURE 1: Southern blot analysis demonstrating the RFLP within the human α_2PI gene. DNA samples (5 μg) from individuals having *A/A*, *A/B*, or *B/B* alleles were digested with *Eco*RI, *Bam*HI, or *Eco*RI plus *Bam*HI; fractionated by size on a 0.7% agarose gel; transferred to a nylon membrane; and hybridized with a ^{32}P-labeled cDNA probe corresponding to exons 7–10 of the α_2PI gene. Molecular size standards were generated by the *Hin*dIII digestion of bacteriophage λ DNA.

clone, pPI39 (Sumi et al., 1986), coding for the carboxyl-terminal half of α_2PI, was labeled by use of [α-^{32}P]dCTP and hexanucleotide primers, and this was used as a probe.

Sequence Analysis. The human α_2PI genomic clone $\lambda\alpha$PN4, used for sequencing allele *A*, was isolated from a patient with a homozygous α_2PI deficiency that was caused by a frameshift mutation near the 3′ end of the coding region of α_2PI in exon 10 (Miura et al., 1989). A genomic clone, λPI2, isolated from a normal individual (Hirosawa et al., 1988) was used for sequencing allele *B*. After the restriction enzyme maps of the two clones were made and compared with each other, appropriate restriction fragments were subcloned into the plasmid pUC18 and sequenced by the dideoxy method using pUC vectors as described (Hattori & Sakaki, 1986). To obtain the appropriate DNA fragments for sequence analysis, the method of unidirectional digestion by exonuclease III was also used as previously described (Henikoff, 1984).

Results

DNA samples were analyzed for RFLP by Southern blot analysis with the cDNA probe encompassing exons 7–10 of the human α_2PI gene. Figure 1 shows the polymorphic patterns seen in the *Eco*RI, *Bam*HI, and *Eco*RI/*Bam*HI digests. This RFLP was also detected with several other restriction enzymes, including *Hin*dIII, *Sac*I, *Xba*I, and *Xho*I (data not shown). We refer to the allele giving rise to the longer fragments in these digests as allele *A* and that giving rise to the shorter fragment as allele *B*. To examine the genotype distribution and to calculate the frequencies of these two alleles, DNA samples from 66 unrelated Caucasian individuals and those from 50 unrelated Japanese individuals were analyzed for this RFLP after *Eco*RI/*Bam*HI digestion. As shown in Table I, the observed genotype distributions do not differ significantly from those expected under the assumption of Hardy–Weinberg equilibrium, and the frequencies for allele *A* and *B* were calculated to be 0.735 and 0.265, respectively, in the Caucasian individuals or 0.510 and 0.490, respectively, in the Japanese individuals.

To elucidate more precisely the molecular genetic basis of this RFLP, we analyzed previously cloned α_2PI genomic clones $\lambda\alpha$PN4 and λPI2, which represent alleles *A* and *B*, respectively, as indicated by the restriction enzyme maps of these two clones (Figure 2) and results of Southern blot analyses (data not shown). Comparison of the restriction maps of these two clones (Figure 2) suggested that allele *B* has an apparent

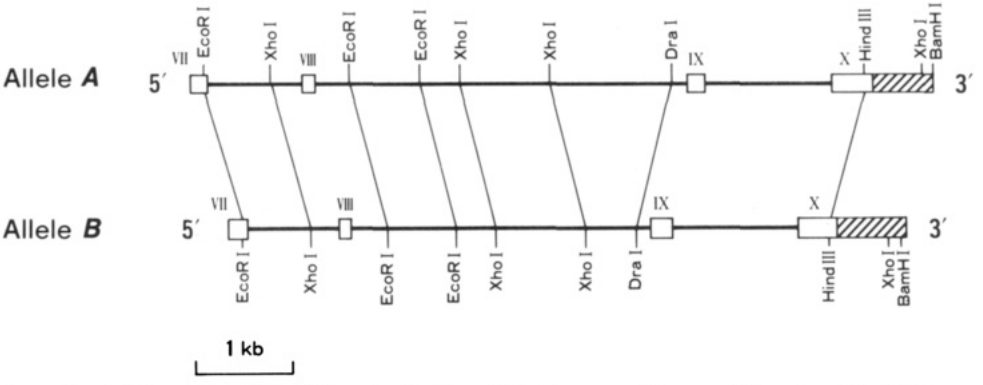

FIGURE 2: Comparison of restriction maps of the 3′ terminal halves of the human α_2PI gene alleles *A* and *B*. Coding regions of the exons are expressed by the open boxes and 3′ nontranslated regions by the hatched area. The exon numbers are indicated by Roman numerals. The map of allele *A* was generated from $\lambda\alpha$PN4 (Miura et al., 1989) and that of allele *B* from λPI2 (Hirosawa et al., 1988).

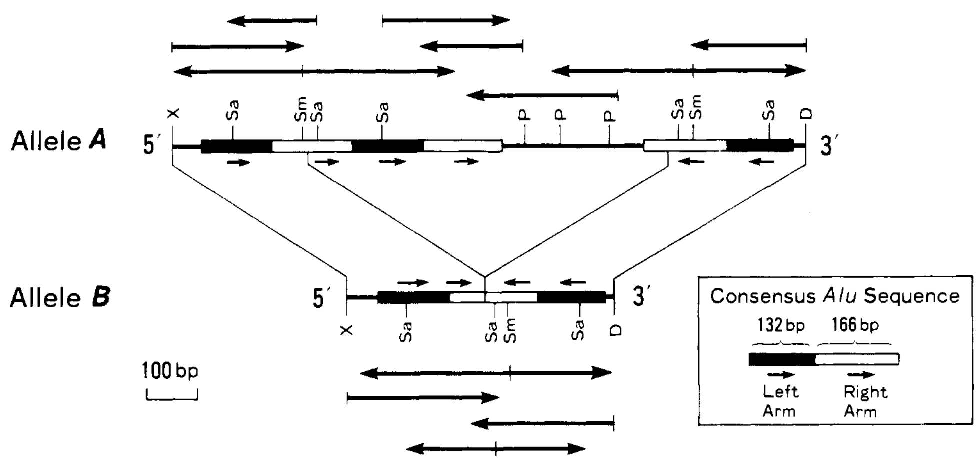

FIGURE 3: Strategy for sequencing the deletion boundaries in allele *A* and the deletion joint in allele *B* and orientation of the relevant *Alu* repetitive sequences. The upper and lower parts of the figure show the relevant restriction fragments in intron 8 of alleles *A* and *B* derived from λαPN4 and λPI2, respectively. The direction and extent of the sequence established in a given experiment are indicated by the long arrows above or below the relevant DNA sequences. The long arrow not coinciding with a restriction endonuclease site indicates the sequence obtained with the unidirectional digestion by exonuclease III. The boxed area shows an *Alu* sequence with left and right tandem repeats (open and closed, respectively), and short arrows indicate their orientations. The structure of a consensus *Alu* repeat is shown in the box. Segments of allele *A* and *B* that correspond to each other are connected by diagonal lines. Abbreviations: D, *Dra*I; P, *Pvu*II; Sa, *Sau*3AI; Sm, *Sma*I; X, *Xho*I.

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                                                                          -150
Allele A  5'  GAGCCCTGTGGTGTCCAATGCTTTTGCTGCAGAATCCCTTCAAATCAAATCTTAATGGCTGGGCACGGTGGCTCACGCCTGTAATCCCAGCACTGTGGGA
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Allele B      GAGCCCTGTGGTGTCCAATGCTTTTGCTGCAGAATCCCTTCAAATCAAATCTTAATGGCTGGGCACGGTGGCTCACGCCTGTAATCCCAGCACTGTGGGA

Allele A  3'  TGGGGCGGGCAGCACTCGGCAGTGGCTCACCCACAGAACCCAGCTGTGGTATGACAGCCAGTGACCCTGTCTGTGGTTACATCTGGAGAGAGATGAGACA

                                      -100                                             -50
Allele A  5'  GGCTGAGGCAGGTGGATCACCTGAGGTCTGGAGTTTGAGACCAGCCTGGCCCACATGGTGAAACCCCGTCTCTACTAAAAATACATCAATTGGCCAGGTG
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Allele B      GGCTGAGGCAGGTGGATCACCTGAGGTCTGGAGTTTGAGACCAGCCTGGCCCACATGGTGAAACCCCGTCTCTACTAAAAATACATCAATTGGCCAGGTG

Allele A  3'  GAGACAGGCGATTCCTTAGCGCCGGGTCACACGGCCAGCTGGTAGCCGGTGAAGAGAGAAAACCACCAGGTATTATTGAGCACCGTCTGTGACAGCGGAC

                                      +1                             +39)↓         +51
Allele A  5'  ATATGGTGAGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCACTTGAACCCGGGTGGCAGAGGTTGCAGTGAGCCAAGATCATGCCAC
              :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Allele B      AGATGGTGAGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCACTTGAACCCGGCTCAAGTGGCATGATCTTGGCTCACTGCAACCTCT
                                                                                    ::::::: ::::::::::::  :::::::::
Allele A  3'  ACTGCTACAATGTTGTTTTTTTTCTTTTTTTGAGACGGATTTTTACTCCTGTCCCCCAGGCTGGAGTGCAGTGGCTTGATCTTGGCTCATGGCAACCTCT
                                                     +101                          ↑(+75               +51

                                      +101                                               -150
Allele A  5'  GCACTCCAGCCTGGACAATAAGAGTGAAACTCTGTCTTGAAAAAAAAAAAAAAAAGAGGCTGGGTGTGGTTTACAGGCTCATGCTTAAATCCCAGCACTTTG

Allele B      GCCTCCCGGGTCAAGCAATTCTCCTGCCTCAGCCTCCCGAGTGCTGCGATTGACAAGCTCCCGTCACCACAGAAGCCCAGCTCATTTTTGTATTTTTAGT
              :::::::::::::::::::::::::::::::::::::::::::::::::::::::::: :::::::::::::::::::::::::::::::::::::::::
Allele A  3'  GCCTCCCGGGTCAAGCAATTCTCCTGCCTCAGCCTCCCGAGTGCTGCGATTGACAAGCGCCCGTCACCACAGAAGCCCAGCTCATTTTTGTATTTTTAGT
                                                         +1                                                 -50
                                       -100                                               -50
Allele A  5'  GGAGGCCAAGGCAGGTGGATCACGAGGTCAGGAGATTGAGACCATCCTGGCTAACNCGGTGAAACCCATCTCTTCTAAAAATACAAAAAATTAGCCGGGC

Allele B      AGAGATGAGGTTTTGCCATGTTGGTCAGGCTGGTCTTGAACTCCTGACCTCA-GTGATCTGCCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAGCGTGG
              ::::::::::::::::::::::::::::: :::::::::::::::::::::: :::::::::::::::::::::::::::::::::::::::::::::::
Allele A  3'  AGAGATGAGGTTTTGCCATGTTGGTCAGGGTGGTCTTGAACTCCTGACCTCAGGTGATCTGCCTGCCTCGGCCTCCCAAAGTGCTAGGATTACAGCGTGG
                                                          -100                                              -150
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FIGURE 4: Nucleotide sequences across the deletion junction of allele *B* and corresponding 5′ and 3′ regions of allele *A*. The sequence of allele *B* across the deletion junction (allele *B*) is aligned with the corresponding sequences of allele *A* surrounding the 5′ breakpoint (allele *A* 5′) and 3′ breakpoint (allele *A* 3′). Colons denote matches in the sequence, and a dash between nucleotides indicates a gap. Thick and thin horizontal long arrows indicate the portions homologous to the left and the right arms of the consensus *Alu* sequence, respectively. The *Alu* sequences are numbered according to the scheme used for the consensus *Alu* sequence (Deininger et al., 1981). The flanking repeat sequences (9 bp) of the first two and the third *Alu* repeats are underlined. The thick vertical arrows denote the positions of the probable deletion joints.

deletion of about 700 bp between an *Xho*I site and a *Dra*I site in intron 8. Thus the *Xho*I/*Dra*I fragments of the two clones were subcloned into pUC18 and subjected to sequence analysis.

The sequence data revealed that the *Xho*I/*Dra*I fragment of allele *A* contains three copies of *Alu* repetitive sequences, the positions of which are illustrated in Figure 3. The first two

Alu repeats are present in a head-to-tail orientation located 60 nucleotides downstream from the *Xho*I site. These two repeats, with no DNA in between, have 9-bp short flanking repeats on their 5′ and 3′ ends, as shown in Figure 4. Located 277 nucleotides downstream from these *Alu* sequences, the third *Alu* repeat is present in an inverted orientation with its own 9-bp flanking repeats. The sequence data also showed that about a 720-bp sequence in the middle of this *Xho*I/*Dra*I fragment of allele *A* is deleted in allele *B*, as shown in Figure 3. The nucleotide sequence of about 500 bases surrounding the deletion joint in allele *B* and the corresponding sequences in allele *A* are shown in Figure 4, with the *Alu* sequences numbered according to the scheme used for the consensus *Alu* sequence (Deininger et al., 1981). These sequences indicate that the 5′ breakpoint in allele *B* is located at nucleotide consensus position 39 in the right arm of the most upstream *Alu* sequence and that this has been joined to consensus position 75 in the right arm of the most downstream *Alu* sequence, which is inversely oriented. Four additional nucleotides have been inserted between these breakpoints.

Discussion

In this paper, we have reported an RFLP in the human α_2PI gene, which corresponds to a deletion of about 720 bp in intron 8. As this RFLP was observed among both Caucasians and Japanese, the deletion which caused this RFLP should have occurred before the divergence of human races. The Mendelian inheritance of the two alleles was ascertained in two families with congenital α_2PI deficiency, in which this RFLP provided information about the state of the deficiency in individual family members (Miura et al., 1989). In addition to evaluating individuals with these and other pedigrees of congenital α_2PI deficiency, this RFLP should be valuable for mapping genes or detecting allelic deletions on chromosome 18 in neoplasms such as colorectal tumors, in which a specific region of chromosome 18 is frequently deleted (Vogelstein et al., 1988), since the location of the α_2PI gene has already been assigned to 18p11.1-q11.2 by in situ chromosomal hybridization (Kato et al., 1988).

The sequence data revealed that the shorter allele, allele *B*, arises from a deletion of about 720 bp between the right arms of two *Alu* sequences that are oriented in opposite directions. Two similar deletions have been reported by Lehrman et al. in the LDL receptor genes of patients with familial hypercholesterolemia: one of the deletions was between two inversely oriented *Alu* sequences (Lehrman et al., 1985), and the other was between an *Alu* sequence and a non-*Alu* sequence showing significant complementarity to an inversely oriented *Alu* sequence (Lehrman et al., 1986). They have hypothesized formation of complex double stem–loop structures as the mechanism for these deletions and also for a similar deletion in the β-globin gene cluster in a patient with δ,β-thalassemia that had been previously reported (Jagadeeswaran et al., 1982). As shown in Figure 5, a similar stem–loop structure can also be proposed for the deletion generating the RFLP in the α_2PI gene. The first long inverted repeat, which involves the two inversely oriented *Alu* sequences, consists of a 170/208 nucleotide match on the whole with a 34/38 nucleotide match in the portion proximal to the deletion junctions. The second inverted repeat, a 8/12 nucleotide match, involves the right arm of the most 3′ *Alu* sequence, whose direction is opposite to those of the other two *Alu* sequences. Formation of this double stem–loop structure in allele *A* would bring the DNA at the points of the deletion into close proximity, thus making it possible to nick and religate the DNA at this point to produce the observed 720-bp deletion. However, this mechanism

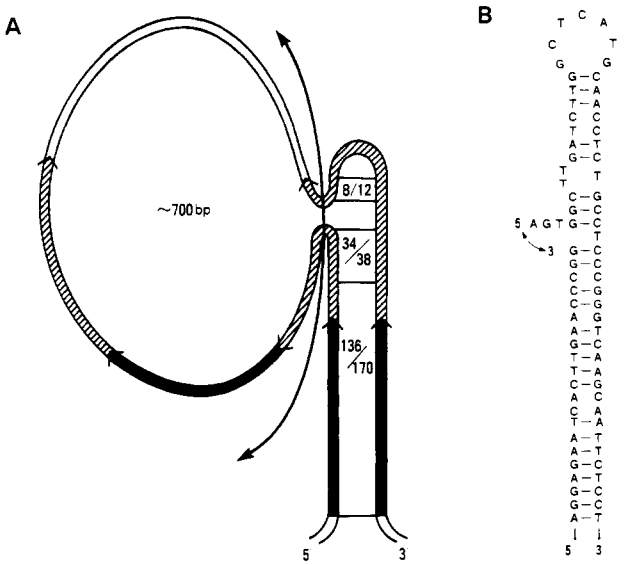

FIGURE 5: Potential mechanism for the deletion generating the RFLP in the α_2PI gene. In panel A, a potential double stem–loop structure of the *Xho*I/*Dra*I region in intron 8 of allele *A* is shown with the left and the right arms of the *Alu* sequences as closed and striped arrows. The lengths of the DNA sequences are not precisely drawn to scale. The positions of inverted repeats are shown by boxed areas, and the fractions inside the boxes denote the number of paired nucleotides divided by the number of paired plus unpaired nucleotides. The size of the deleted segment is indicated in the center of the loop, and the position of the deletion is denoted by a double arrow. In panel B, potential homologous base pairings near the probable deletion points are shown.

would not explain the insertion of four new nucleotides at the deletion joint observed in this case or the duplication of the 11-bp sequence near the deletion joint in the LDL receptor gene reported by Lehrman et al. (1986). Thus, the repair process should be more complex in these cases than in the first reported case of the deletion in the LDL receptor gene (Lehrman et al., 1985), in which it was supposed that repair enzymes filled a single nucleotide gap between the deletion junctions.

Besides these inversely oriented *Alu* sequences, *Alu* sequences that are oriented in the same direction (Lehrman et al., 1987a,b; Horsthemke et al., 1987; Myerowitz & Hogikyan, 1987; Markert et al., 1988) or present singly (Jagadeeswaran et al., 1982; Ottolenghi & Giglioni, 1982; Vanin et al., 1983; Henthorn et al., 1986) have als been implicated to mediate recombinations to cause deletions or a duplication in various genes. Lehrman et al. (1987a,b) have indicated that the majority of these recombination events have involved the left half of the *Alu* repeat sequence, particularly the region between the A and B sequences that are homologous to the two halves of the RNA polymerase III split promoter. In this respect, the deletion reported in this paper is rather exceptional, as both of the deletion points have involved the right arms of *Alu* sequences. However, it is worthy to note that one of the deletion points in this case (position +75) coincides with the breakpoint (between positions +71 and +75) of one of the only two deletions that have been reported to involve the right arms of *Alu* sequences (Henthorn et al., 1986); thus, this region might be also prone to recombination, although proof of this supposition requires further examples of similar recombinations.

One of the *Alu* sequences involved in the deletion in the present case was found to be arranged in a head-to-tail orientation with another *Alu* sequence without any DNA in between. Another example of such *Alu* sequences in a direct tandem repeat has been reported in the human prothrombin

gene (Degen et al., 1983). We do not know if this exceptional arrangement of the *Alu* sequences has any significance in the recombination event. However, in this respect, it is of interest to note that some *Alu* sequences may be intrinsically unstable, as Lehrman et al. (1987a) have suggested to explain many mutations caused by recombinations involving the *Alu* sequences in the LDL receptor gene.

Similar RFLPs generated by deletions have been reported in the chicken c-erbB gene (Raines et al., 1985), the human c-*abl* gene (Xu et al., 1985), and the human c-*fms* gene (Xu & Galibert, 1986). Xu and Galibert (1986) have hence suggested that this type of RFLP might affect cellular oncogenes more frequently than other genes. However, in these RFLPs of cellular oncogenes, the precise boundaries and the mechanisms of the deletions have not been well defined. It will therefore be of interest to determine whether the similar mechanism, the recombination between repetitive sequences such as *Alu* sequences, has caused these or any other RFLPs.

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REFERENCES

Aoki, N. (1986) *J. Protein Chem. 5*, 269–277.

Aoki, N., & Harpel, P. C. (1984) *Semin. Thromb. Hemostasis 10*, 42–50.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980) *Am. J. Hum. Genet. 32*, 314–331.

Degen, S. J. F., MacGillivray, R. T. A., & Davie, E. W. (1983) *Biochemistry 22*, 2087–2097.

Deininger, P. L., Jolly, D. J., Rubin, C. M., Friedman, T., & Schmid, C. W. (1981) *J. Mol. Biol. 151*, 17–33.

Gusella, J. F. (1986) *Annu. Rev. Biochem. 55*, 831–854.

Hattori, M., & Sakaki, Y. (1986) *Anal. Biochem. 152*, 232–238.

Henikoff, S. (1984) *Gene 28*, 351–359.

Henthorn, P. S., Mager, D. L., Huisman, T. H. J., & Smithies, O. (1986) *Proc. Natl. Sci. U.S.A. 83*, 5194–5198.

Hirosawa, S., Nakamura, Y., Miura, O., Sumi, Y., & Aoki, N. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6836–6840.

Hobbs, H. H., Lehrman, M. A., Yamamoto, T., & Russell, D. W. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7651–7655.

Horsthemke, B., Beisiegel, U., Dunning, A., Havinga, J. R., & Williamson, R. (1987) *Eur. J. Biochem. 164*, 77–81.

Jagadeeswaran, P., Tuan, D., Forget, B. G., & Weissman, S. M. (1982) *Nature 296*, 469–470.

Kato, A., Nakamura, Y., Miura, O., Hirosawa, S., Sumi, Y., & Aoki, N. (1988) *Cytogenet. Cell Genet. 47*, 209–211.

Lehrman, M. A., Schneider, W. J., Südhof, T. C., Brown, M. S., Goldstein, J. L., & Russell, D. W. (1985) *Science 227*, 140–146.

Lehrman, M. A., Russell, D. W., Goldstein, J. L., & Brown, M. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3679–3683.

Lehrman, M. A., Russell, D. W., Goldstein, J. L., & Brown, M. S. (1987a) *J. Biol. Chem. 262*, 3354–3361.

Lehrman, M. A., Goldstein, J. L., Russell, D. W., & Brown, M. S. (1987b) *Cell 48*, 827–835.

Maniatis, T., Fritsch, E. J., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Markert, M. L., Hutton, J. J., Wiginton, D. A., States, J. C., & Kaufman, R. E. (1988) *J. Clin. Invest. 81*, 1323–1327.

Miura, O., Hirosawa, S., Kato, A., & Aoki, N. (1989) *J. Clin. Invest.* (in press).

Myerowitz, R., & Hogikyan, D. (1987) *J. Biol. Chem. 232*, 15396–15399.

Ottolenghi, S., & Giglioni, B. (1982) *Nature 300*, 770–771.

Raines, M. A., Lewis, W. G., Crittenden, L. B., & Kung, H. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2287–2291.

Rouyer, F., Simmler, M.-C., Page, D. C., & Weissenbach, J. (1987) *Cell 51*, 417–425.

Schuler, L. A., Weber, J. L., & Gorski, J. (1983) *Nature 305*, 159–160.

Sumi, Y., Nakamura, Y., Aoki, N., Sakai, M., & Muramatsu, M. (1986) *J. Biochem. 100*, 1399–1402.

Ullu, E., & Tschudi, C. (1984) *Nature 312*, 171–172.

Vanin, E. F., Henthorn, P. S., Kioussis, D., Grosveld, F., & Smithies, O. (1983) *Cell 35*, 701–709.

Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., & Bos, J. L. (1988) *N. Engl. J. Med. 319*, 525–532.

Weiner, A. M., Deininger, L. P., & Efstratiadis, A. (1986) *Annu. Rev. Biochem. 55*, 631–661.

Xu, D. Q., & Galibert, F. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3447–3450.

Xu, D. Q., Guilhot, S., & Galibert, F. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2862–2865.