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A Recombination Hot Spot in the Rh Genes Revealed by Analysis of Unrelated Donors with the Rare D−− Phenotype

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Summary

We have studied the arrangement of Rh (rhesus) genes in donors who are completely null for the products of one of them, RHCE. We show that five of six homozygous individuals with the so-called Rh D−− phenotype, who express no red-cell antigens of the C/c and E/e series, have rearranged RHCE genes in which internal sequences have been replaced by the corresponding sequences from RHD. Moreover, although there is heterogeneity at the 3′ end, the 5′ boundary of this chimerism is within the same small interval around exon 2. This interval is characterized by an exceptionally high degree of sequence homology between RHCE and RHD, a high density of dispersed repetitive elements, and the presence of an alternating purine-pyrimidine copolymer tract. We suggest that these features may explain the mechanistic basis for the origin of the rearrangement.

Introduction

The Rh (rhesus) red-cell antigens are carried on cell-surface glycoproteins encoded by two genes, RHD and RHCE. The large number of different Rh serological types are all thought to derive from sequence variation in these two very similar genes. Clinically, the most significant polymorphism that affects the Rh system is the presence (Rh+) or absence (Rh−) on red cells of Rh D antigen, since D-status incompatibility between transfusion donor and recipient, or between mother and fetus, can cause severe hemolytic reactions. Rh D status is usually determined by the presence or absence of the entire RHD gene (Colin et al. 1991; Arce et al. 1993). Sequence variations in the RHCE gene give rise to amino acid changes in extracellular domains, which result in the expression of the different Rh C-like and Rh E-like antigens. The common C-like antigens result from a serine (C antigen)-to-proline (c antigen) substitution at position 103. These are accompanied by a proline-to-alanine substitution at position 226, which determines E and e antigens, respectively (Mouro et al. 1993; Simsek et al. 1994) (fig. 1*A*). It is believed, but not completely proved, that alternative splicing of RHCE transcripts gives rise to C-like and E-like antigens on two distinct polypeptides (Le Van Kim et al. 1992).

There are rare individuals whose red cells totally fail to react with antisera that define one or more of the Rh antigens. These phenotypes include absence of reactivity with all Rh antisera (referred to as the "Rh null phenotype"), lack of both C-like and E-like reactivity in the presence of D antigen (referred to as "D−−"), or absence of E-like reactivity only (referred to as "Dc−, DCW−"). (Although alternative nomenclatures exist, we use one in which positive reactions with the different antisera are indicated in the order DCE, e.g., DCe refers to an Rh+ donor whose red cells react only with anti-C and anti-e antisera.) We previously published details of a single Icelandic case with the D−− phenotype in which ≥85% of the RHCE gene was absent (Blunt et al. 1994*b*). This was in contrast to the one other published D−− case (Colin et al. 1991; Cherif-Zahar et al. 1994*b*) and to two unrelated cases of the Rh null phenotype (Carritt et al. 1993; Cherif-Zahar et al. 1993), in which the unexpressed RHCE genes were grossly intact. In a recent study, Huang et al. (1995) confirmed that donors with the D−− phenotype sometimes lack part of the RHCE gene. We have now examined five additional unrelated Rh D−− homozygotes and have found that, in four of them, RHCE sequences have been replaced by RHD sequences. The 5′ end of these rearrangements all occur within a 4.2-kb interval around exon 2. There is, however, heterogeneity at the 3′ end of the rearranged genes, indicating that they are not identical by descent, rather that independent recombination events have occurred within a small genomic interval.

Material and Methods

Case Material

Blood-group serology was performed in the MRC Blood Group Unit by using standard techniques and

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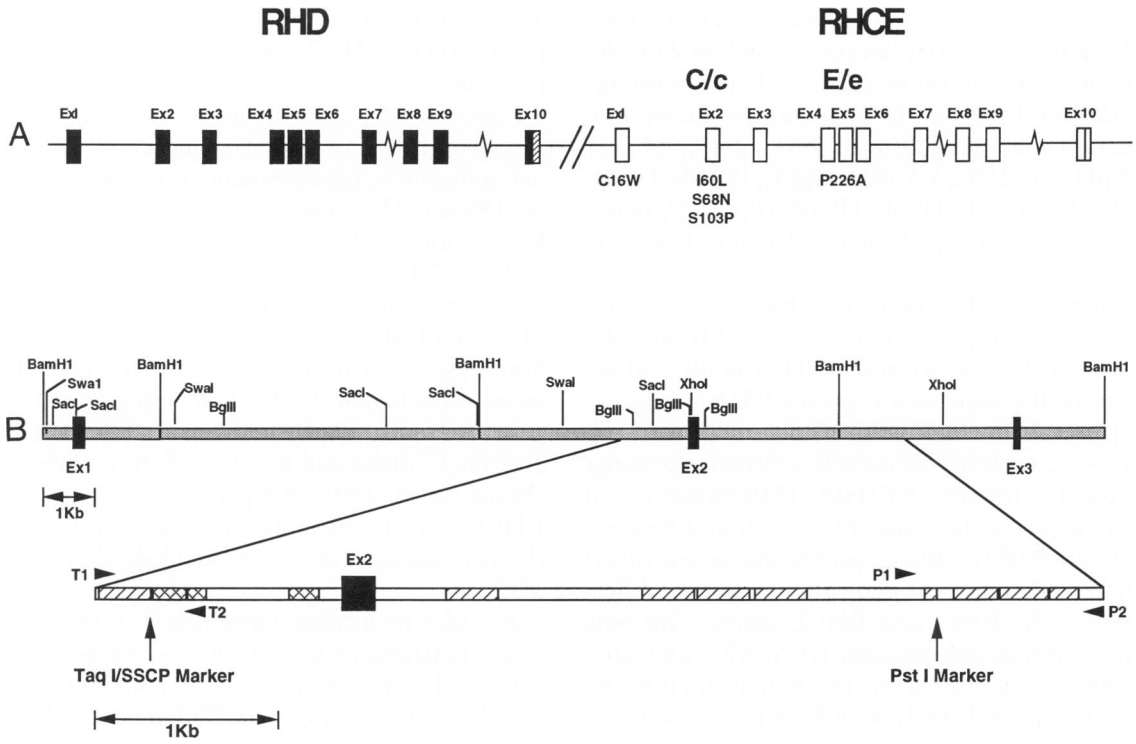

Figure 1 *A,* Diagrammatic representation of RH genes. The arrangement in Rh+ donors is represented. Rh− donors usually lack the entire RHD gene. The sizes of exons are not to scale, and those of the introns only approximately so. The results of our studies of RH gene organization differ slightly from those of Cherif-Zahar et al. (1994*a*), most notably in the apparent size of intron 1 (12 kb vs. 3 kb). The sizes of introns 7 and 9 are not certain but are known to be large. The RHCE gene is orientated 5′−3′, left to right. RHD is classically considered to lie 5′ of RHCE, although experimental support is lacking; the 5′−3′ orientation of RHD with respect to RHCE is not known, nor is the distance between them. We have isolated a 450-kb YAC clone containing both genes (described briefly by Blunt et al. 1994*a*), which indicates they are no further apart than this. The sites of the codon substitutions in exons 2 and 5 that underly C/c and E/e specificity, respectively, are shown. *B,* RHCE gene map spanning exons 1–3. Organization of the RHCE gene was established from analysis of an RHCE cosmid (Ce allele) and plasmid subclones. Exons are represented by black boxes and restriction sites by vertical lines. The expanded region (bottom) represents the 5.5 kb of sequence obtained from subclones of adjacent *Bam*HI fragments which include intron 2. The *Taq*I/SSCP and *Pst*I markers that flank its 4.2-kb region of homology and the primer pairs T1/T2 and P1/P2 are indicated. Alu and mammalian-wide interspersed repeat (MIR) sequences are illustrated by hatched and crosshatched boxes, respectively.

reagents. All the Rh D−− donors had the serological phenotype C−c−D+E−e−, and all were from pedigrees with one or more consanguineous loops. The Icelandic donor SH (Olafsdottir et al. 1983) has been studied before (Blunt et al. 1994*b*), and some of the others have been described at the serological level (JC, Buchanan 1956; HD, Contreras et al. 1979; siblings EmR, ElR, and CR, Mora et al. 1990). LM, one of two unpublished homozygous D−− siblings provided by Dr. G. Reali, Genoa, Italy, and an unpublished U.K. donor (GL) were also included in the survey. One donor (HD) expressed the low-frequency red-cell antigen Evans (Contreras et al. 1979). Little is known about this phenotype, beyond that it is encoded at the RH locus (Race and Sanger 1975).

Analytical Techniques

Locus-specific assays in exon 1, intron 2, exon 4, intron 4, exon 5, exons 6–8, and exon 10 were performed using either Southern blot analysis or PCR as described elsewhere (Blunt et al. 1994*a,* 1994*b;* Arce et al. 1993). One of the intron 2 assays was a PCR-adapted modification (Poulter et al. 1996) of the *Pst*I RFLP published earlier (Carritt et al. 1993). In the present report, we refer to the sense primer as "P1" and the antisense as "P2" (fig. 1*B*). In addition, we used two unpublished PCR assays. First, an RHCE-specific exon 10 PCR assay using the RHCE-specific antisense primer 5′-CCTTGTTTCATTATACATAAGG-3′ duplexed with the RHD-specific assay described by Lo et al. (1993); both antisense primers use the same sense primer at an annealing temperature of 51°C. Second, an *Msp*I restriction-site variation was discovered in a PCR product generated using an antisense primer (5′-GTGAAAAATCTTACCTTCCAGA-3′) based on exon 9/intron 8 splice-acceptor boundary sequence and a sense primer (5′-ATATGTCTTACATGGATCAGCT-3′) lying within intron 8, annealing at 60°C; this variation consistently distinguishes

RHD from RHCE. Unless otherwise stated, we used standard conditions for amplification based on 95°C denaturing and 72°C extension, each for 1 min, with annealing, also for 1 min, at the temperatures shown in a 50-μl reaction containing 20 mM $(NH_4)\ 2SO_4$, 75 mM Tris-HCl pH 9 at 25°C, 1.5 mM $MgCl_2$, 0.01% Tween (w/v), 0.2 mM each dATP, dCTP, dGTP, dTTP, oligonucleotide primers at 2 μM each and 1 unit *Taq* polymerase.

The genomic *Bam*HI fragments that contain either exon 2 or exon 3 (fig. 1*B*), were subcloned from independent RHCE (Ce allele) and RHD cosmids and sequenced, using the Sequenase version 2.0 DNA sequencing kit (Amersham International) as recommended. Sequences were analyzed for repetitive elements by using the computer program PYTHIA (Milosavljevic, in press). Any sequence difference that was found between the RHCE and RHD cosmids could be due to individual variation or could represent a consistent sequence difference between the RHD and RHCE genes. This was tested on a panel of serologically typed Rh+ and Rh− donors, either by exploiting any resulting restriction-site differences or by SSCP analysis of PCR products. Using the sense primer T1 (5′-GTTTAAATCTTGGCTGTAGGC-3′) and the antisense primer T2 (5′-CAGCTTGAGCTCCAGAACG-3′) (fig. 1*B*), annealing at 58°C, we analyzed a consistent sequence difference 5′ of exon 2, by both *Taq*I digestion and by SSCP. SSCP was performed on PCR products that had been heat denatured in 50% formamide-10 mM EDTA and run on 6% nondenaturing polyacrylamide gels. Long-range PCR was carried out using the XL PCR Kit (Perkin-Elmer) and the T1 sense primer, together with the P2 antisense primer described above. The long-range reaction used 30 s annealing, reducing the temperature from 70°C to 60°C by 1°C per cycle for 11 cycles, followed by a further 20 cycles at 60°C and constant temperatures of 95°C for 30 s and 72°C for 5 min for denaturing and extension, respectively.

Results

The Rh D−− donors used in the present study were collected from Italy, Iceland, Canada, England, and Wales. All failed to react with batteries of anti-C, anti-c, anti-E, and anti-e antisera, and all were positive with anti-D and, where tested, were negative with anti-f (anti-ce). All donors tested were positive with saline suspensions of incomplete anti-D, indicating unusually strong D-reactivity. They were unrelated as far as available pedigrees indicate (3–10 generations) and were all from consanguineous families.

Although the RHCE and RHD genes are 96% homologous over their coding regions (Le Van Kim et al. 1992), a limited number of Southern blot and PCR assays distinguish them (table 1), and we used them to investigate RHCE gene structure in the homozygous D−− donors. These assays had previously shown that the genome of a consanguineous Icelandic D−− homozygote (SH) had no RHCE sequences 3′ of a point that lies somewhere between exon 1 and intron 2 (Blunt et al. 1994*b*). The same assays revealed that four of the five additional D−− genes (those in GL, HD, and LM and sibs ElR, EmR, and CR) were indistinguishable at the 5′ end from each other and from the Icelandic case, SH, described earlier (table 1). The exceptional D−− homozygote, of Iroquois descent (JC) and also highly inbred (Buchanan 1956), had a grossly intact RHCE gene. A *Hin*PI (*Hha*I) site is invariably present in exon 1 of the C alleles and in <6% of the c alleles of RHCE (Mouro et al. 1993; Wolper et al. 1994). The residual RHCE exon 1 was in all cases cut by *Hha*I, suggesting the rearranged gene was most likely derived from a C allele.

In order to localize more precisely the 5′ boundary of the rearrangement, 5.5 kb of genomic sequence was generated by primer walking on both RHD and RHCE (Ce allele) cosmids (fig. 1*B*). Within this 5.5 kb, sequence identity was maintained over a 4.2-kb region running from ~1,000 bp 5′ of exon 2 to 3 kb 3′ of exon 2. The 5′ boundary ("*Taq*I/SSCP Marker" in fig. 1*B*) of this region of homology was defined by one of two *Taq*I restriction sites within RHD that were shown to be consistently absent from RHCE. PCR amplification using primers T1 and T2 (fig. 1*B*), which flank both these sites, followed by *Taq*I digestion, revealed the presence of both RHCE and RHD sequences in all D−− donors (fig. 2*A*). This finding was confirmed by SSCP analysis (data not shown). Approximately 3 kb 3′ of exon 2, all C alleles of the RHCE gene contain a 108-bp insertion. The insertion is related to the C/c-associated *Pst*I RFLP described elsewhere (Carritt et al. 1993; Poulter et al. 1996) and distinguishes the C/c alleles from RHD; the PCR products generated using P1 and P2 (fig. 1*B*) are all cut at least twice by *Pst*I, releasing fragments of 322 and 107 bp and a residual fragment that was 748 bp from C alleles and 640 bp from c and from D. The 640-bp c-associated product is reduced further to 390 bp and 250 bp, distinguishing it from RHD. This assay revealed the absence of RHCE sequences in all D−− donors (fig. 2*B*) and defines the 3′ boundary ("*Pst*I Marker") of the 4.2-kb region of homology. In five of six D−− genes, therefore, an RHCE rearrangement occurs in the 4.2 kb that separates these two boundary markers. Alu elements immediately flanking these markers (fig. 1*B*) contained several nucleotide differences, reducing the sequence homology between the Ce allele and RHD to 96%.

Although there is no known relationship between these D−− pedigrees, the similarity of their rearranged

Table 1

Locus-Specific Assays For RH Genes

Region	Assay	Reference	Presence/Absence of RHCE					
			CR	SH	GL	LM	HD	JC
Exon 1	*Hin*dIII Southern (exon 1 probe)	Colin et al. (1991)	+	+	+	+	+	ND
Exon 1	PCR product (*Hha*I digest)[a]	Mouro et al. (1993)	+	+	+	+	+	+
Intron 2	PCR product (*Pst*I digest)	Poulter et al. (1996)	−	−	−	−	−	+
Exon 4	*Bam*HI Southern (cDNA probe)	Blunt et al. (1991*a*)	−	−	−	−	−	ND
Intron 4	PCR product (insertion)	Arce et al. (1993)	−	−	−	−	−	+
Exon 5	PCR product (*Hin*cII digest)	Blunt et al. (1994*b*)	−	−	−	−	−	+
Exon 6	*Bam*HI Southern (cDNA probe)	Blunt et al. (1994*a*)	−	−	−	−	−	ND
Exons 7–8	*Bam*HI Southern (cDNA+Int7 probes)	Blunt et al. (1994*a*)	−	−	−	−	−	ND
Intron 8–Exon 9	PCR product (*Msp*I digest)	Authors' unpublished data	−	−	−	+	+	+
Exon 10	PCR product (locus specific)	Authors' unpublished data	+	+	+	+	+	+

NOTE.—A plus sign (+) indicates the presence of the RHCE gene by the specified assay; a minus sign (−) indicates its absence. ND signifies no data.

[a] Although our data indicate a complete concordance between *Hha*I cleavage and Rh C antigen expression in Caucasian donors, as described by Mouro et al. (1993), the same nucleotide polymorphism in exon 1 generates a reciprocal pattern of cleavage with *Hae*III. In 102 samples, Wolper et al. (1994) found 6 exceptions to the expected association between Rh c expression and *Hae*III cleavage.

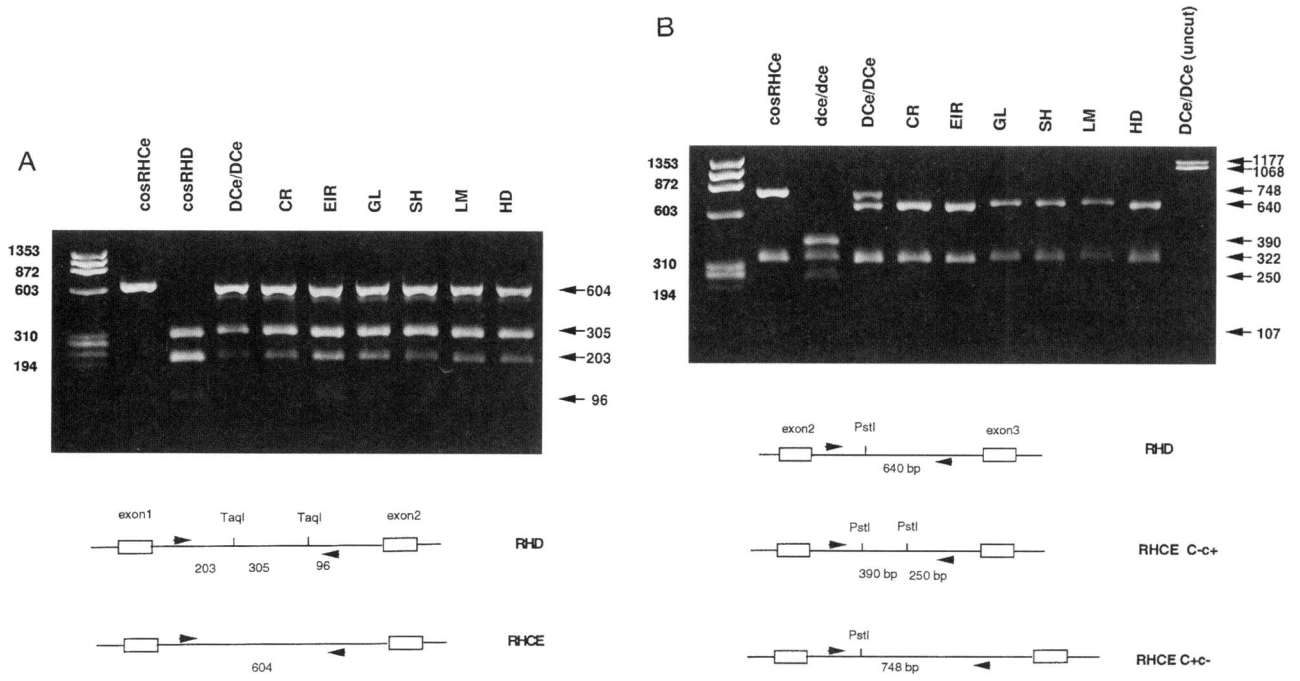

Figure 2 Analysis of the 5′ end of the rearranged RHCE genes in D−− donors. *A*, *TaqI*/SSCP marker lying 5′ to the 4.2-kb region of homology (fig. 1*B*), analyzed by PCR using primers T1 and T2 followed by *TaqI* digestion. The 604-bp product from the RHD gene is cut twice, releasing fragments of 305, 203, and 96 bp. Lane 1, ΦX174 RF/*Hae*III fragment ladder. Lanes 2 and 3, RHCE (Ce allele) and RHD cosmids, respectively. Lane 4, genotyped genomic control illustrating coamplification of both RHCE and RHD genes. Lanes 5–10, D−− donors. The schematic illustrates the principle of the assay. *B*, Marker defining the 3′ end of the 4.2-kb region of homology (fig. 1*B*), a C/c-associated PCR product size difference (1,177 bp vs. 1,068 bp) and a *PstI* RFLP which distinguishes c from D (Poulter et al. 1996). All PCR products generated using primers P1 and P2 are cut at least twice by *PstI*; following digestion, the C-associated products are 748, 322, and 107 bp, in contrast to those associated with D, which are 640, 322, and 107 bp. The c-associated 640-bp *PstI* cleavage product is reduced further to 390 and 250 bp by an additional *PstI* site. Lane 1, ΦX174 RF/*Hae*III fragment ladder. Lane 2, RHCE (Ce allele) cosmid. Lanes 3 and 4, genotyped genomic controls. Lanes 5–10, D−− donors. Lane 11, uncut genotyped control, illustrating the variable product size as described. The principle of the assay is illustrated in the accompanying schematic.

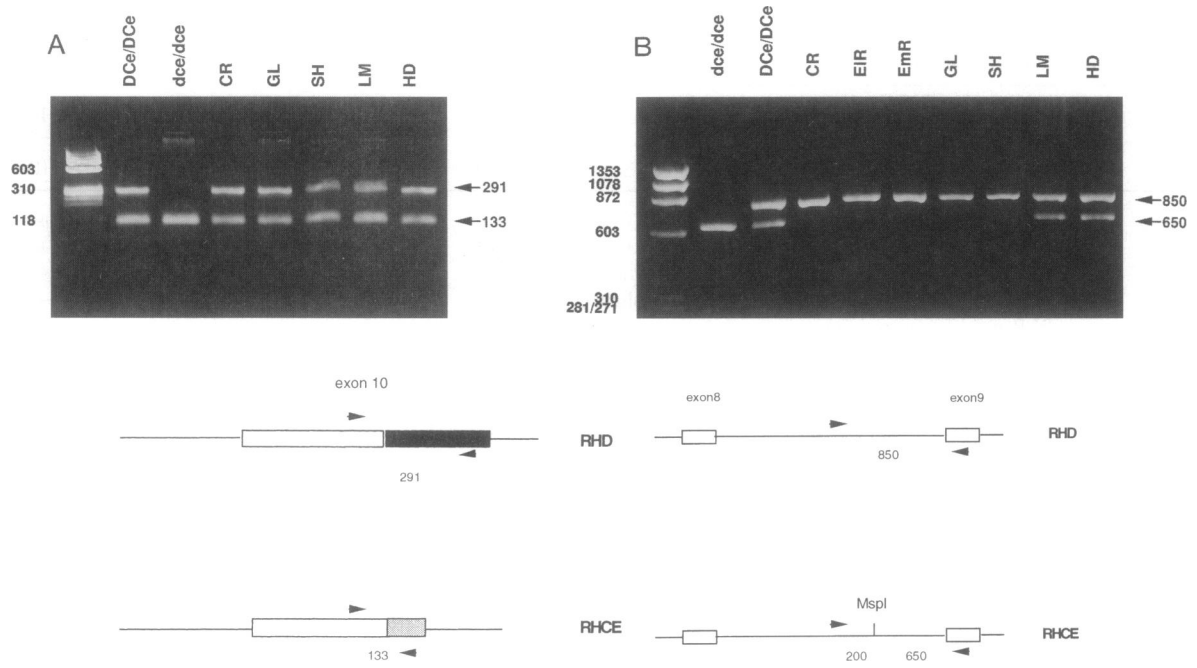

Figure 3 Analysis of the 3′ and of the rearranged RHCE genes in D−− donors. *A*, Exon 10 of the RH genes, analyzed by an RHCE-specific PCR assay duplexed with one that was RHD-specific (Lo et al. 1993). RHCE and RHD templates generated 133 and 291 bp products, respectively. Lane 1, ΦX174 RF/*Hae*III fragment ladder. Lanes 2 and 3, genotyped genomic controls. Lanes 4–8, D−− donors. *B*, Exon 9 sequences in D−− donors, analyzed by generating a PCR product using intron 8 and exon 9 splice acceptor boundary primers. This 850-bp product from the RHCE gene is cut by *Msp*I, releasing fragments of 650 and 200 bp, although the smaller product has been run off the gel in the experiment shown here. Lane 1, ΦX174 RF/*Hae*III fragment ladder. Lanes 2 and 3, genotyped controls. Lanes 4–10, D−− donors. The two assays are illustrated below the figure.

RHCE genes at the 5′ ends raised the possibility that they are identical by descent. However, heterogeneity was detected at their 3′ end. Although all donors have exon 10 sequences from both RHCE and RHD (fig. 3*A*), the unrelated homozygotes LM and HD retained RHCE sequences from the intron 8–exon 9 boundary, whereas these sequences were absent from all homozygous members of the Italian family R and from GL and SH (fig. 3*B*). This heterogeneity, together with the lack of any known relationship between the donors and their geographical separation, suggests that each D−− gene arose independently.

Our failure to detect novel restriction fragments when using intron 1 and exon 2 sequences as probes on genomic DNA blots (data not shown), and the very high degree of homology between the two genes, suggested that the deleted RHCE sequences may have been replaced by the corresponding sequences from RHD. This was tested directly using PCR primers T1 and P2 (fig. 1*B*), which flank the markers defining the boundary of the rearrangement. A product carrying 5′ RHCE sequences can be generated only if the antisense primer P2 can anneal to sequences 3′ to the region of rearrangement. These will be present only if RHCE gene sequences have been replaced by homologous sequences from the RHD gene (i.e., a chimeric gene has been formed). The 5.5-kb product was generated by long-range PCR (fig. 4*A*) and diluted 10^4-fold into a hemi-nested reaction using primers T1 and T2 (fig. 1*B*) specifically to amplify the 5′ end of the first-round product that contains the *Taq*I/SSCP marker. A series of control experiments, including a mock two-stage reaction without added *Taq* polymerase in the first round, confirmed that, under the conditions employed, the second-round product was generated only when the first-round amplification was successful. The 604-bp product of the heminested reaction was subjected to SSCP analysis (fig. 4*B*), which confirmed the presence of RHCE sequence within the 5.5-kb products. It can therefore be concluded that all the D−− donors possess a chimeric gene (fig. 5).

Discussion

The results presented here show that five of the six D−− pedigrees studied have chimeric genes in which different amounts of the RHCE gene have been replaced by RHD sequences. RHD is therefore duplicated over most of its length (fig. 5). It is tempting to suggest that this accounts for the significant overexpression of the

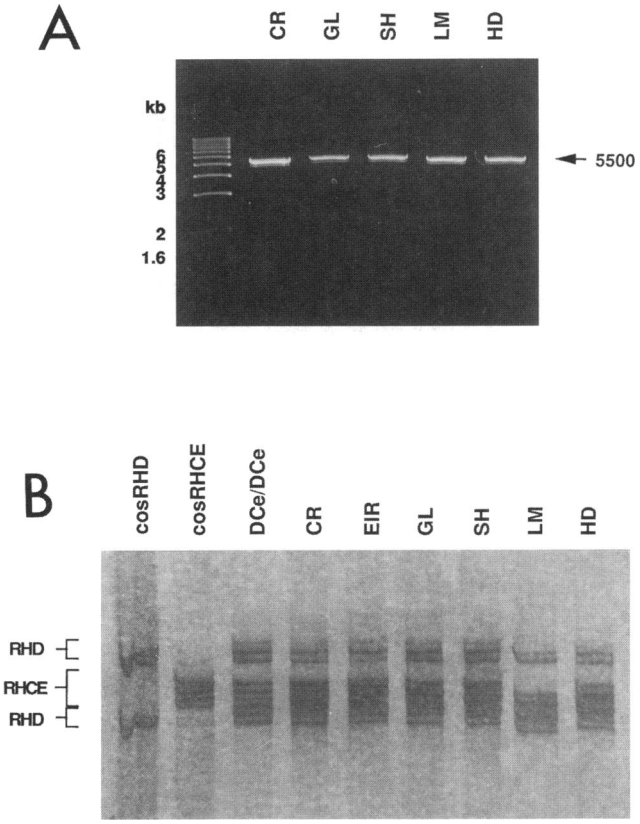

Figure 4 Identification of chimeric genes in D−− donors. *A*, Putative chimeric templates, amplified by long-range PCR using primers T1 and P2 that flank the *TaqI*/SSCP and *PstI* markers defining the 3′ and 5′ limits of the rearrangement, respectively. The 5.5-kb product represents all the region initially sequenced from the RHCE and RHD cosmids (fig. 1*B*). Lane 1, 1.0-kb ladder (BRL). Lanes 2–6, D−− donors. *B*, Products from *A*, diluted 10,000-fold and used in a hemi-nested PCR reaction with primers T1 and T2 to obtain a product containing the *TaqI*/SSCP marker, as described in figure 2*A*. This product was then subjected to SSCP analysis, the silver-stained result of which is illustrated. Lanes 1 and 2, patterns for RHD and RHCE (Ce allele) cosmids, respectively. Lane 3, genotyped control, Lanes 4–9, D−− donors.

Rh D antigen seen in D−− homozygotes, although levels of Rh antigen expression, in general, correlate poorly with gene dosage (Race and Sanger 1975). We have not assayed these donors for chimeric gene expression, but the sequence data of Huang et al. (1995) reveal C-like substitutions at the 5′ and 3′ ends in an mRNA recovered from a homozygous D−− donor who lacks internal RHCE genomic sequences. (Dr. Huang has confirmed that this is donor LM in the present study.) Although support for this conjecture requires the recovery from the same D−− donor of both normal RHD and chimeric gene transcripts, together these findings are consistent with expression from the chimeric gene of an antigen serologically indistinguishable from D. The results presented here are not necessarily in conflict with those of Huang et al. (1995) or Blunt et al. (1994*b*), who concluded that the D−− phenotype resulted from a deletion of the RHCE gene; in the presence of a normal RHD gene, replacement of RHCE sequences with homologous sequences from RHD can only be detected in genomic DNA by the use, as here, of an assay specifically designed to detect possible chimerism.

It is now clear that mechanisms exist in eukaryotes for the generation of polymorphism in gene families that operate in addition to those seen in single-copy genes. Thus, in addition to the point mutations, insertions, and deletions found in single genes, members of gene families also undergo intergenic exchange of various degrees of complexity. These exchanges can be reciprocal or nonreciprocal. They can also be inter- or intrastrand, although, where there is evidence, it appears that, in man, reciprocal intergenic exchanges are the result of interstrand recombination following chromatid misalignment. Single-reciprocal recombination of misaligned repeats generates duplications and deletions, as for example in the PMP22 gene in the CMT1A polyneuropathy and HNPP, respectively (Chance et al. 1994). Nonreciprocal exchange is well documented for some 21-hydroxylase (CYP21A) gene mutations leading to congenital adrenal hyperplasia (Miller 1988). From a mechanistic standpoint, there is no reason at present to distinguish between reciprocal and nonreciprocal recombination, since current models for the recombination process attempt to accommodate both (Whitehouse 1982). The term "gene conversion," originally used to describe aberrant segregation of parental versus recombinant gametes in lower eukaryotes, in mammals has become synonymous with nonreciprocal exchange of genetic information.

It is a reasonable presumption that intergenic recombination generated the chimeric genes described here. Since the chimerism involves only internal sequences, nonreciprocal exchange, i.e., gene conversion, seems more likely, because double reciprocal recombination over the 60–70 kb that separate exons 1 and 9 would not be expected. The chimerism that has been described in connection with partial D antigen expression (the so-called category D phenotypes) (Mouro et al. 1994; Rouillac et al. 1995), in which there is segmental replacement of RHD sequences by RHCE, is also thought to be the result of gene conversion. A striking feature of these replacements is that, in spite of heterogeneity at the 3′ end, their 5′ boundaries are all within the same 4.2-kb interval around exon 2 of RHCE. Because our ability to fix the size of this interval was limited by the identity of the two genes in this region, it is probable that the rearrangement is restricted to a region even smaller than this. The finding of a region of identity within genes that are 96% homologous over their coding region suggests that sequence homology in this region

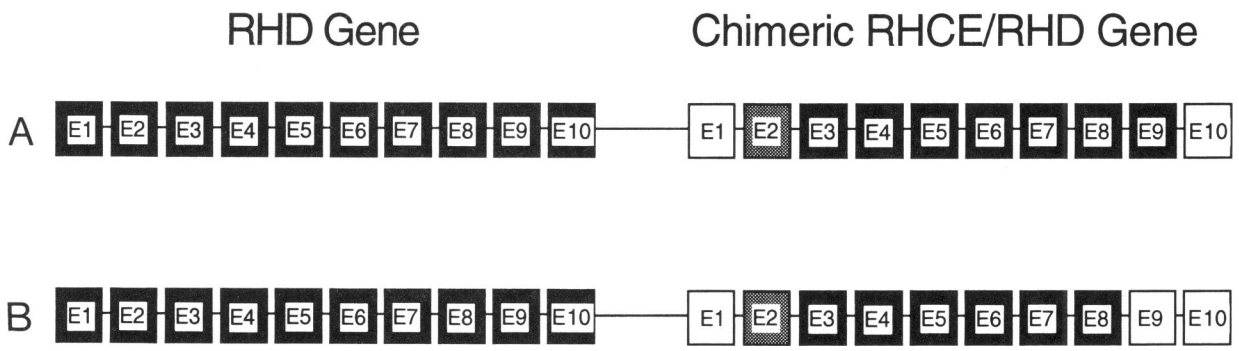

Figure 5 RH locus map of D−− donors. The distribution of genes and their exons is schematically drawn. This schematic represents the simplest interpretation of our results, although they do not exclude the formal possibility that one or more of exons 2–9 of the RHD gene are missing. RHCE exons are represented by plain boxes and RHD exons by black boxes. Gray boxes represent exons within which RH type cannot be determined. *A*, Donors Family R, SH, and GL. *B*, Donors LM and HD.

may have been actively maintained. If, as suggested, intergenic exchange (via conversion) accounts for the origin of the RHD/CE chimerism, it raises the possibility that this may have been a rare aberration in a process that normally operates to maintain sequence homogeneity. The identity of the two genes in this region applies only to the Ce allele; although we have not sequenced this region of the c alleles so extensively, exon 2 of the c alleles differs from both the Ce allele and RHD (Mouro et al. 1993; Simsek et al. 1994), and we have identified additional differences in noncoding sequence (data not shown). This suggests that the proposed homogenization process only involves the C alleles of RHCE. Some support for this comes from the finding, based on the residual RHCE exon 1 sequence, that the RHCE recipient of the presumptive conversion event in all cases most likely carried this allele; the probability of this occurring by chance is the cumulative probability of 5 unrelated individuals being C+, approximately $0.4^5 = 0.01$.

The targeting of five independent recombination events, whether reciprocal or not, to a region of high intergenic homology no larger than 4.2 kb, suggests that it may have been precipitated by some feature of the primary sequence there. We noted a higher-than-expected density of dispersed repetitive elements; there are, for example, eight Alu repeats in 5.5 kb, compared to an expected average density for this repeat of one every 7 kb. There are also three MIR elements (Armour et al. 1989) in the region. Sequence homology between repeats can promote illegitimate recombination (Lehrman et al. 1987*a*, 1987*b*; Rouyer et al. 1987; Markert et al. 1988) although the extent of homology is no less in the single-copy sequence between them, and, moreover, those sequence differences which were found are all within the Alu elements. Of possibly greater significance may be the discovery of an alternating purine-pyrimidine tract of the form (AC).(TG) within intron 2 (authors' unpublished data). It has been suggested that alternating purine-pyrimidine copolymer tracts promote recombination in yeast (Treco and Arnheim 1986), SV40 (Stringer 1985), and lymphoid tumors (Boehm et al. 1989).

Although none of the donors studied here expressed antigens of the C or E type, donor HD expressed the very-low-frequency red-cell antigen Evans (Contreras et al. 1979) and, as such, should strictly be classified phenotypically not as D−− but as D · · . Evans antigen, which has been the cause of severe neonatal hemolytic disease, was shown by segregation analysis to be a product of the RH locus (Race and Sanger 1975) and to be associated with the lack of detectable products of the RHCE gene. Although the allelic provenance of Evans has never been established, a plausible model might regard it as antithetical, i.e., allelic to C and/or E. The finding that HD was indistinguishable at the molecular level from true D−− donors very strongly argues that Evans antigen is expressed from RHD sequences. Whether expression is from the intact or the chimeric RHD gene is not known, although the less-marked elevation of D antigen in D · · as compared to D−− (G. Daniels, personal communication) donors may suggest it is the latter.

Acknowledgments

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