

ADVANCES IN HEMATOLOGY

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The Red Cell Membrane, Part 1: The Role of the Red Cell Membrane



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This is part 1 of a 3-part series on the red cell membrane.

H&O What challenges do red cells face as they pass through the capillaries?

SRP The red cell is approximately 8 μm in diameter when circulating through large vessels. It must, however, squeeze through capillaries that are only 3 μm wide and through slits in the reticuloendothelial system that are only 0.5 to 1 μm wide. Thus, the erythrocyte must be able to undergo extensive deformation safely and reversibly without fragmenting. Failure to achieve this deformation results in damage to red cells and their removal from the circulation by the spleen. In fact, this is the mechanism by which aging red cells—which can no longer flex like their younger counterparts—are selectively removed from the circulation.

H&O What characteristics do red cells require in order to accomplish this deformation?

SRP The red cell achieves its deformity through its unique shape, the structural characteristics and composition of the red cell membrane, and the viscosity of the contents of the cell.

The red cell is a biconcave disc. This shape allows it to possess enough volume (90 fL) to contain sufficient hemoglobin for its oxygen-carrying function, and enough surface area (approximately 140 μm^2) to allow deformability. The high surface area allows the red cell to undergo extensive shape

changes without damage. If the red cell were a sphere, the much lower surface area would prevent it from deforming.

Cellular viscosity, which is determined by intracellular hemoglobin and water content, also regulates cellular deformability. The red cell membrane is deformable, mediated in part by the elastic properties (coiling and uncoiling) of the structural proteins that maintain its shape and stability.

H&O What are the functions of the red cell membrane?

SRP One function is to maintain structure and contain the contents of the red cell—chiefly, hemoglobin. Far from being just a casing, however, the red cell membrane plays a critical role in maintaining cellular functions in the only non-nucleated cell in the body (the cellular organelles are lost during red cell maturation). Thus, the red cell membrane accomplishes vital tasks in the transport of salts and nutrients.

H&O What are the properties of the red cell membrane that allow it to carry out these functions?

SRP The red cell membrane achieves its key structural properties by being deformable but stable, and by having a unique biconcave shape with a high surface area to volume ratio. It achieves its transport role through the range

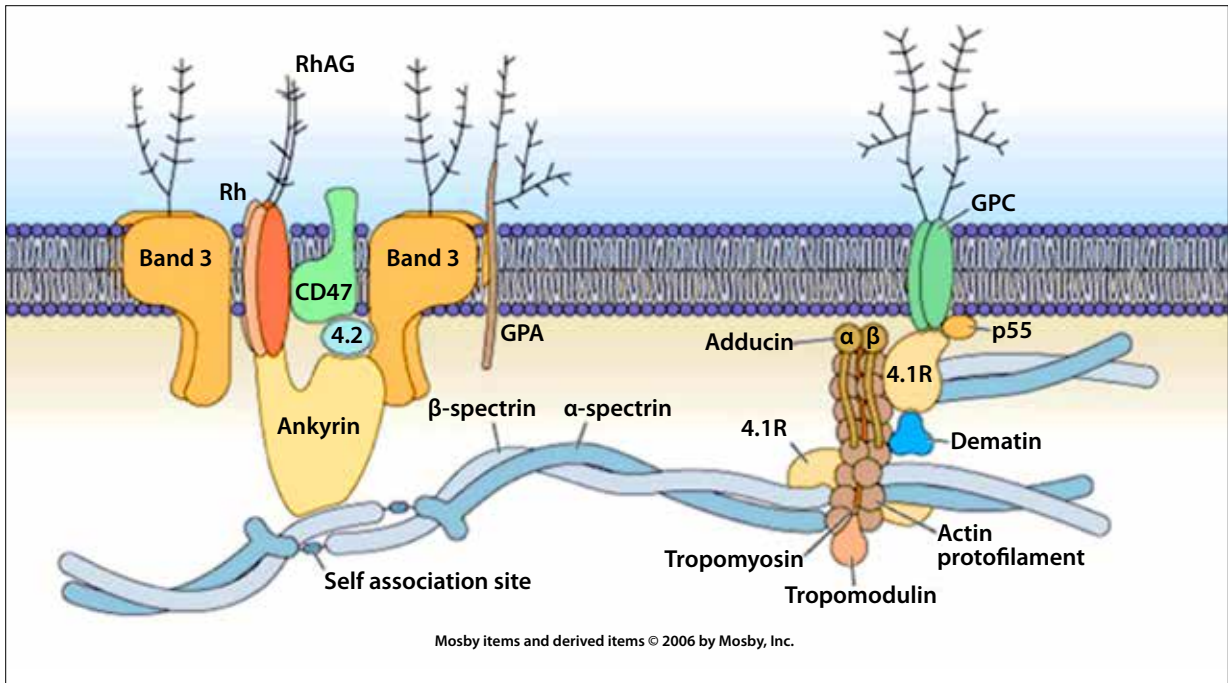


Figure. A schematic model of the organization of the red cell membrane (the proteins and lipids are not drawn to scale).

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of proteins that span it and are embedded in it, although transport through passive diffusion also occurs.

H&O What is the structure of the normal red cell membrane?

SRP The red cell membrane can be considered a lipid membrane tethered to an underlying skeleton. About 52% of the membrane mass is protein, 40% is lipid, and 8% is carbohydrate. The lipid bilayer comprises phospholipids (chiefly phosphatidylcholine and sphingomyelin in the outer layer, and phosphatidylethanolamine and phosphatidylserine in the inner layer), with unesterified cholesterol in the intervening space. Disorganization of the specific location of the phospholipids can result in immunogenicity of the red cell. One result can be the attachment of macrophages for removal.

H&O What is the structure of the membrane skeleton, and what role does it play in supporting red cells?

SRP The proteins responsible for imparting the membrane skeleton are tethered to the cytoplasmic surface of the membrane, and are responsible for the elasticity and stability of the red cell membrane. The key proteins that make up the membrane skeleton are spectrin, actin, protein

4.1, pallidin, and ankyrin, although many other proteins are involved. Spectrin alpha and beta subunits are entwined to form dimers, and dimers associate head to head to form tetramers; spectrin tetramers form hexagonal units that form a mesh-like structure. Spectrin tetramers are bound together by junctional complexes comprising actin, protein 4.1, and other proteins. The skeleton is coupled to the lipid membrane by ankyrin, which binds to band 3, strengthened by band 4.2. Proteins forming horizontal and vertical interactions maintain the integrity and shape of the red cell. The vertical interactions (including spectrin-ankyrin-band 3 interactions, spectrin-protein 4.1R-junctional complex linkages, and interactions between skeletal proteins and negatively charged proteins of the inner component of the lipid bilayer) stabilize the lipid membrane, and the horizontal interactions (involving the spectrin heterodimer associations) support the structural integrity of the red cell, including after exposure to shearing.

The integral proteins, which are embedded in and span the lipid membrane, have necessary functions for red cell and membrane homeostasis and also interact with the skeleton to tether it to the lipid membrane. Band 3, an anion exchanger, plays a critical role in maintaining red cell hydration; however, it also tethers the red cell skeleton to the lipid membrane via interactions with ankyrin, protein 4.2, 4.1 and other proteins. Glycophorins (A, B, C, and D) are glycoproteins rich in sialic acid that impart a net

negative charge to the cell surface, preventing excessive cell-cell and cell-endothelium interactions. The glycoporphins appear to serve as a receptor for, and be important in, plasmodium invasion of red cells. Other integral membrane proteins include a range of ion, water, and gas transport channels, and proteins for which the function has not yet been fully elucidated.

H&O What antigens does the red cell membrane contain?

SRP The red cell membrane expresses the key carbohydrate and protein antigens that are critical for red cell immunophenotyping, and hence are critical for transfusion medicine. Most of these antigens also play important roles in red cell physiology, although the function of some is still uncertain. Most of the red cell antigens are integral membrane proteins. These include the Rh group antigens, as well as Duffy, Kell, Kidd, and Lewis. The Colton blood group represents a polymorphism in the aquaporin 1 protein. The glycoporphins carry the antigens of the MNSs system. The major ABO group of red cell antigens are not integral to the red cell membrane, but are actually carbohydrates that protrude from it.

H&O What have we have learned about the red cell membrane in recent years?

SRP Important discoveries have been made with regard to the interactions between the red cell membrane and malaria. The specific antigens expressed upon infection by *P falciparum* (*Plasmodium falciparum* erythrocyte membrane protein [PfEMP], STEVOR) bind to receptors on the red cell surface (eg, glycoporphin C), mediating invasion, or are expressed on the surface of infected erythrocytes. An interesting new technology that has been reported by a growing number of publications is bioengineering to coopt the red cell membrane as a potential drug delivery system, with therapeutic agents either contained in the cytoplasm or bound to the membrane surface.

H&O What are some of the advances that have made these discoveries possible?

SRP An important approach to the discovery of novel components and function of the red cell membrane has been the capacity to identify new genetic lesions in patients and families with red cell morphology suggestive of a red cell membrane abnormality but hitherto unrecognized mutations, using targeted or massive sequencing; new polymorphisms have been identified and their putative effects on gene expression, amino acid sequence, and protein structure modeled.

Secondly, modern proteomic discovery methods have enabled a detailed analysis and description of the proteins associated with the red cell membrane. A recent analysis identified 340 proteins involved with the membrane, including 105 integral membrane proteins, 54 that were membrane-associated or bound, and 5 that were anchored by glycosylphosphatidylinositol (GPI), and 40 cytoskeletal. The role of most of these proteins within the existing framework of understanding of the membrane has not yet been elucidated.

H&O Is there anything you would like to add?

SRP I would like to briefly address the pathophysiology of disorders of the red cell membrane (spherocytosis, elliptocytosis, ovalocytosis) to link the previous discussion to disease.

Hereditary spherocytosis is caused by lesions in genes encoding proteins involved in the vertical interactions that tether the red cell membrane to the skeleton. Thus, mutations in spectrin (α or β), ankyrin, band 4.2, or band 3 result in inadequate tethering of the red cell membrane to the skeleton, a loss of membrane surface area, and spherocytosis. Protein analysis of the red cell membrane is likely to identify spectrin deficiency, even if mutations in spectrin are not present, as loss of the vertical interactions results in impaired assembly of the spectrin complex. Spherocytosis causes reduced deformability, resulting in entrapment and removal of cells by the spleen, and cases with severe anemia can be greatly improved by splenectomy. Hereditary elliptocytosis is most often caused by mutations in α spectrin, β spectrin, and band 4.1. Hereditary ovalocytosis (South East Asian ovalocytosis) is characteristically associated with heterozygous band 3 mutations, and is generally asymptomatic; homozygous forms are usually lethal. Acquired spherocytosis (for example, with autoimmune hemolytic anemia) is also associated with loss of red cell membrane area.

Suggested Readings

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