

Red cell disorders: Diagnosis and treatment of common red cell defects - Section 16

Red cell membrane proteins

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Take home messages

- The human red blood cell membrane is packed with a regular array of proteins and protein complexes attached at intervals to the underlying cytoskeleton.
- The membrane proteins form large complexes and work synergistically, increasing the efficiency of the metabolic and transport processes of the individual proteins.
- Proteomic analysis and whole genome sequencing are beginning to provide a better understanding of the structural and functional changes in the membrane through erythropoiesis.

Introduction

Proteins of the human red cell membrane have been extensively characterized. In these enucleate cells, plasma membranes were easily isolated and the major membrane proteins identified, using SDS-PAGE with Coomassie Blue and Periodic Acid Schiff (PAS) staining techniques. Investigators used various techniques to assess whether these proteins were single-spanning, multi-spanning or peripheral (eg, hydropathy plots, determining glycosylation, proteolytic sites, or antibody binding sites), and/or associated into complexes (eg, using elution methods, non-ionic detergents, or co-immunoprecipitation).^{**1,2} Further advances were made by linking the expression of RBC antigens to individual membrane proteins and advances in molecular biology began to reveal the molecular basis of these antigens.^{3,4} Studies of variant null RBCs revealed a hierarchy of protein association. For example, Rhnull cells, where Rh-associated glycoprotein (RhAG) is absent, lack all the proteins of the Rh complex (RhAG, Rh proteins (RhD, RhCE), glycophorin B (GPB), CD47 and LW;

band 3 null cells have little or no expression of protein 4.2, proteins of the Rh complex or glycophorin A (GPA); Glycophorin C null RBC membranes lack p55 and showed reduced protein 4.1 binding.^{**5,**6,7} These early data defined the core components of the 2 main membrane complexes that tether the membrane to the underlying cytoskeleton (Fig. 1, part a) and contribute to the formation of the unique biconcave disc shape of this highly deformable cell.

Current state-of-the-art

Study of RBC membrane variants that caused haemolytic anaemia, affecting the morphology or deformability of the RBC, has led to further advances in our understanding of the structure of the membrane as reviewed in “HS, HE and allied disorders”, R Russo (below).

A key RBC membrane protein is anion exchanger 1 (AE1, SLC4A1, band 3), present at over 1 million copies per cell. Band 3 exists in 2 forms, the tetrameric form which associates with ankyrin to form a macrocomplex, and a dimeric form that can either be associated with the protein 4.1/junctional complex or unattached.^{**1} The band 3/ankyrin macrocomplex has 2 main roles: a structural role, ankyrin tethers the macrocomplex to the cytoskeleton via β -spectrin, and an essential role in red cell metabolism and gas exchange.^{**8}

The transport function of band 3 is to exchange chloride ions for bicarbonate ions. Bicarbonate ions are formed in the RBC by the action of carbonic anhydrase II (CAII):



CAII associates with the C-terminal domain of band 3 promoting this reaction.⁹ Movement of bicarbonate out of the RBC via band 3 allows the full volume of the blood to carry carbon dioxide from the tissues to the lungs. The proton produced attaches to haemoglobin encouraging the release of oxygen in the tissues. Both RhAG and aquaporin 1 (AQP1; the water channel)

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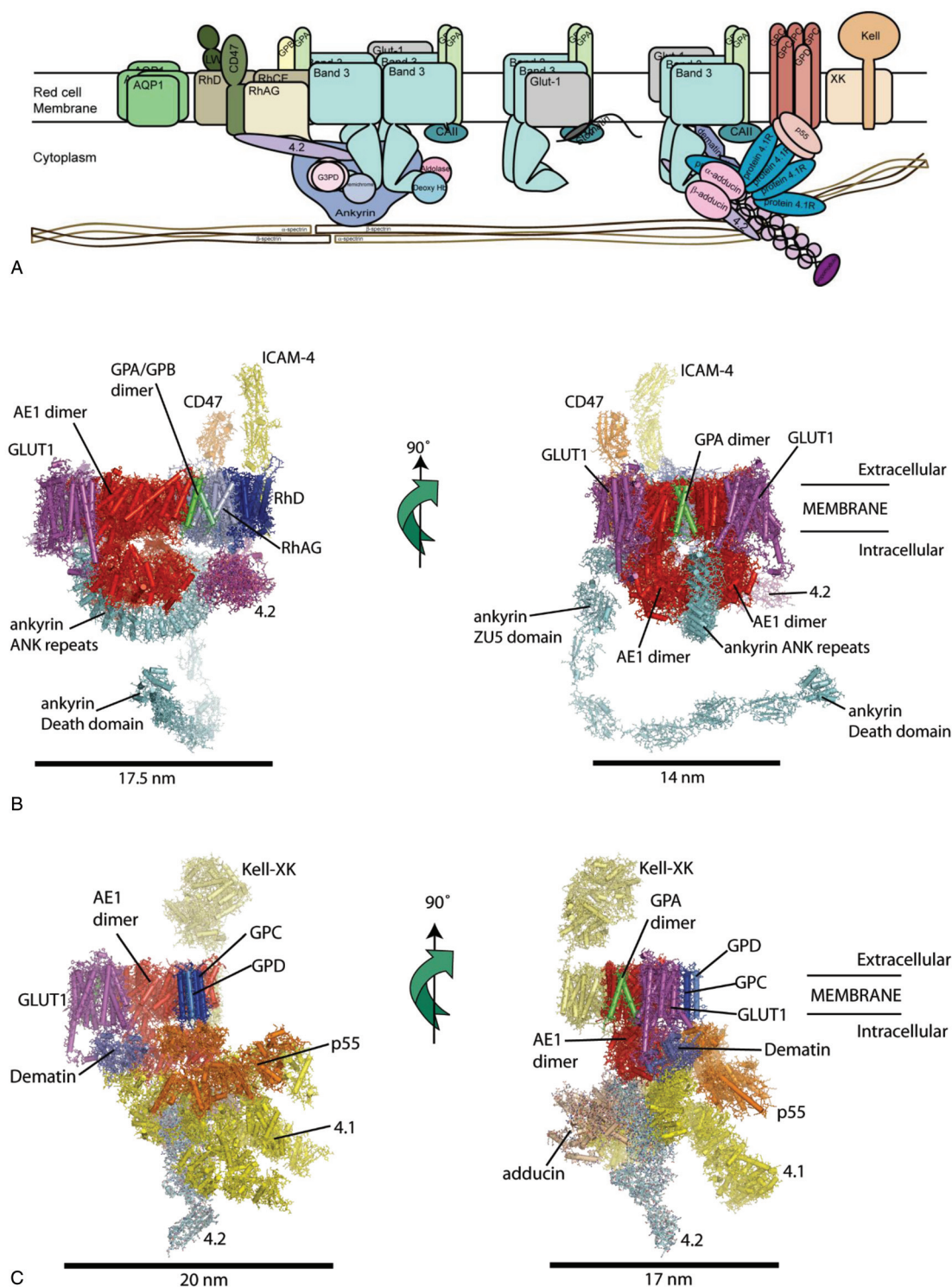


Figure 1. Band 3 complexes. Band 3 forms part of each of the 2 main complexes of the RBC membrane. (A) is a cartoon diagram of the proteins known to associate in these complexes. (B) and (C) show highly speculative molecular models of these complexes. (A) Tetrameric band 3 associates with ankyrin, linking the macrocomplex to the cytoskeleton. Dimeric band 3 associates with b-adducin in the junctional complex. (B) Model of the band 3 macrocomplex showing 2 band 3 homodimers (red), 1 Rh/RhAG heterotrimer (RhAG light blue, RhCE mid-blue, RhD dark blue), one GPA homodimer (green), 1 GPA/GPB heterodimer (green), 1 ICAM-4 monomer (yellow), 1 CD47 monomer (orange), 1 glut1 monomer (purple), one protein 4.2 monomer (magenta) and 1 ankyrin monomer (cyan). (C) Model of the junctional complex showing 1 band 3 homodimer (red), a cluster of 4 GPC monomers (dark blue) and 1 GPD monomer (light blue), 1 GPA dimer (green), 1 glut1 monomer (purple), 1 Kell/Kx heterodimer (pale yellow), 1 protein 4.2 monomer (cyan), one dematin homotrimer (violet), 1 a/b adducin heterodimer (beige), 1 p55 monomer (orange) and 5 protein 4.1 monomers (yellow). All components are shown with secondary structure elements highlighted as cartoons and residues also displayed in an all-atom stick representation. The figure is adapted from Burton and Bruce¹.

have been reported to act as gas channels, and there is evidence to suggest they both associate with band 3.^{6,**10} Thus, the macrocomplex acts as an integrated metabolon promoting efficient gas transport around the body.^{**8}

The role of the junctional complex is thought to be mainly structural, tethering the membrane to the spectrin/actin cytoskeleton and contributing to the deformability of the cell. However, over recent years numerous other proteins have been found to associate at these junctions suggesting other roles for this complex. Study of protein 4.1 null cells showed that the urea transporter, XK, Kell and Duffy associate with this complex, although the copy number of these proteins ($10\text{--}15 \times 10^3/\text{cell}$) suggests that they cannot be present in every junctional complex ($\sim 40 \times 10^3/\text{cell}$).^{**11} Band 3 dimers were shown to associate with the junctional complex via an interaction with adducin, and GLUT1 via dematin.^{12,13}

A proportion of band 3 dimers also exist as 'freely' mobile units, unattached to the cytoskeleton, and floating in the corrals confined by the hexameric arrays of spectrin tetramers in the cytoskeleton.¹ Some crystal structures have been solved for RBC membrane proteins, or for their homologues, allowing models of the main RBC complexes to be compiled (Fig. 1b and c).¹ These models demonstrate how tightly packed the membrane is with protein; domains extending far above and below the membrane bilayer (Fig. 1b and c).¹

Recent proteomic studies show that there are hundreds of RBC membrane proteins; 'new' proteins are being identified regularly.^{14,15} Some of these proteins have been found to carry antigens, defining new blood group systems.¹⁶ Currently, a total of 36 human blood group systems and 360 antigens are recognized by the International Society of Blood Transfusion (ISBT).¹⁶ Other proteins have shed light on the regulation of RBC hydration. PIEZO1, a mechano-sensitive cation channel can, when stimulated, result in calcium influx which then activates the Gardos channel causing potassium efflux and dehydration.¹⁷ This protein is mutated and overactive in many patients with hereditary dehydrated stomatocytosis (DHSt).

Future perspectives

There is still so much to learn, with so many RBC proteins of unknown function. Some of these may be residual proteins, left over from reticulocyte maturation. Others though have roles that have yet to be ascertained. Quantitative proteomics studies, using sorted erythrocyte and reticulocyte populations, begin to provide a more accurate picture of the red cell proteome.¹⁸ In silico methods of predicting protein-protein interactions are an emerging, powerful tool for investigating the red cell interactome. Whole genome sequencing is revealing SNPs associated with RBC membrane properties.¹⁹

There is a need to investigate how the composition of the membrane and cytoskeleton change through erythropoiesis. The processes of differentiation, enucleation and reticulocyte maturation involve extensive rearrangements of the membrane and cytoskeleton. Greater understanding of these processes is required to increase efficiency of the in vitro culture of RBCs. Currently, cultured RBCs have less than optimal rates of enucleation and cannot be matured to erythrocytes. If these cultured RBCs are ever to become a diagnostic or clinical component then their maturation, and stability during storage, needs to be improved. Already, proteomic studies are comparing the proteins in different hematopoietic cell types, for example, in cord, adult and retic RBCs aiming to respond to this challenge.²⁰

References

- ^{**1}. Burton NM, Bruce LJ. Modelling the structure of the red cell membrane. *Biochem Cell Biol.* 2011;89:200–215.

This paper is a much more extensive, relatively recent review of the proteins of the red cell membrane.

2. Steck TL. The organization of proteins in the human red blood cell membrane. A review. *J Cell Biol.* 1974;62:1–19.
3. Anstee DJ. Blood group-active surface molecules of the human red blood cell. *Vox Sang.* 1990;58:1–20.
4. Cartron JP, Bailly P, Le Van Kim C, et al. Insights into the structure and function of membrane polypeptides carrying blood group antigens. *Vox Sang.* 1998;74 (Suppl 2):29–64.
- ^{**5}. Anstee DJ, Tanner MJ. Blood group antigen deficiencies associated with abnormal red cell shape. *Blood Rev.* 1988;2:115–120.

This review was one of the first to describe how loss of glycophorin C or Rh-associated glycoprotein affects the complexes that they associate with and their interaction with the cytoskeleton, and thereby the morphology and deformability of RBCs.

- ^{**6}. Bruce LJ, Beckmann R, Ribeiro ML, et al. A band 3-based macrocomplex of integral and peripheral proteins in the RBC membrane. *Blood.* 2003;101:4180–4188.

This paper defined the proteins that make up the band 3/ankyrin macrocomplex and first suggested the integrated function of the macrocomplex.

7. Hemming NJ, Anstee DJ, Mawby WJ, et al. Localization of the protein 4.1-binding site on human erythrocyte glycophorins C and D. *Biochem J.* 1994;299 (Pt 1):191–196.
- ^{**8}. Chu H, McKenna MM, Krump NA, et al. Reversible binding of hemoglobin to band 3 constitutes the molecular switch that mediates O₂ regulation of erythrocyte properties. *Blood.* 2016;128:2708–2716.

This paper describes how the binding of haemoglobin to the cytoplasmic domain of band 3 mediates oxygen regulation of the red cell.

9. Sterling D, Reithmeier RA, Casey JR. A transport metabolon. Functional interaction of carbonic anhydrase II and chloride/bicarbonate exchangers. *J Biol Chem.* 2001;276:47886–47894.
10. Hsu K. Exploring the potential roles of band 3 and Aquaporin-1 in Blood CO₂ transport-inspired by comparative studies of Glycophorin B-A-B Hybrid Protein GP.Mur. *Front Physiol.* 2018;9:733.
- ^{**11}. Azouzi S, Collec E, Mohandas N, et al. The human Kell blood group binds the erythroid 4.1R protein: new insights into the 4.1R-dependent red cell membrane complex. *Br J Haematol.* 2015;171:862–871.

This paper describes the proteins that are dependent on protein 4.1, refining our understanding of the junctional complex.

12. Franco T, Chu H, Low PS. Identification of adducin-binding residues on the cytoplasmic domain of erythrocyte membrane protein, band 3. *Biochem J.* 2016;473:3147–3158.
13. Khan AA, Hanada T, Mohseni M, et al. Dematin and adducin provide a novel link between the spectrin cytoskeleton and human erythrocyte membrane by directly interacting with glucose transporter-1. *J Biol Chem.* 2008;283:14600–14609.
14. Bryk AH, Wisniewski JR. Quantitative analysis of human red blood cell proteome. *J Proteome Res.* 2017;16:2752–2761.
15. D'Alessandro A, Zolla L. Proteomic analysis of red blood cells and the potential for the clinic: what have we learned so far? *Expert Rev Proteomics.* 2017;14:243–252.
16. Storry JR, Clausen FB, Castilho L, et al. International society of blood transfusion working party on red cell immunogenetics and blood group terminology: report of the Dubai, Copenhagen and Toronto meetings. *Vox Sang.* 2019;114:95–102.
17. Badens C, Guizouarn H. Advances in understanding the pathogenesis of the red cell volume disorders. *Br J Haematol.* 2016;174:674–685.
18. Gautier EF, Leduc M, Cochet S, et al. Absolute proteome quantification of highly purified populations of circulating reticulocytes and mature erythrocytes. *Blood Adv.* 2018;2: 2646–2657.
19. van der Harst P, Zhang W, Mateo Leach I, et al. Seventy-five genetic loci influencing the human red blood cell. *Nature.* 2012;492:369–375.
20. Wilson MC, Trakarnsanga K, Heesom KJ, et al. Comparison of the proteome of adult and cord erythroid cells, and changes in the proteome following reticulocyte maturation. *Mol Cell Proteomics.* 2016;15:1938–1946.