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Review: the molecular basis of the Rh blood group phenotypes

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The Rh blood group system is the most complex blood group system known.[1] Currently, 48 antigens are distinguished. Even this number does not fully reflect the serologic diversity of the Rh blood group, because this list of antigens disregards the complexity of the D antigen revealed by monoclonal antibodies binding to different epitopes[2,3] and by the anti-D formed in carriers of partial D phenotypes.[4] This review presents an overview of the molecular structures shaping the serologic complexity of the Rh blood group system. We outline the general principles underlying the relationship of Rh molecular structure and phenotype.

Rh proteins

The antigens of the Rh blood group system are located on two proteins.[5,6] RhD carries the D (Rh1) antigen, and RhCE carries the C, c, E, and e (Rh2 to Rh5) antigens. Both proteins are composed of 417 amino acids.[5,7] Current structural models predict 6 extracellular loops and 12 transmembranous and 7 intracellular protein segments.[8,9] Both C- and N-terminal protein ends are intracellular (Fig.1).Depending on the *RHCE* allele considered, RhD and RhCE differ in 34 to 37 amino acids. These differences are dispersed throughout the amino acid sequence of the protein. Only a limited number of these differences are located exofacially; such exofacial differences are restricted to loop 3 encoded by exon 4, loop 4 encoded by exon 5, and loop 6 encoded by exon 7. In loop 2 encoded by exon 2, the *c* allele but not the *C* allele of *RHCE* differs from RhD (Fig. 1).

In the RBC membrane, the Rh proteins form a complex with Rh-associated glycoprotein (RhAG), previously known as RH50.[10] This “Rh complex” is tightly linked to the cytoskeleton.[11] Several additional proteins, such as CD47, LW, and the Duffy glycoprotein, are associated with the Rh complex but not necessary for Rh expression. The membrane expression of Rh depends on functional RhAG: mutations in RhAG could be shown to underly the “regulator form” of the Rh_{null} phenotype characterized by lack of all Rh antigens.[12]

The RhAG and Rh proteins share homologies with ammonia transport proteins and have been shown to transport ammonia.[13–15] Currently, it is unknown whether this represents their sole function; indirect data[16] feed speculation on transport functions for other gases more relevant to the RBCs, such as CO_2 or O_2. Furthermore, the distorted RBC shape in the Rh_{null} phenotype indicates the importance of the correct interaction of the Rh complex with the cytoskeleton.[11]

RH gene locus

The two *RH* genes, *RHD* and *RHCE*, are each composed of 10 exons[17] and are spread along about 60,000 bp genomic sequence each. The genes have opposite orientation,[18,19] face each other by their 3′ ends, and are separated by only about 30,000 bp.[18,19] A third gene, *SMP1*, is interspersed between *RHD* and *RHCE*.[18] There is no indication that *SMP1* is functionally related to *RH* or expressed on the RBC surface; rather *SMP1* is considerably more conserved throughout evolution than *RH*[20] and mainly expressed in the cytoplasm.[21] Based on a comparison of the *RH* loci of man and mice, *RHD* is the duplicated gene,[20] whereas *RHCE* with its close proximity to *SMP1* represents the ancestral position (Fig. 2).

The *RHD* gene is flanked by two DNA segments of 9000 bp, called *Rhesus boxes*.[18] The D− phenotype in Whites is usually caused by the homozygous presence of a haplotype in which all the *RHD* gene is deleted.[22] This deletion occurred in the *Rhesus boxes*,[18] probably by an unequal crossing over. Hence, the *RH* locus of the *RHD* negative haplotype is almost identical to the ancestral *RH* locus before the duplication event.

The characterization of the deletion site was instrumental for the specific detection of the *RHD* deletion.[18] Since then, the *RHD* deletion may be detected even in heterozygous form, i.e., if it occurs *in trans* to the normal *RHD* allele. Thus, it became possible to distinguish D+ individuals with two *RHD* genes from D+ individuals with one *RHD* gene and one *RHD* deletion. For instance, it is important to predict the probability of a D+ pregnancy if the mother is D−.[23] Such determination cannot be achieved by serology. However, even current molecular methods for the detection of the *RHD* deletion are not yet reliable in people of African descent.[24,25]

Mechanisms contributing to the molecular variability of the *RH* locus

Several different mechanisms contributed to the large number of *RH* alleles. Many of these mechanisms are shared by most other genes and contributed to the complexity of other blood groups, like KEL and LU. A single nucleotide substitution may cause a change of the encoded amino acid (missense mutation), leading to a single amino acid substitution; introduce a premature stop codon (nonsense mutation), leading to a truncated protein; or destroy the splice consensus sequence (splice site mutation), preventing the correct splicing of the allele. Insertions or deletions of one or a few nucleotides usually lead to a frameshift resulting in completely aberrant amino acid sequences. Finally, recombinations between different alleles lead to alleles sharing peculiarities of both parent alleles.

Two mechanisms that are favored by the structure of the *RH* locus are rare in other blood group systems. The two highly similar *Rhesus boxes* flanking the *RHD* gene[18] allowed the deletion of *RHD* by an unequal crossing over (Fig. 2) in the common *RHD* negative haplotype. Obviously, a similar mechanism is not possible for *RHCE*, and there is no common *RHCE* negative haplotype.

The high similarity of both genes and their opposite orientation favored gene conversions *in cis* (Fig. 3), in which internal parts of one gene are replaced by the corresponding parts of the other gene.[26] The results were *RHD-CE-D* or *RHCE-D-CE* hybrid alleles. Some

haplotypes (e.g., *DC*^{w}–^{27}) are composed of two single hybrid alleles of type *RHD-CE* and *RHCE-D*.[28,29] Such haplotypes are best explained by gene conversions involving more than one gene (Fig. 3).

Molecular basis of the antigen D

Independent of the exact binding site, any anti-RBC antibody binding to an Rh protein is considered an anti-D if the antibody binds to RhD but not to RhCE. With a few exceptions, the presence of D antigen may be equated to the presence of RhD-specific amino acids in any of the exofacial loops 3, 4, or 6. These three loops are the only exofacial protein segments that differ between RhD and RhCE. Monoclonal antibodies binding to different parts of RhD usually differ in their ability to bind to aberrant forms of RhD; this phenomenon was instrumental for establishing the serologic classification of D epitopes.[30]

Aberrant *RHCE* alleles encoding D-specific amino acids in the extracellular loops 3, 4, and 6 often express some D epitopes. The best known example is *DHAR* caused by an *RHCE-D(5)-CE* hybrid allele[31] that encodes D epitope 6 (epD6)[2] and is often typed as D+ by using commercial, licensed monoclonal anti-D.

Recently, the observation of the *RHCE* allele *ceRT*[32] has added to the complexity of D antigen expression: the *ceRT* allele encodes part of epD6 without encoding any D-specific amino acid. This observation provided direct evidence that the Ser to Thr missense mutation at codon 154 forms structural features that are present in RhD but not in standard RhCE. This conclusion is in congruence with the notion that most D epitopes represent three-dimensional structures rather than linear protein segments.[33]

Molecular basis of the D− phenotype and of D_{el}

The D− phenotype may be caused by the lack of functional RhD protein or by the presence of aberrant forms of RhD that do not express D antigen.

The most frequent cause for the absence of a functional RhD protein is a deletion of the whole *RHD* gene.[18,22] This *D*− haplotype represents 40 percent of all haplotypes in Whites, and even in Africans, it may be more often the cause of D− than all other causes combined.[18,25] The *RHD* deletion resulted from an unequal crossing over of the *Rhesus boxes* and is characterized by the presence of a "hybrid *Rhesus box*"[18] (Fig. 2). The absence of the *RHD* gene in most D− Whites was a fortunate coincidence fostering the analyses of the molecular basis of D antigen: Whites with weak D or partial D often carry the *RHD* deletion *in trans*, a fact that much simplified the analyses of their single aberrant *RHD* alleles. Likewise, almost any *RHD*-specific polymorphism is a good predictor of D antigen in Whites.

Other observed sources of non-functional RhD proteins are nonsense mutations leading to premature stop codons, insertions and deletions leading to frameshifts, and splice site mutations that prevent the correct splicing of the RhD mRNA. These nonfunctional alleles are important for any genotyping approach[34] including *RHD* genotyping by PCR: unless the mutation is detected specifically, *RHD* PCR will give a falsely positive prediction

for D antigen.[26] The most important allele of this type is *RHDΨ*,[35] which is about as frequent as the *RHD* deletion among some African populations.[35] Therefore, a correct D antigen prediction by PCR in Africans became possible only after the characterization of the structure of *RHDΨ*,[35] which harbors a 37-bp insertion at the intron 3/exon 4 junction, a stop codon in exon 6, and several missense mutations. Today, a specific detection of *RHDΨ* is considered mandatory for any D antigen prediction by PCR.[26]

Some splice site mutations are permissive for the expression of minute traces of D antigen resulting in a D_{el} phenotype, in which D antigen may only be demonstrated by adsorption and elution of anti-D. The most important example is *RHD*(G1227A),[26] the most frequent D_{el} allele in Japanese and Chinese,[36] and the second most frequent D_{el} allele among Whites.[26]

Other forms of aberrant *RHD* alleles can express RhD proteins that however do not carry D antigen. Such RhD proteins are encoded by *RHD-CE-D* hybrid alleles in which the *RHCE* segments must encompass at least exons 4 to 7.[26] This causes exofacial loops 3 to 6 to resemble their RhCE counterparts. Since loops 1 and 2 do not differ between RhD and RhCE, the whole exofacial protein part of these hybrid proteins is identical to RhCE, and hence these aberrant RhD proteins cannot express D antigen. The two most important examples of such alleles are $dCce^{s}$,[37] a *RHD-CE(4–7)-D* hybrid allele with some additional substitutions, and *RHD-CE(2–9)-D*.[27] The $dCce^{s}$ allele is the third most common cause of D− phenotypes among Africans,[25] and *RHD-CE(2–9)-D* is a major cause for D− phenotypes in Chinese.[36] It should be kept in mind that, although these hybrid alleles do not express D antigen, they are present in the RBC membrane and may carry other antigens, albeit often in modified, e.g., weakened, forms. For example, the RhD protein of $dCce^{s}$ carries C antigen.[38]

Partial D caused by hybrid proteins

What happens if some, but not all, RhD-specific extracellular loops are replaced by their RhCE counterparts? Depending on their exact binding site on the RhD protein, some monoclonal anti-D antibodies will be able to bind to such hybrid proteins, other anti-D will not be able to bind. Thus, these hybrid RhD proteins present themselves serologically as a partial D phenotype whose hallmark is the lack of some, but not all, distinct D epitopes (epD), which are defined by monoclonal anti-D. Using polyclonal anti-D, these partial D RBCs are typed as D+, but carriers of these alleles may form anti-D antibodies, which bind to those parts of normal RhD that are lacking in the aberrant RhD protein.

Similar to the *RHD-CE-D* hybrids represented by $dCce^{s}$, the partial D phenotype is mainly determined by the RhD- or RhCE-specificity of the exofacial loops 3, 4, and 6 and influenced by the RhE/Rhe origin of loop 4.[39] There are six possible combinations of the segments encoding exofacial loops 3, 4, and 6 (Table 1). Substitutions in nonexofacial protein segments have generally minor effects on the phenotype.[40] Alleles that differ in the exact extent of the substitution but express the same exofacial protein segments form clusters of alleles that share similar phenotypes.[39–41] Three of these clusters (DIVb, DVa, and DVI) were recognized early on and form three major groups of the D category classification.[42,43]

The other possible combinations are represented by DFR,[43] DHAR,[31] and DBT[44] and have been recognized only since the mid 1980s.

The unique combinations of RhD- and RhCE-specific extracellular loops found in hybrid *RHD-CE-D* alleles often are antigenic and may explain the low-frequency antigens expressed by those alleles, e.g., *FPTT* in DFR[45] and *BARC* in D category VI.[46]

Of course, there are a few exceptions to this general outline, which are exemplified by two known partial D phenotypes that involve segmental substitutions other than loops 3 to 6: (1) DIIIb is caused by a substitution of exon 2 corresponding to loop 2[47] and (2) DIIIc by a substitution of exon 3[48] that presumably does not affect any exofacial amino acid. Although these phenotypes may become immunized to normal D, the alterations of the D antigen are much more limited than in the other hybrids, and their serologic detection using monoclonal antibodies is difficult.

Partial D caused by missense mutations affecting the exofacial protein segments

Missense mutations affecting the exofacial protein segments of RhD generally lead to a partial D phenotype. Because there are many more possible missense mutations than segmental substitutions, the phenotypic changes are much more diverse than those observed in partial D caused by hybrid proteins (Table 2). However, the phenotypic changes are often limited, correlating with an often low anti-D immunization risk and a difficult serologic detection. Based on the frequency of anti-D immunization events,[49] DNB[50] and DVII[51] may be the two most important partial D of this type.

Similar to hybrid alleles, the presence of aberrant amino acids in the RhD sequence may cause the expression of low-frequency antigens. The most important example is the Tar (RH40) antigen accompanying DVII[46] that is caused by a leucine to a proline substitution at position 110.

Weak D caused by missense mutations affecting the nonexofacial protein segments

If a missense mutation affects a nonexofacial segment of the RhD protein, the influence on the D antigen is limited. However, such mutations tend to interfere with membrane integration of the RhD protein and are causing the vast majority of the weak D phenotypes.[9] Although each allele has a distinct phenotype,[52] a purely serologic discrimination of the many alleles is almost impossible, because the phenotypic differences are most often minute. The weak D types are designated by numbers according to their molecular structure; the lowest numbers were given to the most frequent types in the initial study,[9] which has been proved since then to be representative for White populations in general (Table 3).[53–55] No allo-anti-D immunization has been reported for weak D type 1 to type 3.[49,52] Caution should be applied when considering the transfusion strategy for the less frequent weak D types, because, at the moment, it is not possible to exclude the possibility that changes in nonexofacial protein segments may allow allo-anti-D immunization.

The mechanisms underlying reduced RhD expression by weak D alleles are not completely understood and may differ depending on the allele concerned.[56] Almost all the involved single point mutations relate to amino acids conserved throughout species.[57] The missense mutations seem to occur in clusters[9] (Fig.4), which might hint to regions important for the correct integration of the Rh proteins in the membrane or the correct interaction with RhAG.

Dispersed mutations, “African” alleles and the phylogeny of *RHD* alleles

A few partial D[43,49,52,58–60] first detected among individuals of African descent are characterized by a multitude of missense mutations dispersed throughout the amino acid sequence of the RhD protein. The substitutions often are typical for RhCE but do not form a continuous stretch of RhCE sequence within RhD. The observation of these alleles is best explained assuming an *RHD* phylogeny in which almost all *RHD* alleles detected in Eurasians form just one of four branches.[60] The multitude of missense mutations in “African” alleles simply reflects a longer phylogenetic distance from standard RhD (Fig. 5). The lack of “African” alleles in Eurasians is probably the result of a bottleneck during the migration out of Africa. Only a few “African” alleles, such as *weak D type 4*[9] and *DAU-0*,[60] are occasionally observed among Whites. It is currently unknown whether these alleles were present during the primary bottleneck or entered the Eurasian allele pool by secondary migrations. Among Africans, alleles of all four clusters, including the alleles of the “Eurasian” cluster, are frequent.

Molecular basis of c, E, and G antigens

These three antigens are strongly correlated with the presence of a specific amino acid in an Rh protein: a c antigen is determined by Pro at position 103,[61,62] G antigen by Ser at position 103,[63] and E antigen by Pro at position 226.[61] Ser at position 103 is present both in RhD and in the *C* allele of RhCE, therefore, both *C+* and *D+* haplotypes generally also express G antigen.

The mechanisms leading to partial D, weak D, and D− phenotypes may also occur in RhCE and lead to aberrant *RhCE* alleles carrying partial antigens, weakened antigens, or low-frequency antigens or not expressing the specific antigen despite PCR prediction of the corresponding allele (Table 4). However, these phenomena are recognized less frequently, because the antigens are less immunogenic than D and there are fewer monoclonal antibodies that could unravel lacking epitopes.

Molecular basis of e and C antigens

The molecular bases of e and C antigens are a bit more complicated, because the amino acids characterizing these alleles of RhCE are also present in RhD. While the e antigen is associated with the presence of alanine at position 226,[61] which is also found in RhD, e antigen is only expressed if its typical alanine occurs in an “RhCE context.” The minimal necessary “RhCE” context is unknown: *RHCED(5)-CE* alleles like R_0^{Har} lack *RHCE* exon 5 but express some e antigen. These alleles may cause a falsely negative e antigen prediction by PCR.[64]

The hallmark of alleles expressing C antigen is the presence of *RHD* exon 2 (encoding exofacial loop 2) in an *RHCE* context.[61] Two mechanisms have led to such proteins: the "standard" *C* allele (Ce) differs from "standard" ce by an *RHD*-like type exon 2 that resulted from a gene conversion with *RHD*[65]; a small duplication at the insertion point was found to be the most reliable molecular polymorphism to predict this mechanism of C antigen expression.[66] In addition, in this type of *C+* allele, a cysteine must be present at position 16 encoded by exon 1. This cysteine is necessary for expression of C antigen,[67] although it is not C-specific as it is shared by many *ce* alleles of the *Dce* haplotype.[68,69] A different mechanism leads to the C antigen of *dCce*^{s}: this C antigen is carried by an aberrant RhD protein[38] encoded by an *RHD-CE(4–7)-D* hybrid allele with several additional substitutions,[70] including an RHCE-like threonine at position 152.The *dCce*^{s} haplotype codes for both C and c, because the accompanying RhCE protein carries c antigen.

Large *RHCE-D-CE* hybrids and the D– – phenotype

Similar to the loss of D antigen in *RHD-CE-D* hybrid alleles with a *CE* segment ranging from exons 4 to 7, substitution of all or almost all RhCE-specific extracellular protein segments with RhD sequence results in RhCE proteins that do not express CE antigens or parts thereof.[71] Depending on the extent of the substitution, three forms of RhCE proteins lacking CE antigens, dubbed "*CE*-silent haplotypes,"[72] result: substitution of exons 2 to 7 leads to the absence of all CE antigens in D– –.[73,74] If exon 7 retains *RHCE*, the resulting phenotype is D··,[75] which differs from D– – by the presence of the low-frequency antigen Evans (Rh37) and the high-frequency antigen Dav (RH47). Third, if a c-type exon 2, which encodes loop 2, is present, a cD− phenotype will result.[76,77] In the C^{W}D−phenotype,[27,77] exon 2 is *RHD* but the C^{W}-specific mutation in exon 1 is present. There is no frequent *CE*-negative haplotype, therefore *CE*-silent haplotypes are only detected if they occur in homozygous or compound heterozygous form or become apparent by family studies. The often enhanced expression of D antigen probably derives from the additional expression of D antigen in the aberrant RhCE protein.

"African" *RHCE* alleles

In analogy to the *RHD* allele of African origin, several *RHCE* alleles are frequent in Africans but rare in Europeans and often differ by a multitude of mutations (Table 5).[37,59,70,78] These alleles code for RhCE proteins that lack some high-frequency antigens. Immunizations to these antigens may pose serious logistic problems, especially if they occur in individuals depending on chronic transfusion support because of inherited anemias.[78]

Rh$_{null}$ of the amorph type

Carriers of the "amorph" type of Rh$_{null}$ were shown to lack any functional *RHCE* and any functional *RHD* genes. Generally, they are caused by nonsense mutations in *RHCE* in an *RHD* negative background.[79,80]

Unresolved issues of *RH* genotype and phenotype

The models presented in this review correlate the Rh phenotype with the type of the extracellular protein segments and missense, nonsense, and splice site mutations. These models are powerful in explaining much of the allelic and antigenic variation observed in vivo (Table 6). However, it is important to realize that the exact relationship of D epitopes (epD) and RhD structures as well as the molecular basis of several Rh antigens remains unresolved. In addition, there may be some *RHD* alleles that lack D antigen without obvious changes in the *RHD* gene.[55] Detection of the *RHD* deletion by PCR is still hampered by falsely negative and falsely positive results in Africans, indicating a variability in *Rhesus boxes* in Africans that may well surpass the variation observed among Whites. Finally, while the function of Rh proteins in the Rh complex is only emerging, the functional role of different Rh variants and a possible selection pressure generating and maintaining the astounding Rh antigenic variability is a remaining mystery and poses an opportunity for continuing research.

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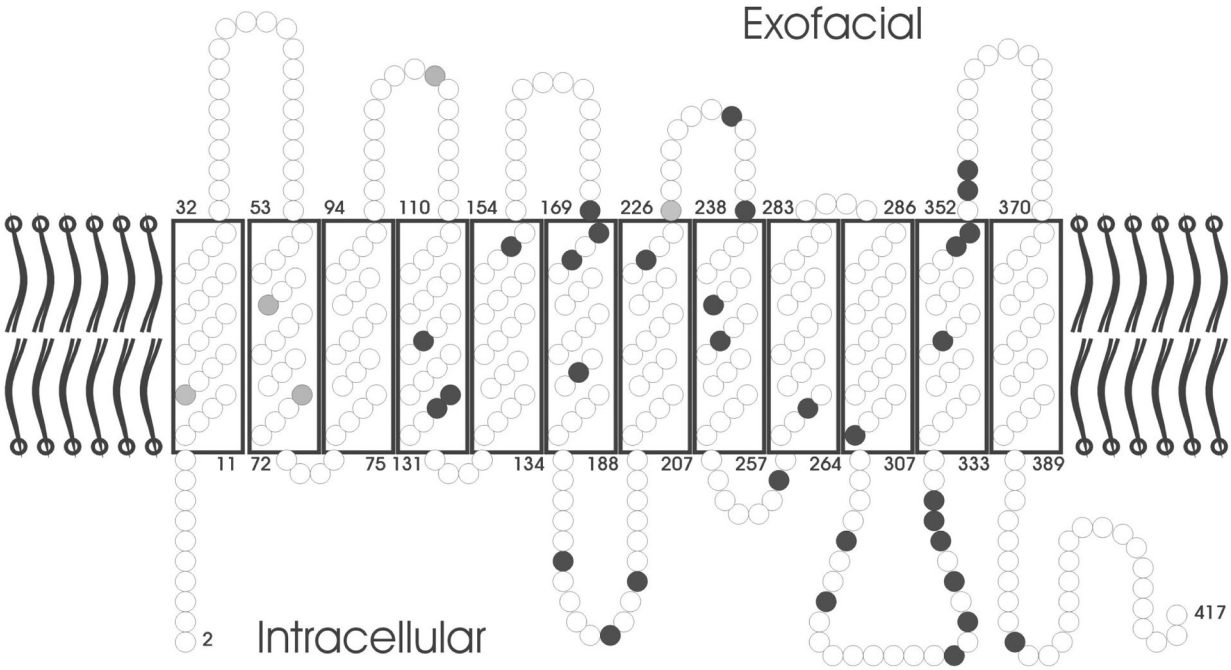

Fig. 1.

Rh topology in the RBC membrane. The protein is assumed to possess 12 transmembranous segments, 6 extracellular loops, and 5 intracellular loops. Both the C- and N-terminal ends of the protein are intracellular. Each amino acid is depicted by a circle; black circles indicate positions that differ between RhD and RhCE in all frequent alleles, grey circles indicate positions that differ between RhD and RhCE only in some alleles. Most differences are located in transmembranous or intracellular segments; among the extracellular loops, only loops 3, 4, and 6 differ between RhD and RhCE. The latter fact is most important for D antigen expression and is discussed in detail in the text.

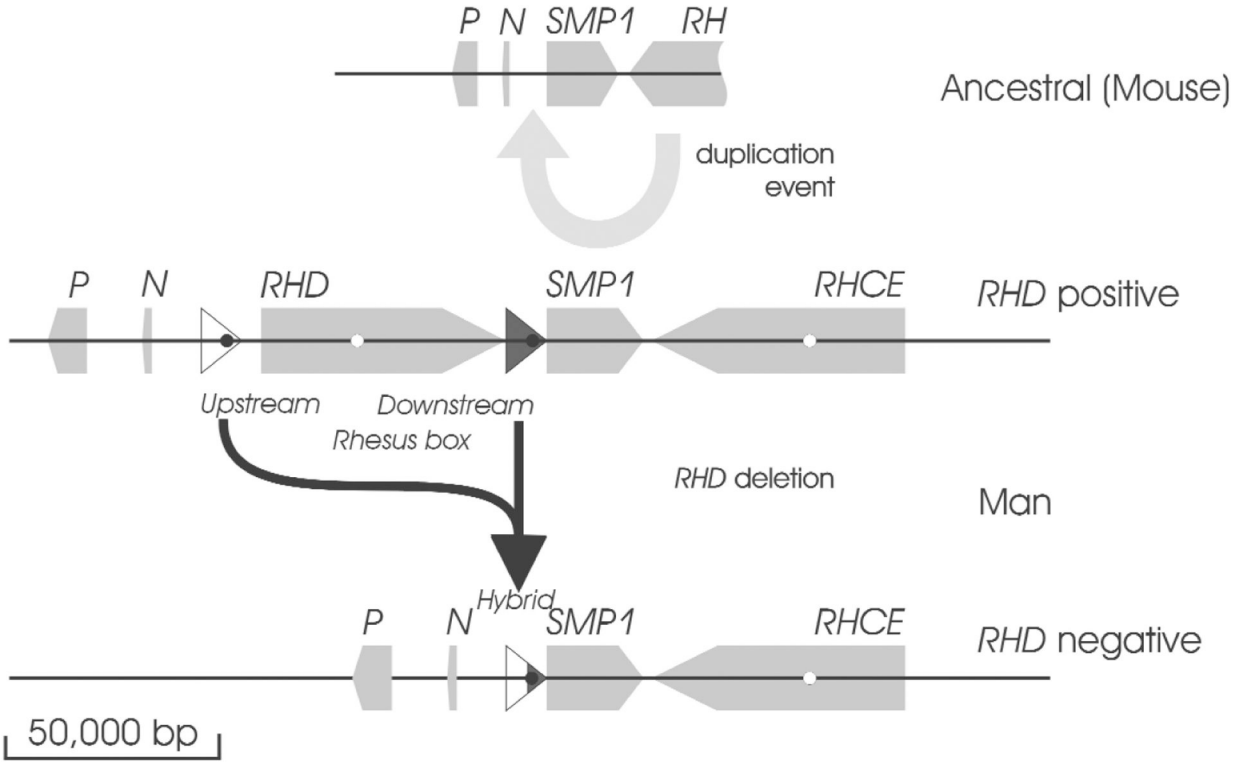

Fig. 2.

RH duplication and deletion. In the ancestral state (deduced from the mouse *RH* locus), a single *RH* gene is in close proximity to the *SMP1* gene. Two other genes, the *P29-associated protein* (P) and *NPD014* (N) are upstream of *SMP1*. In the duplication event, an inversed *RH* gene is introduced between *NPD014* and *SMP1*. At the insertion point, a 9000-bp DNA segment is duplicated, resulting in the formation of upstream and downstream *Rhesus boxes*, which flank the *RHD* gene. The *RHD* deletion occurred by a recombination of the upstream and downstream *Rhesus boxes* and led to a *RH* locus, which closely resembles the ancestral state before the *RH* duplication occurred.

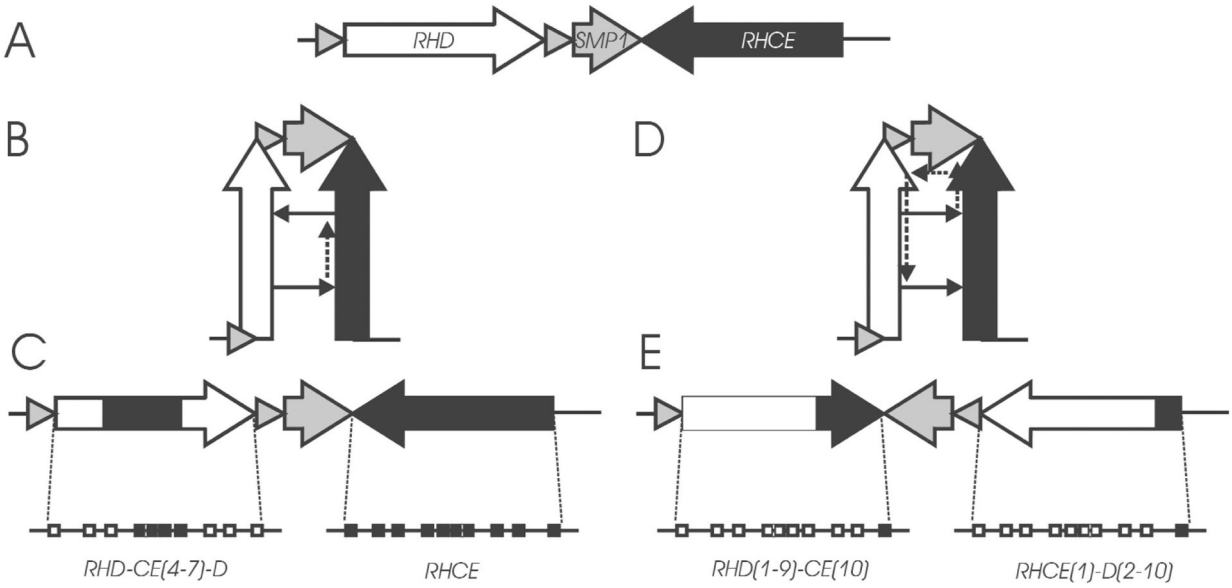

Fig. 3.

Gene conversions *in cis*. Panel A:The two *RH* genes have opposite orientation. Panel B:A gene conversion *in cis* might be favored by a hairpin-like structure. Panel C:As a result of the gene conversion, part of one *RH* gene is replaced by the corresponding segments of the other gene. Panel D: If transcription continues on the wrong template throughout *SMP1*, it may return to the correct template when it enters the second *RH* gene. Panel E:The result of this multi-gene-conversion are haplotypes with two "single hybrid" alleles, as described for $C^{W}D-$,[27] *EKH*,[28] and some $D\cdot\cdot$:[29]

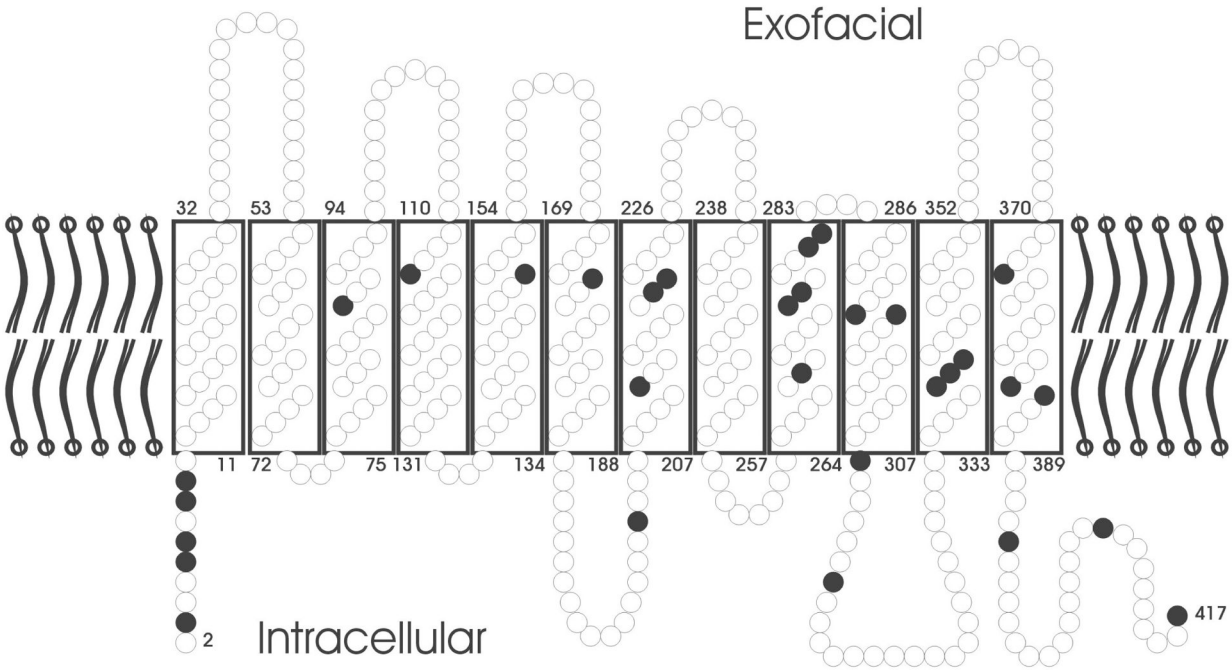

Fig. 4.

The predicted topology of RhD in the RBC membrane is shown. Amino acids are depicted as circles. Black circles indicate amino acid substitutions, each of which was correlated with a molecularly distinct weak D type.

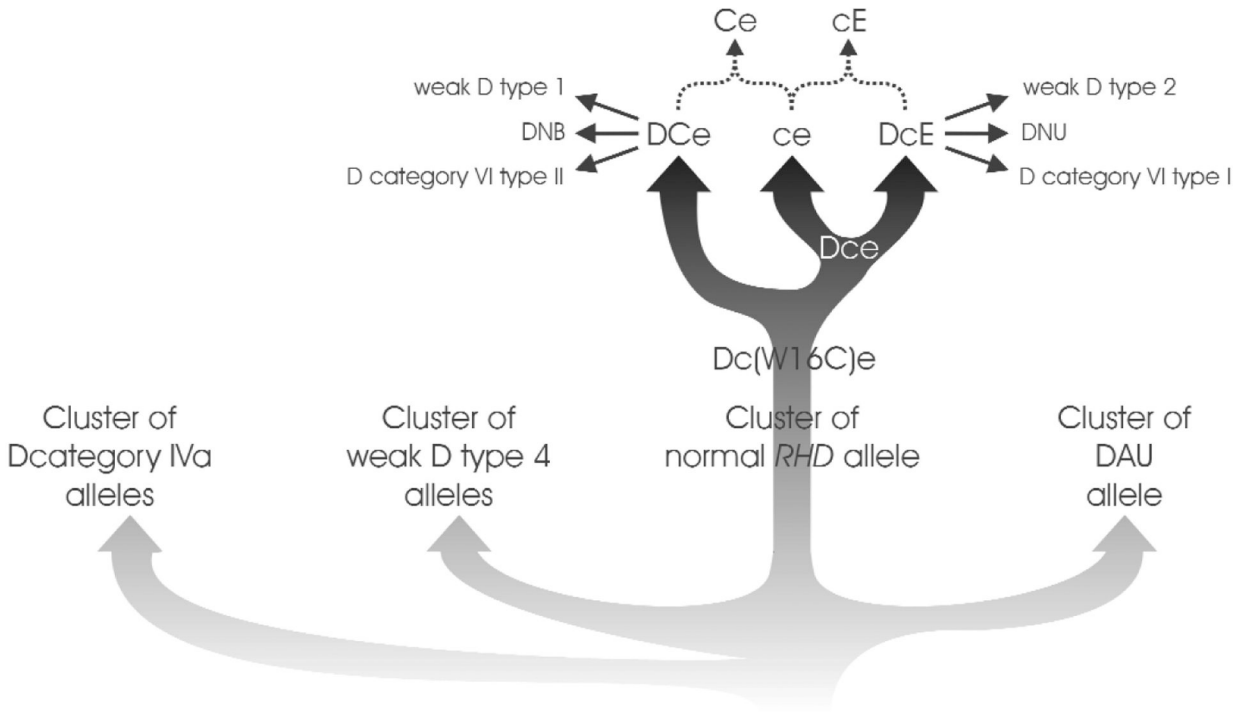

Fig. 5.

Phylogenetic tree of *RHD*. There are four independent branches of *RHD* alleles: The D category *IVa* cluster, the *weak D type 4* cluster, the *Eurasian D* cluster, and the *DAU* cluster. The alleles of the *D category IVa, weak D type 4*, and *DAU* clusters are largely confined to individuals of African ancestry and generally occur in *Dce* haplotypes. The alleles of the *Eurasian D* cluster are predominant in Eurasian populations and most often occur in *DCe* and *DcE* haplotypes. The vast majority of aberrant alleles detected in individuals of Eurasian populations belong to the *Eurasian D* cluster and may be derived from "standard" *RHD* by a single molecular event (i.e., single nucleotide substitution or single gene conversion). In contrast, most alleles of the "African" clusters differ from "standard" (Eurasian) *RHD* by more than a single molecular event.

The phylogenic relationship between the four clusters is not completely resolved, the depicted topology is just one possibility. Likewise, it should be noted that the *RHCE* variation present among "African" haplotypes is not depicted.

Table 1.

Phenotype of *RHD-CE-D* and *RHCE-D-CE* hybrid alleles with segmental substitutions in exofacial loops 3 to 6

Loop 3 (exon 4*)	Loop 4 (exon 5)	Loop 6 (exon 7)	Phenotype	Antigens expressed	
				D epitopes	Low-frequency antigens
RhCE†	RhD	RhD	DFR	(1),(2),3,4,(5),(6/7),9	FPTT (Rh50)
RhD	RhCE	RhD	DVa‡	2,3,4,6/7,8,9	D^{W} (Rh23)
RhD	RhD	RhCE	DIVb	5,6/7,8	Evans (Rh37)
RhCE	RhCE	RhD	DVI	3,4,9	BARC (Rh52)
RhCE	RhD	RhCE	DHAR	(5),(6/7)	RH33, FPTT (Rh50)
RhD	RhCE	RhCE	DBT	(6/7),8	Rh32

*The polymorphic parts of loops 3, 4, and 6 are encoded by exons 4, 5, and 7, respectively.

†RhD indicates RhD-like sequence in the loop, RhCE RhCE-like sequence.

‡For the expression of a DVa phenotype, the RhD-specific alanine at position 226 must be retained (donor allele e). A proline at 226 (donor allele E) leads to a different phenotype, e.g. in DBS.

Table 2.

Partial D caused by missense mutations

Loop involved	Position involved	Substitution	Trivial name
1*	54	Leu to Pro	DMH[81]
2	103	Ser to Pro	(G negative RhD)[63]
	110	Leu to Pro	DVII[51]
3	166	His to Pro	DFW[82]
4	229	Arg to Lys	DHR[83]
	233	Glu to Lys	DHK[28]
	234	Arg to Trp	DYU†
	235	Lys to Thr	DHO[53]
5	283	Thr to Ile	DHMi[84]
	284	Ser to Leu	"Sample A"[85]
	285	Cys to Tyr	DIM[52]
6	353	Gly to Arg	DNU[86]
	354	Ala to Asp	DII[86]
	355	Gly to Ser	DNB[50]
	358	Met to Thr	DWI[87]

*Depending on the model used, this amino acid position is considered transmembranous closely adjacent to the RBC surface.

†GenBank entry AJ557827

Table 3.

Examples of clinically important and well defined weak D types *

Weak D type	Position involved	Substitution	Intracellular/transmembranous
1	270	Val to Gly[9]	transmembranous
2	385	Gly to Ala[9]	transmembranous
3	3	Ser to Cys[9]	intracellular
4.0	201	Thr to Arg[9]	transmembranous
	223	Phe to Val[9]	transmembranous
4.1	16	Trp to Cys[50]	transmembranous
	201	Thr to Arg	transmembranous
	223	Phe to Val	transmembranous
4.2 †	201	Thr to Arg[50]	transmembranous
	223	Phe to Val	transmembranous
	342	Ile to Thr	transmembranous
5	149	Ala to Asp[9]	transmembranous
11	295	Met to Ile[9]	transmembranous
15	282	Gly to Asp[9]	transmembranous
20	417	Phe to Ser[88]	intracellular

* A regularly updated list can be found at the RhesusBase: http://www.uniulm.de/~wflegel/Rh/.

† The protein sequence of weak D type 4.2 is identical to the partial D DAR.[59]

Table 4.

Examples of partial and weak antigens caused by aberrant *RHCE* alleles

Mechanism	Example	"Parent allele"	Mutation	Antigens lost	Antigens expressed
Gene conversion	*E category II*[89]	*cE*	Ce-D(2–3)-ce		
	$\overline{\overline{R}}^{N}$ [90]	*Ce*	*RHCE-D(4)-CE*	Rh46	Rh32
		Ce	*RHCE-D(3 partial-4)-CE*		
	R_0^{Har} [31]	*ce**	*RHCE-D(5)-CE*		Rh33, Rh50
	CeVA[91]	*Ce*	*RHCE-D(5)-CE*		Rh33, Rh50
Missense mutation (exofacial)	C^X [92]	*Ce*	A36T	MAR	C^X
	C^W [92]	*Ce*	Q41R	MAR	C^W
	RH:-26[93]	*ce*	G96S	Rh26	
	CeMA	*Ce*	R114W		(Har*)
	E cat V = EHK[28]	*cE*	R154T	E epitopes	
	ceRT[32]	*ce*	R154T		D epitope 6
	E cat I[89]	*E*	M167K	E epitopes	
	E cat III[89]	*E*	Q233E, M238V	E epitopes	
Missense mutation (non-exofacial)	*E cat IV*[89]	*E*	R201T	E epitopes ?	
	VS[70]	*ce(W16C)*†	L245V		VS
In-frame-deletion	e^U [95]		Del 229	Very weak e	

*RBC samples harboring this allele react with human serum Har. It is unknown whether the target antigen of this serum is identical to a known low-frequency antigen of the Rh blood group

†ce(W16C) indicates *ce* allele encoding cysteine at codon 16.This allele is generally associated with a *Dce* haplotype[69] and encodes a weakened e antigen.[94] Most "African" *RHCE* alleles derive from this allele.

Table 5.

"African" *RHCE* alleles*

Allele type	Allele name	Antigens lacking	"New" antigens	Molecular bases†
RH:-18,-19 (Hr^S-,hr^S-)	ceEK[78]	RH18, RH19 (Hr^S, hr^S)		Trp16Cys, Met238Val, Arg263Gly, Met267Lys
	ceBI[78]	RH18, RH19 (Hr^S, hr^S)		Trp16Cys, Met238Val, Ala273Val, Leu378Val
	ceAR[59]	RH18, RH19 (Hr^S, hr^S)		Trp16Cys, Met238Val, Leu245Val, Arg263Gly, Met267Lys, Ile306Val
RH: −19 (hr^S-), Partial e	ceMO[96]	RH19 (hr^S)		Trp16Cys, Val223Phe
RH:−34 (Hr^B-)	*Ccde^s* [37]	RH34 (Hr^B)	RH20 (VS)	*dCce^s*
Partial e	ce^s(340CT)[78]			Arg114Trp, Leu245Val
RH:32,−46	$\overline{\overline{R}}^N_{90}$	RH46	RH32†	*RHCE-D(4)-CE*
	$\overline{\overline{R}}^N_{90}$	RH46	RH32	*RHCE-D(3 partial-4)-CE*
Weak e	ce(W16C)[94]			Trp16Cys
	ce^s [70]		RH10, RH20 (V,VS)	Trp16Cys, Leu245Val
	ceCF[97]		RH20, RH43 (VS, Crawford)	Trp16Cys, Gln233Glu, Leu245Val

*Only important examples relevant to specific antigens are given. Several additional *RHCE* alleles have been described in individuals of African ancestry but often still await a full serologic characterization.

†Among Africans, R_0^{Har} appears to be linked to a D+ allele.

Table 6.

Most likely explanations for Rh antigens using a loop-centered approach *

Antigen Number	Antigen symbol	Molecular basis (protein)	Molecular basis (DNA/Codons)
1	D	RhD exofacial loops 3, 4, and 6	*RHD* exon 4, 5, 7
2	C	RhD-like loop 2 in RhCE with Cys 16	*RHD* exon 2 in *RHCE*
3	E	Pro 226 in loop 4	226 Pro in exon 5
4	c	Pro 103 in loop 2	103 Pro in exon 2
5	e	Ala 226 in RhCE loop 4	226 Ala in *RHCE* exon 5
6	f	Pro103 in loop 2 and Ala 226 in loop 4	103 Pro in exon 2 and 226 Ala in exon 5
7	Ce	RhD-like loop 2 in RhCE and Ala 226 in loop 4 and Leu 245 (transmembranous)	*RHD* exon 2 and 226 Ala, 245 Leu in exon 5 *RHCE*
8	C^W	Arg 41 in loop 1	41 Arg in exon 1
9	C^X	Thr 36 in loop 1	36 Thr in exon 1
10	V	Val 245 and Gly 336 (transmembranous location)	245 Val in exon 5 and 336 Gly in exon 7
11	E^W		
12	G	Ser 103 in loop 2	103 Ser in exon 2 (both *RHD* and *RHCE* possible)
17	Hr_0	RhCE exofacial loops 3, 4 and 6	*RHCE* exons 4,5,7
18	Hr	Met 238 in RhCE loop 4	238 Met in *RHCE* exon 5
19	hr^s	Ala 226 and Met 238 in RhCE loop 4	226 Ala and 238 Met in *RHCE* exon 5
20	VS	Val 245 (transmembranous location?)	245 Val in exon 5
21	C^G	Ser 103 in RhCE loop 2	103 Ser in *RHCE* exon 2
22	CE	Ser 103 in loop 2 and Pro 226 in loop 4	103 Ser in exon 2 and 226 Pro in exon 5 (*RHCE*)
23	D^W	RhCE loop 4 (Gln 233) and RhD loop 3 and 6	*RHCE* exon 5 and *RHD* exons 4 and 7
26	c-like	Gly 96 and Pro 103 in RhCE loop 2	96 Gly and 103 Pro in *RHCE* exon 2
27	c^E	Pro 103 in loop 2 and Pro 226 in loop 4	103 Pro in exon 2 and 226 Pro in exon 5
28	hr^H		
29	Total RH	Any Rh protein	Any expressed *RH* gene
30	Go^a	Carried by DIVa †	Carried by DIVa
31	hr^B		
32	Rh32	RhD loop 3 together with RhCE loop 4 and 6	*RHD* exon 4 together with *RHCE* exon 5 and 7
33	Har	Probably RhD loop 4 together with RhCE loop 3 and 6	*RHD* exon 5 together with *RHCE* exon 4 and 7

Antigen Number	Antigen symbol	Molecular basis (protein)	Molecular basis (DNA/Codons)
34	Hr^B	Cys 336 in RhDE	336 Cys in *RHDE*
35	1114		
36	Be^a		
37	Evans	RhD loops 3 and 4 with RhCE loop 6	*RHD* exon 4 and 5 together with *RHCE* exon 7
39	C-like		
40	Tar	Pro 110 in RhDE loop 2	110 Pro in *RHDE* exon 2
41	Ce-like	RhD-like loop 2 in RhCE and 226 Ala in loop 4 and normal loop 1 (Gln 41)	*RHD* exon 2,226 Ala in exon 5, normal exon 1
42	Ce^s	Carried by *dCce*$^{s\,\dagger}$	
43	Crawford	Gln 233 in loop 4 in VS-like allele	233 Gln in exon 5 of VS-like allele
44	Nou		
45	Riv	Carried by DIVa(C)-†	
46	Sec	RhCE loop 3	*RHCE* exon 4
47	Dav	Probably RhCE loop 6	*RHCE* exon 7
48	JAL		
49	STEM		
50	FPTT	RhCE loop 3 together with RhD loop 4	*RHCE* exon 4 together with *RHD* exon 5
51	MAR	Normal RhCE loop 1 (Ala 37 and Gln 41)	Normal *RHCE* exon 1 (37 Ala and 41 Gln)
52	BARC	RhCE loop 3 and 4 (with Ala 226) together with RhD loop 6;	*RHCE* exon 4 and 5 (with 226 Ala) together with *RHD* exon 7
53	JAHK	RhD loop 2 in RhCE without Cys 16	*RHD* exon 2 in *RHCE* without 16 Cys
54	DAK	Carried by DIIIa, DOL, and $\overline{\overline{R}}^N$	
55	LOCR		

*All explanations are tentative and based on the published distribution of the antigens. These interpretations may need modifications, if additional haplotypes encoding or not encoding the antigens are identified.

†The region of Rh relevant for the antigen cannot be deduced from the published distribution of the antigen among *RH* haplotypes.