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REVIEW

Erythrocyte membrane proteins and membrane skeleton

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Abstract Considerable advances in the research field of erythrocyte membrane were achieved in the recent two decades. New findings in the structure-function correlation and interactions of erythrocyte membrane proteins have attracted extensive attention. Interesting progress was also made in the molecular pathogenesis of erythrocyte membrane disorders. Advances in the composition, function and interaction of erythrocyte membrane proteins, erythrocyte membrane skeleton, and relevant diseases are briefly described and summarized here on the basis of domestic and world literatures.

Keywords erythrocyte, membrane proteins, interaction, functional disorders, membrane skeleton

1 Introduction

An important function undertaken by erythrocytes in blood is to discharge the useless and intake the useful. The oxygen is transported from the lungs to tissues, whereas the CO_2 produced in tissues is transported back to the lungs and then exhaled. The erythrocyte is coated externally with a single layer of plasma membrane, without inner membranes and a nucleus. Its cytoplasmic contents are hemoglobin (Hb) and a small amount of intracellular proteins. A normal erythrocyte displays the shape of biconcave disc, with a diameter of 7 μm. The flexible morphology of erythrocytes permits them to pass through capillaries of smaller caliber under the squeezing pressure. In the circulating blood, they resist the shearing force from blood vessels and visceral walls, thus avoiding fragmentation. Their characteristic flexibility and deformability originate from the support of membrane structures and cytoskeleton.

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Significant advances in the structure–function correlation of erythrocyte membrane proteins and studies on the molecular basis of erythrocyte membranopathies were achieved in the recent two decades. In this review, the status quo of the following four aspects is concisely discussed, human erythrocytes are explored as its main line, erythrocytes of some mammals are also involved.

2 Erythrocyte membrane proteins (Lu and Liu, 1987, 1990; Tse and Lux, 1999)

Erythrocyte membrane is a kind of complex structure containing multiple elements. It belongs to the bilayer membrane composed of various proteins and lipids. The nomenclatures and ordinal numbers of the erythrocyte membrane proteins are assigned according to the mobility of their bands during separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1).

In general, erythrocyte membrane proteins can be divided into two groups.

2.1 Peripheral proteins

2.1.1 Spectrin (Gallagher and Forget, 1993)

Spectrin (Sp) comprises 25%–30% of total membrane proteins and is composed of equal quantity of two subunits, that is α-Sp (band1) and β-Sp (band 2) encoded by different genes. Each erythrocyte contains about 200,000 copies of α and β Sp. α and β-Sp chains intertwine in an antiparallel fashion, forming the heterodimer.

Two molecules of this heterodimer self-associate from head to head to produce a tetramer (Fig. 2A). Sp heterodimer manifests a rod-like flexible filament, approximately 100 nm in length. The molecular weight of α-Sp is 240 kDa, carrying with 20 homologous 106-amino acid repeat segments and two non-homologous segments: while the molecular weight of β-Sp is 220 kDa, carrying with 17 homologous 106-amino acid repeat segments, a non-homologous amino terminal

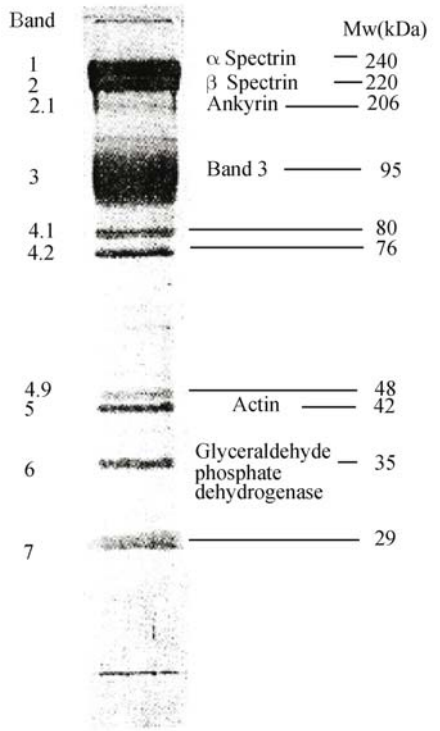
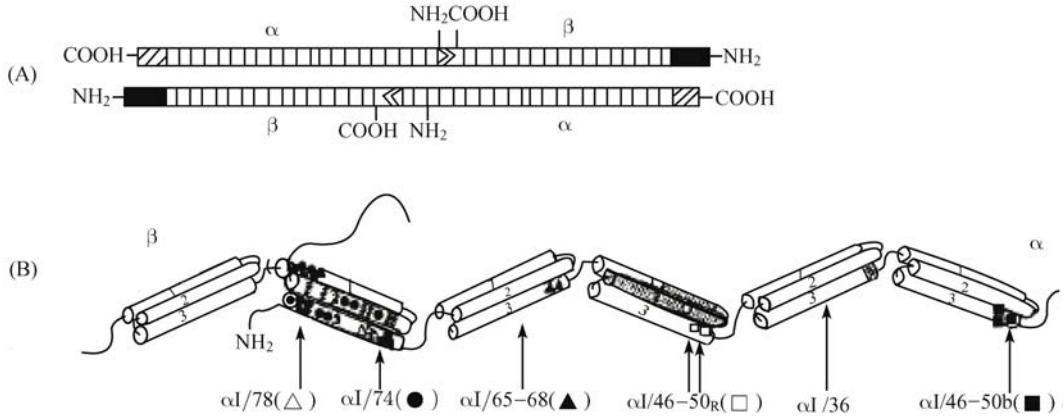


Fig. 1 SDS–polyacrylamide gel electrophoretic pattern of the erythrocyte membrane proteins stained with Coomassie blue (glycophorins are not shown) (Stryer, 1988).

segment containing an actin-binding domain, and a short non-homologous carboxyl terminal segment containing a consensus sequence for a casein-phosphorylation site. A limited trypsin cleavage of Sp can separate the α and β-Sp chains into five and four domains (called as α I-αV and β I-β IV), respectively. The homologous 106-amino acid segment of both α and β-Sp can fold into α helical repeats composed of three antiparallel segments, connecting by short non-helical segments (Fig. 2B). In the repeated segments, several amino acid residues seem to be highly conserved, e.g., Tpr-45 and Leu-26 are usually invariable. Other conserved hydrophilic amino acid residues include Arg-22, Asp-38, His-72, and His-101. Dependent on its interaction with membrane proteins, the functions of Sp are normal maintenance of erythrocyte shape, regulation of lateral motion of integral membrane proteins, and offering structural support to the lipid bilayer. In non-erythroid cells, Sp may be related to the promotion and maintenance of local concentration of plasma membrane proteins, participation in the early stages of cell junction formation and regulation of entrance of secretory vesicles into the plasma membrane. The full-length cDNAs encoding human erythrocyte α and β-Sp have been isolated and identified. The chromosomal location of α-Sp gene is at 1q22–q23, with its length around 80 kb. There are a total of 52 exons encoding 2429 amino acids, the predicted molecular weight of the expressed peptide is 280 kDa. The chromosomal location of β-Sp gene is at 14q23–q24-2, its length exceeds 100 kb. There are about 32 exons encoding 2137 amino acids, the predicted molecular weight of the expressed peptide is 246 kD.

2.1.2 Ankyrin (or Band 2.1) (Peters and Lux, 1993; Palek, 1995)

Band 2.1 was found in a search for the membrane attachment site of erythrocyte spectrin and named as ankyrin. The entire family, including bands 2.1, 2.2, and 2.3, has been referred as ankyrin by some early authors, but it was disused later on. Human ankyrin belongs to an asymmetrical polar protein composed of 1881 amino acid residues, with a molecular weight of 206 kDa. It is actually a mixture of proteins with different size, containing three functional domains defined by chymotrypsin cleavage: 1) the 89-kDa N-terminal domain extends from Pro-2 to Phe-827 and involves the binding site



(A): Formation of Sp heterodimer. The N-terminus of the α unit interacts with the β unit in an antiparallel fashion to form Sp heterodimer. Two Sp dimmers interact in a head-to-head fashion to form a Sp tetramer (Gallagher and Forget, 1993). (B): Triple helical model of the αβ-Sp self-association contact site and four adjacent α-Sp repeats. Symbols (e.g. □□,▲▲, etc.) denote genetic defects identified in patients with hereditary elliptocytosis (HE) or hereditary pyropoikilocytosis (HPP). The abnormal tryptic cleavage sites in Sp associated with different mutations are shown by arrows (Gallagher, 2004).

Fig. 2 A schematic model of spectrin heterodimer self-association

for band 3 and tubulin; 2) the central 62-kDa domain begins at Lys-828 and ends at either Leu-1382 or Tyr-1386 or both. It contains the binding sites for β-Sp and the intermediate filament proteins vimentin and desmin. Sp is anchored on the cell membrane via these bindings; 3) a functional 55-kDa domain formed from the remaining 495 th–499 th C-terminal amino acids. It participates in the widely selective processing of mRNA and modulates the interaction of ankyrin with Sp and band 3. This domain can affect the hydrodynamic properties of ankyrin and is extremely sensitive to proteases, suggesting that it may be more loosely assembled and its last 20-kDa segment possibly extends from the ankyrin molecule like a tail.

2.1.3 Protein 4.1

Protein 4.1 amounts to about 6% of the total membrane proteins and can be separated by PAGE into two isoforms (4.1a and 4.1b) with molecular weights of 80 kDa and 78 kDa, respectively. Protein 4.1 gene is located on chromosome 1 adjacent to Rh protein locus, and usually encounters alternative mRNA processing that shows cell differentiation and tissue-specific nature. Protein 4.1 possesses two important functions: 1) its 10 kDa-Sp-binding domain interacts with the N-terminal region of β-Sp to promote the binding of Sp with actin; 2) its N-terminal domain binds with GPC and the negatively charged lipids in the inner hemi-leaflets of lipid bilayer, thus causing the distal ends of Sp tetramer to attach to the erythrocyte membrane.

2.1.4 Actin (or band 5)

Actin is an important protein required for muscular contraction and cell motion. Each erythrocyte contains $40\sim50 \times 10^4$ actin monomers, which can be packed into short protofilaments of F-actin composed of 12–14 monomers within erythrocyte membrane, 30–40 nm in length. β-actin takes part in the formation of junctional complex (JC) (refer to the next section).

2.1.5 Protein 4.2

Protein 4.2 is present in almost same number of copies as Sp or ankyrin. It is associated with the cytoplasmic domain of band 3, ankyrin, and protein 4.1, but its function is unknown. A complete deficiency of protein 4.2 may cause spheroovalocytosis and hemolytic anemia, suggesting that it plays a certain role in the maintenance of erythrocyte membrane integrity.

2.2 Integral proteins

2.2.1 Band 3 (Lu and Liu, 1987; Palek, 1995; Gallagher and Forget, 2001)

Band 3 is the major transmembrane protein of erythrocytes, comprising about 25% of the total membrane proteins. Each erythrocyte contains 10^6 copies of band 3 protomer (molecular weight 95 kDa). Various forms of band 3 exist in erythrocyte membrane, including homodimer, tetramer or oligomer; its polymorphism appears approximately in 6% of population. Two essential functions of band 3 are 1) transmembranous transport of anions: with the help of "chloride shift", Cl^-/HCO_3^- exchange across the erythrocyte membrane results from the "ping-pong" mechanism of conformational conversion involving a membrane transporter; hence, the CO_2 released in tissue metabolism is conveyed to lungs and 2) it provides the binding sites for ankyrin, protein 4.1, and 4.2. Moreover, it binds with some glycolytic enzymes, Hb, and hemichromes. These two functions described above are mediated by two specific domains: The 40 kDa N-terminal ankyrin-binding domain is located within the cytoplasm, whereas the C-terminal 55 kDa anion transport portion spans across the erythrocyte membrane for 13 to 14 times. The N-terminus of band 3 hides deeply inside the cytoplasm, with its C-terminus extended outside the membrane. Band 3 is glycosylated at a single external site (Asn-642).

2.2.2 Glycophorin (Lu and Liu, 1987; Palek, 1995; Gallagher and Forget, 2001)

Sixty percent of Glycophorin (GP) molecules consist of saccharides. The N-terminus of its polypeptide chain protrudes beyond the erythrocyte membrane. The majority of 16 scattered oligosaccharides contain trioses and tetroses on their side chains, with sialic acid as end sugar residues. Using periodic acid-Schiff (PAS) staining after electrophoretic separation, erythrocyte membrane of healthy subjects displays 4~5 GP bands. Human GPs belong to transmembrane sialoglycoprotein families, including GPs A, B, C, D (or called α, δ, β, γ GPs), and GP E. GPs A, B, and E carry the MNSs blood group antigens: while GPs C and D carry the Gerbich (Ge) blood group antigens. The structures of all five GPs and their encoding genes were elucidated, with molecular weights in a range of 17–36 kDa. High degree of homology is found in the amino acid sequences of primary structures among GPs A, B, and E. Comparing the first 26 amino acid residues in the extracellular region, GP B and GP E are identical with GP A^N and GP A^M, respectively. Both of them only carry an extracellular region shorter than that of GP A and their cytoplasmic sequences are entirely missing. GP E is a truncated GPB lacking of S/s antigenic segment. The α, δ and ε genes encoding GPs A, B, and E are located on q31-34 of chromosome 4. They pertain to the autosomes of codominant heredity, which are organized in clusters, with spanning sequence of approximate 330 kb in order of 5′–GP A–GP B–GP E–3′. According to the information reported by world literatures, 25 variants were detected in GP families, most of them originate from unequal homologous recombination or micro-conversion between α GP genes and δ GP genes. Five types of GP variants were also identified in Southern China (Lu and Liu, 1999).

Table 1 Major erythrocyte membrane proteins and their genes (Palek, 1995; Gallagher and Forget, 2001)

Band	Memb. Proteins	Mw(gel) /kDa	Mw (calc.) /kDa	Copies per cell /($\times 10^3$)	% of total	Gene symbol	Chrom. localization	Amino acids	Gene size/kb	Number of exons	Involvement in HA
1	α-Spectrin	200	280	240	16	SPTA11	1q22-q23	2 429	80	52	HE, HS, HPP
2	β-Spectrin	220	246	240	14	SPTB14	14q23-q24.2	2 137	>100	~32	HE, HS
2.1	Ankyrin (2.1 isoform*)	210	206	120	4.5	ANK18	8p11.2	1 881	>100	40	HS
	α-Adducin	103	81	30		4	Chrom. 4				N
	β-Adducin	97	80	30							N
3	Band 3	90–100	102	1 200	27	EPB3.17	q21-qter	911	17	20	HS, SAO
4.1	Protein 4.1	80	66	200	5	EL11	1p33-p34.2	588	>100		HE
4.2	Pallidin	72	77	200	5	EPB42.15	q15-q21	691	20	13	HS
4.9	Dematin	48 + 52		40	1						N
5	β-Actin	43		400–500	5.5	ACTB7	pter-q22				N
	Tropomodulin	43	41	30		TMOD 9	q22	359			N
6	G-3P-D	35	37		3.5	GAPD 12	p13.31−p13.1				N
7	Stomatin	31	32		2.5				12	9	HST
	Tropomyosin	27+29		80		TPM 31	q31				N
PAS-1	GP A	36		500~1000	85	GYPA4	4q28-q31	131	>40	7	N(E)
PAS-2	GP C	32	14	50~100	4	GYPC2	q14-q21	128	14	4	HE
PAS-3	GP B	20		100~300	10	GYPB4	4q28-q31	72	>30	5	N
	GP D	23		20	1	GYPC2	q14-q21	107			N
	GP E					GYPE4	4q28-q31	59	>30	4	N

Notes: Mw: molecular weight; HS: hereditary spherocytosis; HA: hemolytic anemia; HE: Hereditary elliptocytosis; (E): one case of elliptocytosis associated with an abnormal GP A was reported (Lu et al., 1992); HPP: hereditary pyropoikilocytosis; SAO: Southeast Asian ovalocytosis; HST: hereditary stomatocytosis; G-3P-D: glyceraldehyde-3-phosphate dehydrogenase; N: hematological abnormality not reported:* Bands 2.1, 2.2, 2.3 and 2.6 are protein isoforms of erythroid ankyrin.

The major erythrocyte membrane proteins are listed in Table 1.

3 Erythrocyte membrane skeleton (Gilligan and Bennett, 1993; Palek, 1995; Gallagher and Forget, 2001)

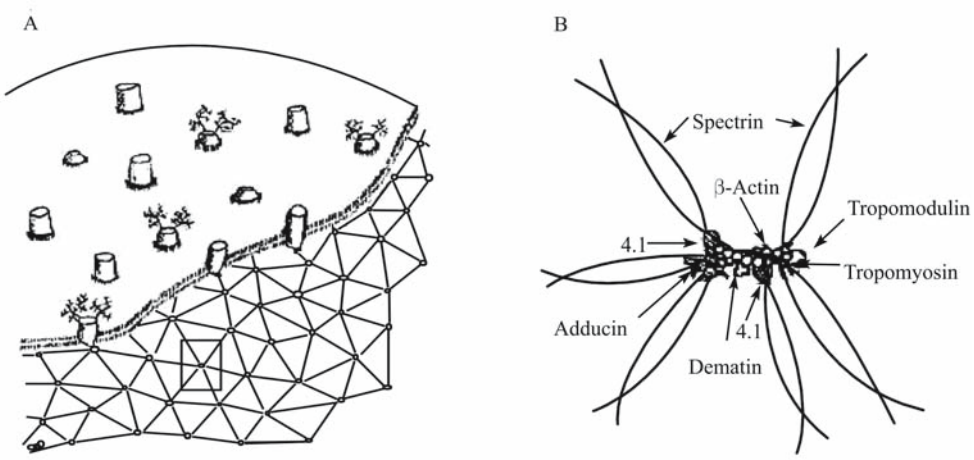
In the intact membrane, almost 60% of the lipid bilayer is directly covered by the underlying membrane skeleton. If the membrane skeleton is evenly spread out, the individual skeletal proteins would display a hexagonal lattice in distinct order. The basic structure of erythrocyte membrane skeleton is a polygonal crystal lattice (mainly as hexagon) composed of Sp tetramers arranged in long chain, cross-bridging the profilaments of short actin oligomers. Globular structures formed on the vertices of two-dimensional polygonal arrays are called junctional complexes (JC). The vertices are linked each another by the Sp tetramers, each JC can bind with 4~7 Sp molecules and 12 actin molecules. This complex connects with GP via protein 4.1, whereas Sp binds to band 3 through ankyrin. The stability of F-actin is strengthened with the help of tropomyosin. Owing to the participation of accessory proteins, such as adducin, tropomodulin and dematin, the crystal lattice mentioned above is firmly anchored on the molecules of integral membrane proteins (Fig. 3). Adducin is composed of equal amounts of an α subunit (103 kDa) and a β subunit (97 kDa), undertaking as an assembly factor for construction of the Sp-actin hexagonal array. The structural model of adducin proposed recently is an $\alpha_2\beta_2$ tetramer with a globular head domain at their N-termini and is connected by a neck region to the extended tails of the C-termini. Each erythrocyte contains 30,000 molecules of adducin. The gene encoding human α-adducin is located on chromosome 4, while that for gene encoding β-adducin has not been determined. Originally, dematin is implied to three polypeptides consisting of erythrocyte membrane protein 4.9, they can promote actin bundling. The purified protein 4.9 is actually dematin, containing only two polypeptides with molecular weights of 48 kDa and 52 kDa. Both of them effectively cause actin bundling in vitro (Shen, 1990). All constituents of JC are also found in non-erythroid cells and tissues. Some proteins of JC are essential substrates for phosphorylation, regulating the assembly and disassembly of membrane skeleton.

4 The interactions of erythrocyte membrane proteins (Palek, 1995; Tse and Lux, 1999; Gallagher and Forget, 2001)

According to the interrelation of these membrane proteins with the erythrocyte planes, the interactions can be divided into two categories (Fig. 4).

4.1 Vertical interactions

They are perpendicular to the plane of erythrocyte membrane, participating in the adhesion of skeletal network structure with integral membrane components.



(A): The cell surface is observed from the extracellular space, while the lipid bilayer is cut away to expose the nearly hexagonal array of the underlying membrane skeleton. The boxed area is enlarged in (B). (B): Composition of the JC. The vertex of the hexagonal array is the short actin profilament. Molecules involved in stabilizing the profilament include tropomyosin, tropomodulin, adducin, protein 4.1 and dematin, etc.

Fig. 3 A schematic diagram of erythrocyte membrane skeleton (Gilligan and Bennett, 1993)

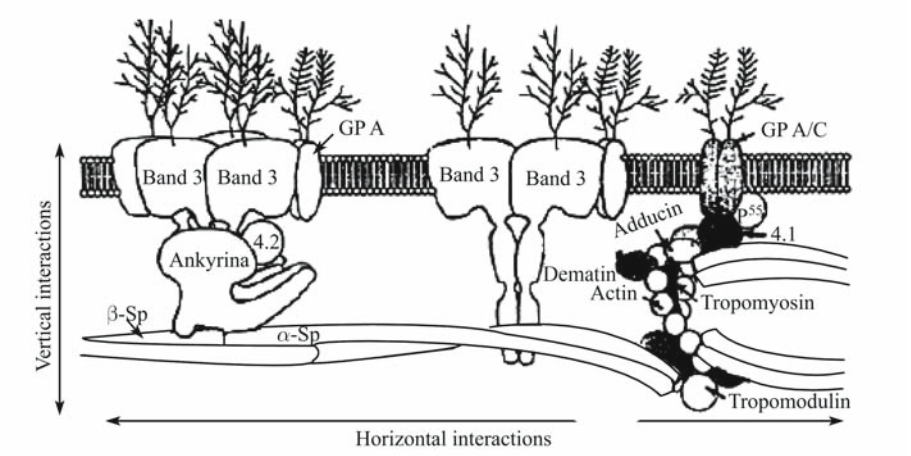


Fig. 4 The interactions and associations of human erythrocyte membrane proteins (Tse and Lux, 1999)

4.1.1 Sp-ankyrin-band 3 contact

Near the central region of Sp tetramers, β-Sp is bound to ankyrin, the 89-kDa domain at N-terminus of the latter further attaches to a specific site in the cytosol of band 3.

4.1.2 Sp-protein 4.1-GP C / A contact

At the distal end of Sp tetramers, Sp binds to the erythrocyte membrane via linkage to the protein 4.1, which is in adhesion to transmembrane GP C or possibly GP A. In spite of the weak bindings of Sp and protein 4.1 with phosphatidyl-serine (PS) located preferentially at the inner leaflets of lipid bilayer, these vertical protein-protein and protein-lipid bilayer connections are very crucial for stabilization of the lipid bilayer, preventing its loss from the erythrocytes.

Three major hypotheses have been proposed recently to explain the role of integral proteins in maintaining membrane stability (Van Dort et al., 2001; Goni, 2002): 1) their ability to anchor the membrane skeleton to the lipid bilayer, e.g. band 3 and GP C; 2) serving as "lipid anchors", they possess the capacity to bind and stabilize membrane lipids. Hence, the spontaneous fragmentation and vesiculation of membrane are avoided; 3) their ability to affect and regulate local membrane curvature. However, the impact from deficiencies of various integral membrane proteins on the membrane morphology and stability are quite different. When all 10^6 copies of band 3 are missing from the cattle erythrocyte membrane, the membrane would become very unstable and vesiculates in response to mechanical stress (Inaba et al., 1996). In human erythrocytes deficient in GPs C and D, their membrane mechanical stability and deformability decrease to 50% and 40% of normal levels, respectively; while those deficient in GPs A and B still maintain normal membrane stability and deformability (Reid et al., 1987). Although addition of 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), an inhibitor

of band 3-mediated anion transport, has changed the morphology of treated erythrocytes, but their membrane mechanical properties are not significantly affected. Because the experimental results of these authors and other investigatior cannot reconcile with any single hypothesis on membrane stability described above, it suggests that more than one hypotheses may provide a reasonable explanation (Van Dort et al., 2001). The dissociation of protein 4.1 from GPC in Sp/actin-protein 4.1-GPC contact is promoted by using five kinds of experimental approaches, the function of stabilizing Sp-actin junction is not interfered (Chang and Low, 2001). Detection of membrane through ektacytometry (Xie et al., 2002) and nickel mesh filtration demonstrates that rupture of the membrane-to-skeleton bridge has little or no impact on the mechanical properties of erythrocytes (Chang and Low, 2001). Band 3 does not regulate the assembly of erythrocyte membrane skeleton in vivo, but it is essential for membrane stability (Peters et al., 1996). Band 3 oligomers are converted slowly to dimmers and ultimately monomers following removal of ankyrin. Addition of excess ankyrin back to these membranes enriched in dissociated band 3 would shift band 3 almost entirely to its tetramer, thus confirming that band 3 tetramer constitutes the preferred oligomeric state for ankyrin binding (Van Dort et al., 1998).

4.2 Horizontal interactions

It runs parallel to the plane of erythrocyte membrane, maintaining the structure of submembranous network in bi-directional continuity.

4.2.1 Sp heterodimer contact (SpD–SpD)

Sp tetramers are formed by the head-to-head association of pairs of heterodimers attached at their ends to JC of several proteins. Although a modest shearing force is well below that experienced by the erythrocytes in the circulation, yet it is sufficient to sever SpD-SpD link in the membrane network. Therefore, this fact implies that erythrocyte membrane accommodates the enormous distortion imposed there during the passage of erythrocytes through the micro-vessels by means of local dissociation of SpD-SpD to dimers. The membrane network in situ is in a dynamic state and undergoes a "breathing" action of tetramer dissociation and reformation (An et al., 2002).

4.2.2 SpD-protein 4.1-actin interaction

With the assistance of protein 4.1 and adducin, SpD binds the JC-containing actin at its ends, forming horizontal interaction to maintain the structural integrity of erythrocytes, so erythrocyte membrane obtains high strength of tension resistance.

5 Several diseases of erythrocyte membrane proteins (Gallagher and Forget, 1993; Palek, 1995; Tse and Lux, 1999; Gallagher and Forget, 2001)

As early as more than 10 years before, Palek and Lux pointed out that defects in horizontal interactions of erythrocyte membrane proteins lead to HE or HPP, whereas defects in their vertical interactions would cause HS. Having identified and followed up the various defects bringing about abnormalities of erythrocyte membrane recently, this hypothetical model of membranopathies is confirmed to be basically correct (Fig. 5).

5.1 Hereditary spherocytosis (HS)

In dominant HS, frameshift and nonsense mutations of ankyrin, band 3 and β-Sp predominate, while recessive HS is

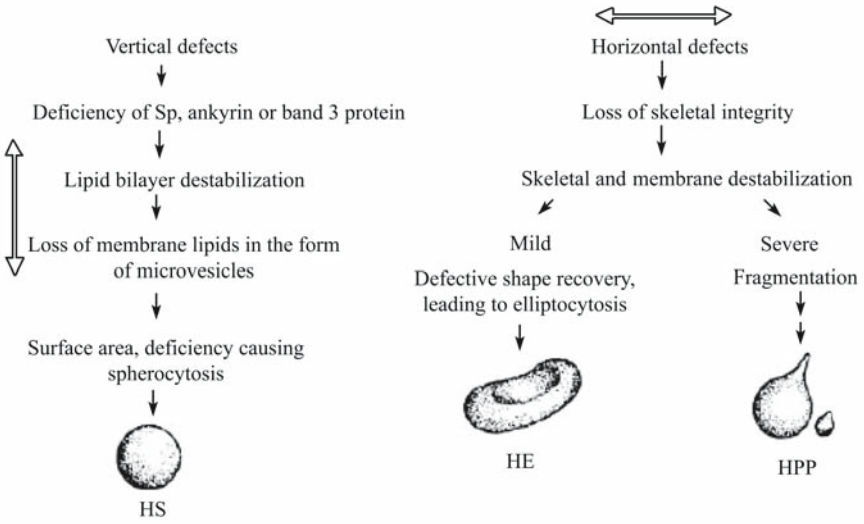


Fig. 5 Schematic illustration of the molecular mechanism occurred in the interactions among the major erythrocyte membrane proteins and a hypothetical model explaining the principle molecular defects in patients with HS, HE and HPP (Palek, 1995)

most often due to the compound heterozygosity of defects in ankyrin, α-Sp or protein 4.2. Their common combinations include a defect in the promoter or 5′-untranslated region of ankyrin accompanied with a missense mutation; a low expression allele of α-SP plus a missense mutation; and various mutations in the gene for protein 4.2 (Fig. 6) (Eber and Lux, 2004). The murine erythroid α-Sp gene (Spnal) is located on chromosome 1. Four mutations of this gene (Sph, Sph^{Dem}, Sph^{2BC} and Sph^{J}) cause severe HS and HE (Wandersee et al., 2003).

Among 20 families of Egyptian children with HS, the combined defects of Sp and ankyrin amount to 85%, single defect of ankyrin is about 10%, and single defect of α-Sp comprises only 5%. Genetic analysis reveals that eight families belong to allelic dominant heredity (AD), one family is grouped as allelic recessive heredity (AR), the hereditary nature of six families is undetermined, four families are possibly AD or AR, and another one family is AD or X-linked chromosomal heredity (Assar et al., 1998). Partial splenectomy was performed for the treatment of 25 children with congenital hemolytic anemia, 16 out of them had HS (Rice et al., 2003). Follow-up ranged from 7 months to 6 years shows prominent therapeutic efficiency. The Hb contents increase in most children, with a decline of reticulocyte counts and bilirubin levels. Their hemolysis is successfully controlled under preserved splenic function.

5.2 Hereditary elliptocytosis (Gallagher, 2004)

Hereditary elliptocytosis is an especially common disease found in population of African and Miditerranean ancestry, presumably with relation to that elliptocytes confer certain resistance to malaria. The principle lesion in HE is mechanical weakness or fragility of erythrocyte membrane skeleton due to defects of α-Sp, β-Sp or protein 4.1. Mutations of the genes encoding these membrane proteins include point mutations, gene deletions and insertions as well as mRNA processing defects. The majority of HE patients are asymptomatic, but some of them may experience hemolytic anemia, splenomegaly and intermittent jaudice. α-Sp mutations in HE associated with HPP frequently occur at codon 28 (a CpG dinucleotide), which is a "hot spot" for mutation. All 27 variants reported pertain to missense mutations, those in the N-terminal region of α-Sp are among the most common type, e.g., Sp Corbeil variant (Tryptic phenotype αI/74, Arg 28→ His). Nineteen types of β-Sp mutations associated with HE and HPP were documented up to now. Those occurred in the C-terminal region of β-Sp are truncations or point mutations, e.g., Sp Paris variant (Tryptic pheotype αI/74, Ala 2023→ Val). A case of elliptocytosis associated with an undescribed abnormal αGP was reported (Lu et al., 1992). Using immunoblotting techniques, a clear-cut minor band 6′ (24 kDa) is detected emerging just behind the δ GP band when probed with anti-α GP antiserum. It also reacts with anti-peptide C antiserum, suggesting that this new band is related to the structural alteration of α GP and not δ GP. In addition, the reaction with anti-protein 4.1 antiserum confirms the increase in proteolytic susceptibility of patient's protein 4.1. Abnormalities of both α GP and protein 4.1 refer to the disorder of Sp-protein 4.1-α GP contact in vertical interactions, thus causing elliptocytosis rather than spherocytosis. Therefore, this case is exceptional to Palek and Lux's hypothetical model (Palek, 1995).

5.3 Hereditary stomatocytosis (HST) (Delaunay, 2004)

The morphological feature of stomatocytes is characterized by a well-demarcated linear unstained area across their centers, instead of the normal circular area of pallor. The erythrocyte membrane of HST patients displays a rare hereditary disorder in permeability to monovalent cations. Their syndromes include hemolytic anemia, macrocytosis and the presence of abnormally shaped erythrocytes, etc.

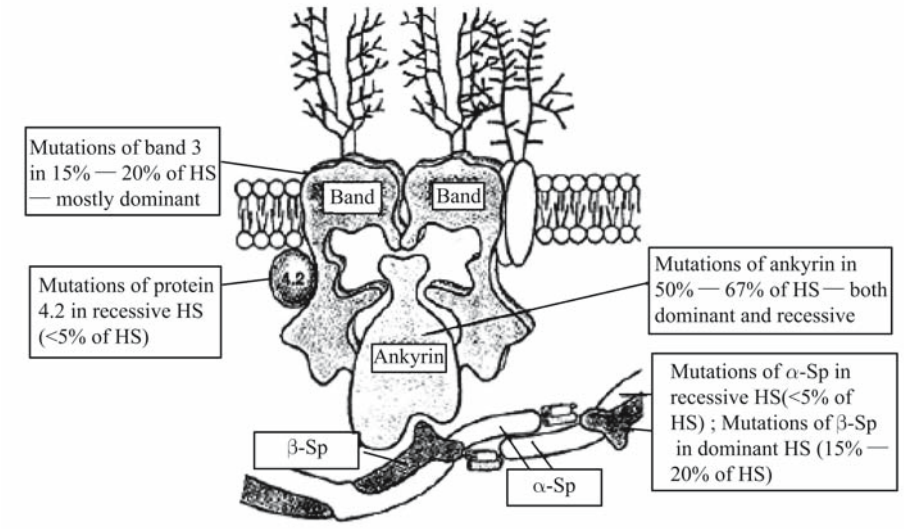


Fig. 6 Membrane defects in HS affect the vertical Interactions connecting the membrane skeleton and lipid bilayer (Eber and Lux, 2004)

Osmotic gradient ektacytometry is the key test for the diagnosis of HST.

5.4 Malaria and erythrocyte membrane (Cooks et al., 2004)

Malaria is a severe and widespread parasitic disease in humans. It makes a considerable impact on the course of human evolution and selection for resistance has led to the occurrence and persistence of numerous hereditary diseases. After merozoite invasion, erythrocytes are progressively modified, new structures appear inside the erythrocytes and many parasitic proteins are exported and associated with the cytoplasm and membrane skeleton of infected erythrocytes. The precise functional roles of these proteins have not been elucidated yet. The biochemical, morphological, and rheological alterations of erythrocytes manifest as increased membrane rigidity, reduced cell deformability, and higher adhesion to vascular endothelium and other blood cells. A total of 15 kinds of *Plasmodium falciparum* (Pf) proteins exported to human erythrocytes are so far found, with their molecular weights of 23–2500 kDa, e.g., the molecular weight of Pf erythrocyte membrane protein 1 (PfEMP1) is 265–285 kDa, its receptors include CD36, intercellular adhesion molecule-1 (ICAM-1) and thrombomodulin (TSP). As for erythrocyte genetic disorders maintained in human subjects exposed to malarial infection, 14 kinds are totally described, e.g., S-s-U blood group deficiency, hereditary ovalocytosis, etc. (Cooks et al., 2004). During invasion of Pf, the infected human erythrocytes preserve appropriate amount of membrane skeletal proteins (ankyrin, band 3, protein 4.1 and 4.2), but also what happens is the intermingling of host GPA, parasite proteins, and the degraded products of both. Four erythrocyte-binding antigens (EBAs-107 K, 115 K, 135 K, and 143 K) are detected in the infected erythrocytes, their saponin lysates and pellets (Liu et al., 1993). When mice of BALB/c species are infected with *Plasmodium chabaudi*, their merozoites also carry multiple EBA-like antigens. These antigens can cross-react with subtertian malaria hyperimmune serum. An intermingling of disintegrated membrane sialoglycoproteins (SGPs) with the EBA-like antigens occurs in the invaded mice erythrocytes (Liu et al., 1994). Three distinct types of GP variants are found among 222 residents in a tertian malaria hyperendemic area of Libo County, Guizhou Province (Lu et al., 1998). They are identified as MiVI GP (δ^N-α-δ^s), MiV (J.L.) GP (α^M–δ^S) and Mi III GP (δ^N-α-δ^s) (Lin et al., 1997; Lu et al., 1998). The frequency of GP variants in this hyperendemic area does not depend upon the severity of malarial prevalence (Lu et al., 1998).

6 Summary

During the past 10 years, several new contributions and substantial progress have been made in the structure and function of erythrocyte membrane proteins and membrane skeleton as well as their roles in the pathogenesis of some human diseases. However, the achievements in this research field lag far behind the final resolution of relevant problems. Facing a heavy task, we have to walk a long way to solve puzzles. Just as Mohandas (2004) emphasized that "the function of a number of important red cell surface molecules, such as the Rh proteins, still needs to be defined. The role of red cells in thrombosis is a novel area of investigation and also requires active exploration. Finally, our understanding of how the malarial parasite commandeers the red cell for its survival is still very rudimentary". As malarial infections continue to become a major public health problem on a global scale, we should remind ourselves to endeavor earnestly for fulfilling the goal.

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