

REVIEW

Red cell membrane and erythropoiesis genetic defects

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In recent years, the frontline of red cell membrane research has shifted. Seeking new mutations in known genes has been taken over by the quest of new genes. It remains that many natural mutations, both in human and mice, have an irreplaceable heuristic value. For example, it is some of these mutations that eventually paved the way to the demonstration that the band 3 and the Rh complexes form a macrocomplex fulfilling, as one may assume, the function of a gas transport metabolon. Mapping and individualization of novel genes have been successful in inherited disorders of the membrane permeability to monovalent cations, such as dehydrated hereditary stomatocytosis and pseudohyperkalemia, and congenital dyserythropoietic anemias (CDAs). We herein included CDAs because the red cell membrane is abnormal in at least CDA I and II. A major breakthrough was the identification of the gene, the mutations of which cause CDA I. It encodes codanin-1. The occurrence of lipid rafts in the red cell starts being documented. GPI-anchored proteins and a number of minor proteins associated with the rafts allow foreseeing a chapter of great novelty in red cell membrane physiology.

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Introduction

By the second half of the last decade, genetic investigation on the red cell membrane changed its scope. The quest for unknown genes took over the elucidation of novel mutations in known genes. This is the reason why the last comprehensive list of mutations, concerning some major membrane proteins, have been published some years ago already.^{1,2} It is a pity because at least a set of natural changes brings about invaluable clues as to the structure–function relation within proteins. In parallel, interest partly shifted towards other diseases. Some of these, such as the hereditary stomatocytoses, stem from the changes within genes encoding membrane proteins which must be transporters of monovalent cations. Others, such as the congenital dyserythropoietic anemias (CDAs), show indirect membrane abnormalities, but the primarily altered genes do not encode membrane proteins, as is now demonstrated at least for CDA I.

Concerning 'classical' knowledge of the red cell membrane structure and abnormalities, the reader is kindly redirected to recent reviews.^{3,4} We will never-

theless mention a few significant mutations in humans, before considering natural or induced mutations in animals. We will then focus on new facts concerning (i) the macrocomplex achieved by the band 3 and the Rh complexes, (ii) the gene whose mutations are responsible for congenital dyserythropoiesis anemia, type I (CDA I), (iii) dehydrated hereditary stomatocytosis (DHS) and related disorders, and (iv) the lipid rafts.

Some remarkable natural mutations of the red cell membrane

Figure 1 is intended to help understand the organization of the red cell membrane.

Human mutations

In hospital practice, red cell membrane protein mutations are no longer elucidated at the gene level. This formidable task, owing to the size of many involved genes, is not warranted by the cost/medical benefit ratio. The molecular knowledge of such mutations also elicits a weaker scientific interest. Only in exceptional cases, when the severity of the disease in a child, for example, may indicate prenatal diagnosis in future pregnancies, is the search of

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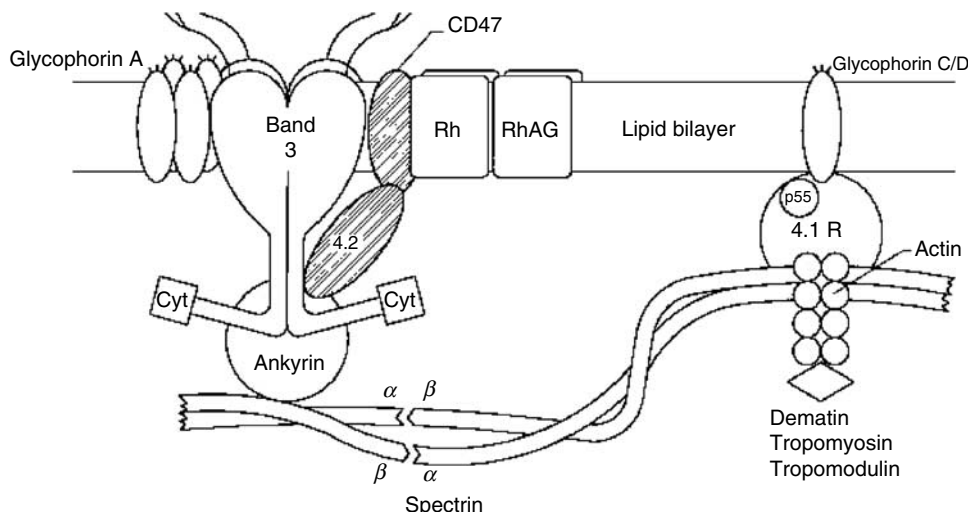


Figure 1 A simplified crosssection of the red cell membrane and skeleton. (A) The band 3 complex, centered by a band 3 tetramer. The bulky part of each monomer represents the transmembrane segment, towered by a long polylectosaminoglycan chain stemming from asparagine residue 642. The stalky, hinged part of band 3 monomer accounts for its cytoplasmic domain and is the anchoring part of the protein. Among the anchored proteins, one finds (i) ankyrin, which also binds to spectrin β -chain (C-terminal region), (ii) protein 4.2 (4.2) and (iii) a number of cytoplasmic proteins (cyt: deoxyhemoglobin, glyceraldehyde-3-phosphate dehydrogenase, aldolase and others). Glycophorin A, which also exists as a tetramer, is covered by numerous short, sialic acid containing glycans. (B) The Rh complex contains the Rh-polypeptides (Rh) and the Rh-associated glycoprotein (RhAG), arranged as a heterotetramer, CD47, glycoprotein B (not shown) and LW (not shown). A major recent finding is that CD47 interacts with protein 4.2, thus connecting the Rh complex and the band 3 complex into a macrocomplex. (C) The protein 4.1 (4.1R) complexes. On one side, 4.1R interacts in a triangular fashion with glycophorin C, covered by numerous short, sialic acid containing glycans and p55. On another side, 4.1R interacts with one extremity of spectrin tetramer through its β -chain (N-terminal region), in a region where actin oligofilaments are also found, as well as a bundle of actin-binding proteins (dematin, tropomyosin, tropomodulin and others (not shown)). (D) Spectrin. The $\alpha_2\beta_2$ tetramer of spectrin forms a dense network on the inner surface of the lipid bilayer. Both α - and β -chains are antiparallel. Two dimers associate side by side, a process initiated at the nucleation sites on both chains, not far from the C-terminal and N-terminal region of the α - and β -chains, respectively. They also associate head-to-head, N-terminal region of α -chain versus C-terminal region of β -chain, at the tetramerization (oligomerization) site in order to form tetramers and oligomers.

the mutation undertaken. Nevertheless, we will mention some recently discovered mutations that are interesting.

Band 3 Walton misses its 11 last amino acids.⁵ Two heterozygous patients were investigated. They presented with distal renal tubular acidosis (DRTA), but they were devoid of hematological signs. This is the paradoxical situation found nearly always with dominantly inherited DRTA mutations. It was shown that band 3 Walton is targeted more efficiently to the membrane in erythroid precursors than in the kidney. Yet the red cell band 3 was reduced by 20% (normal + mutated haploid sets) and this should have been enough to generate a spherocytosis, however mild.

A case of hereditary elliptocytosis was attributed to a double change in spectrin, both standing close to the oligomeric site.⁶ One α -chain mutation was known (CGT \rightarrow TGT; R28C). The other one was novel (GCC \rightarrow GAC; A2018D: β -chain Kuwaitino, altered at the same codon as spectrin Cagliari). It is unlikely that a dimer nor even a tetramer would harbor the two mutations at a time. The interaction of the α - and β -chains is quite alike in a dimer and in a tetramer, and a mutation on each partner chain would probably forbid any binding. Two populations of different tetramers, one mutated on the α -chain and the other on the β -chain, would rather be expected to coexist.

The abolition of the initiation codon of the β -spectrin messenger RNA (AUG \rightarrow GUG; β -spectrin Promissão) was recorded in a case of hereditary spherocytosis.⁷

This is reminiscent of the mutation that cancels the (downstream) initiation codon of protein 4.1 Madrid messenger RNA.

In most proteins, many regions have never been assigned any mutations associated with a pathological state. This has long been the case of the α -spectrin C-terminal segment. In a severe case of hereditary spherocytosis, we observed a splice site mutation in the C-terminal region of this chain. The mutation was 'assisted' by allele α^{LELY} in *cis* and allele α^{LEPRA} in *trans* (unpublished results). This alteration reminds of a natural mutation found in the mouse (see the *sph^l/sph^l* spherocytic mouse, in Table 1).

The latter observation leads us to consider the extreme rarity of severe hereditary spherocytosis cases because of homozygosity or compound heterozygosity for mutations in the *SPTA1* gene. Only one case was duly documented. It was the one in which allele α^{LEPRA} was discovered, precisely.⁸ Other cases, surely, have been overlooked or not fully investigated because of the task of sequencing the *SPTA1* gene (52 exons), which most hospital molecular genetic laboratories cannot afford.

Promoters of the genes encoding many red cell membrane proteins have only started being deciphered. Binding sites for GATA-1 and NF-E2 transcription factors have been identified in the 5'-region of the *SPTA1* gene.⁹ One would expect that it will take some time until promoter mutations in red cell membrane

Table 1 Natural mutations in the mouse

Gene and chromosome	Protein	Mutant strain	Mutation	Disease	Reference
<i>Spna1</i> Chromosome 1	Spectrin α -chain	<i>sph/sph</i>	Base deletion in repeat 5	HS	11
<i>Spna1</i> Chromosome 1	Spectrin α -chain	<i>sph^{Dem}/sph^{Dem}</i>	Insertion of an intracisternal particle element in intron 10. Exon skipping. In-frame deletion of 46 amino acids from repeat 5	HE and thrombophilia	12
<i>Spna1</i> Chromosome 1	Spectrin α -chain	<i>sph^{2BC}/sph^{2BC}</i>	Skipping of exon 41	HS	13
<i>Spna1</i> Chromosome 1	Spectrin α -chain	<i>sph^l/sph^l</i>	C→A transition in exon 52 (TGC→TGA). Missing of the last 13 amino acids	HS	13
<i>Spcb1</i> Chromosome 12	Spectrin β -chain	<i>jaja</i>	A→T; R1160X	HS	14
<i>Ank1</i> Chromosome 8	Ankyrin	<i>nb/nb</i>	Single base deletion	HS	15
?	?	<i>wan/wan</i>	See text	HS	16

HS: hereditary spherocytosis. HE: hereditary elliptocytosis.

genetic diseases are ascertained. Nevertheless, *in vitro* evidence has been gathered that a recessive form of hereditary spherocytosis could result from the T108C mutation in the *ANK1* gene promoter.¹⁰

Animals

Mouse natural mutations, most of which have been recently elucidated, may yield an important functional information (Table 1).

One remarkable finding is the thrombophilia associated with elliptocytosis in the *sph^{Dem}/sph^{Dem}* mouse. This may be brought together with the hypercoagulable state causing widespread thrombosis¹⁷ in a mouse invalidated for the *Slc4a1* gene (chromosome 11) encoding band 3.¹⁸ In the latter case, it was postulated that thrombophilia was because of a change in the lipid composition of the membrane leading to the exposure of phosphatidylserines on the outer leaflet, and allowing enhanced activation of the prothrombinase complex. We will see below the culmination of the hypercoagulable state in dehydrated hereditary stomatocytosis following splenectomy.

The *wan/wan* mouse¹⁶ is a natural variant strain of another mouse invalidated for the *Slc4a1* gene¹⁹ (band 3). An additional mutation, not yet located, lowers the reticulocytes count. It acts as a genetic modifier, aggravating the anemia, hence the name given to the strain.

Very elegant studies have been carried out in the Zebra fish. Identifying orthologs of human genes may be delicate. In a remote biological context, these orthologs are bound to play different roles and natural mutations will produce phenotypes distinct from phenotypes in humans. In Zebra fish *retsina*, the mutation alters the ortholog for human *SLC4A1* gene and produces a dyserythropoiesis.²⁰ No case of dyserythropoiesis have ever been attributed to mutations of the *SLC4A1* gene in man. In Zebra fish *merlot/chablis*, the mutation alters the ortholog of human *EPB41* gene, encoding protein 4.1R, and produces severe congenital anemia.²¹ However, what is the relation of this condition with the so well individualized 4.1 (–) hereditary elliptocytosis in man? In Zebra fish *riesling*, the

mutation lies in the ortholog of human *SPTB* gene that encodes β -spectrin, and produces ‘hereditary spherocytosis’.²² Again, how remote from the human hereditary spherocytosis is the hematological phenotype in the fish?

Fragments of some proteins have been obtained in a crystallographic form: the 30 kDa domain of protein 4.1R²³ and the cytoplasmic domain of band 3.²⁴ A tight symmetric dimer is formed stabilized by interlocked dimerization arms contributed by each monomer. The 3D location of several binding sites for peripheral proteins has started being assessed. Localization of mutations leading to altered cell shape and anion transport have also started being defined in 3D.²⁵

Study on mutations in hereditary spherocytosis leads to the concept of a macrocomplex resulting from the combination of the band 3 and the Rh complexes

It has long been assumed that the complexes formed by band 3 (including ankyrin, spectrin, protein 4.2, among others) and the Rh polypeptides (comprised, in particular, of the Rh-associated glycoprotein (RhAG), glycophorin B, LW and CD47) were linked. This linkage was proved recently and at least one of the linking proteins identified. The initial observation was made by Bruce *et al.*²⁶ A Pakistani patient whose parents are consanguineous presented with mild hereditary spherocytosis associated with the complete lack of protein 4.2. The latter resulted from a previously undescribed 41 bp frameshift deletion that removed the 5' region of exon 11 (allele Hammersmith of the *EPB42* gene). It stemmed from a cryptic acceptor splice site created by the G→T substitution at position 1747 (cDNA numbering). CD47, a protein associated with the Rh complex, was reduced to about 1% (and to 65% in the heterozygous mother) of its normal content. The Rh polypeptides level was normal. RhAG migrated with an apparently higher molecular weight, suggesting an overglycosylation state. It was surmised that CD47 was the (or one of the) long-sought link(s) between band 3 and the Rh complexes. On a stoichiometric basis (200 000 protein 4.2 molecules versus ca 20 000 CD47 molecules), it was anticipated that missing protein 4.2 was the primary event. As a matter of fact, no mutations were found in

CD47 (nor in band 3). The abnormal glycosylation of RhAG would suggest that it is chaperoned by protein 4.2 and/or CD47 throughout its way to the membrane.

Independently, Mouro-Chanteloup *et al.*²⁷ found very similar results, studying patients who carried allele Lisboa or allele Nancy of the *EPB42* gene (see Gallagher and Forget¹), both in the homozygous state and both bearing a one-base frame shift deletion. As in the previous case, CD47 was reduced (up to 60%, that is, not as much as in the preceding work). RhAG was also found overglycosylated. Curiously enough, these changes would not show in the 4.2^{-/-} mice.²⁸ Conversely, some humans carrying RhCE gene variants (D⁻, D[.] and R^N) and CD47^{-/-} mice²⁹ had no detectable effect on protein 4.2 and RhAG expression.

The membrane from a patient nearly completely devoid of band 3 (mutation Coimbra, V488M)³⁰ and from the band 3^{-/-} mice¹⁹ creates an earthquake in the band 3 complex but also in the Rh complex.³¹ Protein 4.2 is missing. Rh polypeptides, RhAG, CD47 and glycophorin B are reduced, and LW is absent. Rh polypeptides and RhAG coimmunoprecipitated with band 3 in human normal membranes. These results bring yet stronger evidence that the band 3 and the Rh complexes are organized as a macrocomplex. There must be a functional counterpart to this. It is suggested that the macrocomplex works as a CO₂/O₂ exchange unit metabolon. It must be recalled that carbonic anhydrase II itself binds to the 'small' cytoplasmic domain (C-terminal region) of band 3.

Things might be even more complicated. The protein 4.1R-glycophorin C-p55 protein complex appeared spared in membranes lacking protein 4.2, band 3 or both. p55 belongs to the family of signaling and scaffolding proteins MAGUKs and contains, among others, a guanylate kinase domain that might have evolved towards nonenzymatic functions. Using a two-hybrid screen of a human bone marrow cDNA library using p55 as a bait, Kuchay *et al.*³² observed that protein 4.2 was a binding partner. Deletional mutagenesis and yeast cotransfection showed that the guanylate kinase domain of p55 and the 240 C-terminal residues of protein 4.2 (opposite the binding site of protein 4.2 for band 3) were the region involved in the binding. These results indicate that there is some connection between p55 and protein 4.2. This link might not work in the assembled membrane, but during the targeting and sorting of protein 4.2, MAGUK proteins being mediators of intracellular trafficking.

In order to further emphasize the role of anchor protein played by band 3, we will mention that the latter is also the anchor for SHP-2 tyrosine phosphatase.³³ Tyrosine phosphorylation of band 3 elicited by pervanadate, *N*-ethylmaleimide or diamide enhances the recruitment of SHP-2 tyrosine phosphatase by band 3, through its SH2 domain. Docking of the SHP-2 tyrosine phosphatase to band 3 is a prerequisite for dephosphorylation of the latter.

It is classically held that hereditary spherocytosis can stem from mutations within five genes: *SLC4A1*, *ANK1*, *SPTA1*, *SPTB* and *EPB42*, encoding band 3, ankyrin,

α -spectrin chain, β -spectrin chain and protein 4.2, respectively. 4.2⁻ hereditary spherocytosis has slightly unusual features (ektacytometric profile, in particular). This can be brought together with its marginal position within the band 3 complex and its more pronounced closeness to the Rh complex (to which it is linked through CD47 as we have said). The Rh null syndrome may include a hemolytic anemia and abnormally shaped red cells. The latter alterations lie between spherocytosis and stomatocytosis, and display some increase of osmotic fragility;³⁴ however, these stigmata have not been thoroughly investigated. A number of associated mutations affect the *RhAG* gene which, therefore, appears as a sixth possible gene whose mutations can be viewed as causing HS, atypical as it might be.

Congenital dyserythropoietic anemia

Congenital dyserythropoietic anemia, type I, is the first disease of this group to be related to a gene

CDA I is a very rare genetic condition. It includes dysplastic changes in the late erythroid precursors: internuclear chromatin bridges, spongy heterochromatin, and invagination of the nuclear envelope carrying cytoplasmic organelles into the nucleus. The bone marrow is hyperactive, but the output of red cells is decreased. CDA I may be associated with a series of dysmorphologies of the skeleton. It is a heavily 'iron-loading' condition.

Red cells that nonetheless undergo egress into the circulation show a number of altered shapes, such as elliptocytosis, dacryocytosis and poikilocytosis. This indicates that the mature erythrocytes are themselves abnormal and account for the hyperhemolysis that aggravates the anemia of central origin. The red cell morphological changes are associated with a small but significant reduction of protein 4.1R (unpublished data). It is sufficient to alter the erythrocyte shape. It is part of the dyserythropoietic process but must occur quite downstream in the chain of events stemming from the primary genetic lesion. Membrane changes are the reason why we introduced CDA I in the minireview. They provide an unknown yet simple and reliable help to the diagnosis.

The *CDANI* gene whose mutations are responsible for CDA I was mapped to q15.1–15.3 by Tamary *et al.*³⁵ Homozygosity mapping was used in a cluster of 45 highly inbred Israeli Bedouins living in the Negev Desert and constituting an isolate. Recently, the *CDANI* gene was identified.³⁶ It is comprised of 28 exons and encodes a ubiquitously expressed mRNA of 4738 bases. The encoded protein has been termed codanin-1 and contains 1226 amino acids. It is expected to be *O*-glycosylated. The first 150 residues of codanin-1 display sequence similarities with collagens and contain two short segments showing a weak resemblance with microtubule-associated proteins, MAP1B (neuraxin) and synapsin. Most Bedouins were homozygous for

mutation C3238T (exon 24; R1040W); however, a few of them were compound heterozygotes for this mutations and mutation G2719A (exon 19; V867M). A Polynesian patient carried the C3503T mutation (P1129L) in the homozygous state. Patients of European origin were usually compound heterozygotes. For the time being, the second mutation remained elusive in one patient and no mutations were found in monozygotic twins. Putative mouse and *Fugu* orthologs of codanin-1 were identified.

In a different line, a treatment of CDA I has been found serendipitously.³⁷ α -Interferon, used to cure hepatitis C in a patient, turned out to be efficient in the CDA I which the patient also suffered from. Thereafter, a number of reports confirmed the efficiency of α -interferon at different ages of life,^{38,39} and references quoted thereof. During its periods of efficiency, which show intermittence,⁴⁰ transfusion needs are suppressed, a precious advantage in this heavily 'iron-loading' condition. How long α -interferon treatment can go on without complications is a pending issue.

Congenital dyserythropoietic anemia, type II, and at least some congenital disorders of glycosylation share an abnormal glycosylation of band 3

It has long been known that CDA II is a very rare genetic condition. There are binucleated erythroblasts in the bone marrow. CDA II, which is a purely hematological condition, displays major changes in the N-glycosylation of band 3.⁴¹ Such a stigmata is easy to show and is a pathognomonic sign of CDA II. This is the reason, again, why we introduced CDA II in this minireview. The *CDAN2* gene has been mapped,⁴² but not yet identified. Given the glycosylation abnormalities of band 3 and of membrane glycosphingolipids, too,⁴¹ it has been thought that some enzyme involved in the building up of the glycan moiety were the primarily mutated proteins. Despite many efforts and the exclusion of some genes,⁴³ the question remains pending.

A contrasted parallel can be drawn, tentatively, between CDA II and the congenital disorders of glycosylation (CDGs). CDGs designate a group of rare inherited conditions with multisystemic lesions, particularly deficiencies of the central and the peripheral nervous systems.⁴⁴ The CDG I group refers to the defects in the assembly of the dolichol-linked glycan and its transfer to the proteins (endoplasmic reticulum). The CDG II group deals with the defects in the processing of protein-bound glycans. There are no known hematological symptoms in CDGs.

In the latest case of CDG I discovered, CDG Ig, associated with a homozygous mutation in the human ortholog of yeast gene *alg12*, encoding a dolichol-P-Man:Man₇GlcNAc₂-PP-dolichol α ,1-6mannosyltransferase of the endoplasmic reticulum, a major electrophoresis abnormality of band 3 was recorded, still in the absence of any hematological symptoms.⁴⁵ Glycosylation abnormalities of band 3 have also been reported in cases of CDG Ia⁴⁶ and CDG IIa.⁴⁷ Supposing that CDA

II results from mutations in a gene involved in glycosylation, there would be a sheer contrast between this purely hematological condition in which band 3 is misglycosylated, and these multisystemic CDGs in which band 3 is misglycosylated, although differently, without yielding any hematological signs. Boldly speaking, would not CDA II be a CDG restricted to the erythroid cell line?

Dehydrated hereditary stomatocytosis is part of a pleiotropic syndrome that extends beyond haematology

Dehydrated hereditary stomatocytoses (DHS)⁴⁸ is a rare hemolytic anemia characterized by abnormally shaped red cells, a substantial increase of the permeability, also termed passive leak, to monovalent cations across the membrane, a typical shape of the curve: leak versus temperature, and a highly specific ektacytometric profile. For routine diagnostic purposes, ektacytometry represents a very discriminative, reliable and straightforward tool. It shows an increase of the osmotic resistance and a state of cellular dehydration.

If DHS is a paradigm of genetic disorders of membrane permeability to monovalent cations, these conditions also include overhydrated hereditary stomatocytosis (OHS) and familial pseudohyperkalemias (FP). OHS is an extremely rare disease, which should be regarded as very different from DHS on a pathophysiological basis, presumably. We still ignore where the corresponding gene maps. The long recorded lack of band 7.2b, or stomatin, encoded by the *EPB72* gene (9q23.2), is a consequence of the primary alteration (absence and/or qualitative change of another protein, yet to be found). Mice invalidated for the murine ortholog of the *EPB72* gene lack band 7.2 but show no OHS.⁴⁹ Here, we will not deal further with OHS. Let us recall that pseudohyperkalemias are characterized by an increase of kalemia when freshly drawn blood is allowed to stand at room temperature for some hours, whereas kalemia is normal *in vivo*.

We will now show that DHS and FP represent a group ongoing permanent nosological fusion and fragmentation at a time. As for the fusion, DHS1 and FP1, as we will call them from now on (see below), are thought to be different clinical forms of the same genetic entity. Both DHS1 and FP1 map to 16q23-qter, as was found in an Irish family⁵⁰ and a Scottish kindred,⁵¹ respectively. Besides, DHS1 may be associated with pseudohyperkalemia, of the essence of FP1 as we assume, and consequently named pseudohyperkalemia 1. As for the fragmentation, at least in one case of DHS (with pseudohyperkalemia),⁵² and in a case of FP, discovered in a French family of Flemish descent, mapping to 16q23-qter was excluded (unpublished data). These novel genetic entities have been tentatively coined DHS2 and FP2, respectively. The '2' digit is misleading, however, because DHS2 and FP2 map to different chromosomes (unpublished data) and are

definitely different entities. In addition, a number of DHS and/of FP cases have been found with distinct phenotypical features, concerning mostly the curve of the leak as a function of temperature.^{53–56} This inflation of phenotypes is puzzling. It sounds doubtful whether there is such an inflation in tow at the gene level. On the whole, the number of genes that cause DHS and/or FP is urgent to establish. All the conditions mentioned above have a dominant inheritance pattern.

We will now see that mutations in the 16q23-qter locus produce, more broadly, a pleiotropic syndrome in which DHS1, in some kindreds, is associated with pseudohyperkalemia 1, as has been mentioned, and/or a completely unexpected manifestation: various perinatal fetal fluid effusions. Following earlier observations, Grootenboer *et al.*⁵⁷ showed that DHS1, pseudohyperkalemia 1 and perinatal fetal fluid effusions constitute a novel pleiotropic syndrome (registered as Mendelian Inheritance in Man #603528). Edema is a transient manifestation that spontaneously dries out within weeks following birth if not prior to it. It thus stands in sheer contrast with life-long DHS1 and pseudohyperkalemia 1, if present. The severity of the fluid effusions is variable, ranging from a mere sonographic discovery⁵⁸ to a severe⁵⁹ or very severe pictures (unpublished data). *In utero* transfusions are of no avail. The effusions are a phenotypical equivalent of anemia. They do not result from anemia because anemia is not pronounced enough. Fluid effusions are to be drained in order to avoid mechanical hindrances.

An important observation was made by Stewart *et al.*⁶⁰ in DHS. Splenectomy, which is readily indicated in severe hemolytic anemias, has highly deleterious effects in DHS1 (and, puzzlingly enough, in OHS too). It nearly systematically leads to thromboembolic accidents, which may be lethal. In the kindreds investigated by Grootenboer *et al.*,⁵⁷ all splenectomized DHS1 patients had presented with such complications. Recently, we dealt with a patient with DHS1 and heterozygous hemoglobin S. Ironically, she had to be splenectomized in emergency because of a spleen infarction having occurred during a flight. After 19 years, repeated pulmonary embolies had ended up in a cor pulmonale. A heart–lung transplantation was successfully carried out (unpublished data).

Lipids rafts

Lipid rafts are membrane microdomains. Their sizes are ill-defined and would range between 70 and 300 nm. Rafts are insoluble in nonionic detergents ('detergent-resistant membrane' or DRM). They are rich in

sphingolipids, which can pack to one another because they contain long and largely saturated acyl chains and cholesterol.⁶¹ They participate in signal transduction in hematopoietic cells and in sorting of glycosylphosphatidylinositol (GPI)-anchored proteins. In the red cell membrane, they harbor an increased amount of GPI-proteins, as well as stomatin, flotillins 1 and 2.⁶² Flotillins 1 and 2, known as lipid raft proteins in neurons and as caveolae-associated proteins in A498 kidney cells, thus also exist in the red cell membrane. Stomatin and the flotillins proteins are organized in higher-order oligomers. They might act as separate scalloping components at the cytoplasmic surface of lipid rafts. Flotillins 1 and 2 are normal in OHS.⁶²

The elevation of cytoplasmic $[Ca^{2+}]$ activates the phospholipid scramblase and inhibits the aminophospholipid translocase, thus leading to the disruption of phospholipid asymmetry. The Ca^{2+} -activated K^+ efflux via the Gardos channel causes loss of cell water. The increase triggers the shedding of microvesicles (ca 150 nm diameter) and of nanovesicles (ca 60 nm diameter) from the red cell membrane. Red cell vesiculation process might be a protection strategy against the destruction by complement. Attack by C5b through C9 triggers local Ca^{2+} influx, to which the erythrocyte responds by the release of vesicles containing the attack complex.

The vesicles contain stomatin-specific lipid rafts.⁶³ They are rich in GPI-proteins (acetylcholinesterase, decay accelerating factor (CD55), transmembrane complement receptor I (CD35)), but are devoid of skeletal proteins and of most of the transmembrane proteins. They also contain synexin (annexin VII), a critical protein for membrane fusion and consequently for vesiculation, and sorcin. Red cells of patients with paroxysmal nocturnal hemoglobinuria have an impaired ability to vesiculate.⁶⁴

Future lines of research

The search of unknown genes appears as a priority, opening a large field for molecular and cellular biology investigations under normal and abnormal conditions. The supramolecular organization within the red cell membrane will eventually lead to a very complex and intricate picture. There is much to decipher in the molecular events underlying erythropoiesis and the factors that induce it. No doubt that the membrane of erythroid precursors is deeply involved all along. The lipid rafts are leading to an area of high conceptual novelty.

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