Membrane assembly during erythropoiesis

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Purpose of review

In this review, we summarize our current knowledge on the expression patterns of various proteins during erythropoiesis and discuss how this information can lead to development of detailed understanding of membrane biogenesis during erythropoiesis.

Recent findings

Changes in expression pattern of more than 30 red cell membrane proteins during terminal erythroid differentiation and during reticulocyte maturation was discerned. During maturation of murine reticulocytes, tubulin and cytosolic actin were lost, while the membrane-associated levels of myosin, tropomyosin, ICAM-4, GLUT4, Na/K-ATPase, NHE1, GPA, CD47, Duffy, and Kell were reduced. During murine terminal erythroid differentiation, expression levels of all major transmembrane and skeletal proteins of the mature red blood cell increased, while those of various adhesion molecules decreased. A 30-fold decrease in expression of the adhesive protein CD44 was noted during differentiation of murine proerythroblast to orthochromatic erythroblast. These changing protein expression patterns were used to devise an effective strategy to distinguish erythroblasts at distinct stages of development.

Summary

All major red cell membrane proteins undergo dynamic changes during terminal erythroid differentiation. Use of CD44 in conjunction with TER119 and cell size enabled the development of a method for distinguishing distinct stages of erythroblasts during murine erythropoiesis. These findings should enable development of detailed understanding of membrane biogenesis during erythropoiesis and obtain mechanistic insights into disordered erythropoiesis in various red cell disorders.

Keywords

CD44, erythroblast, erythrocyte, erythropoiesis, membrane proteins, red cell

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Introduction

The process of erythropoiesis includes all steps of hematopoiesis starting with the multipotential hematopoietic stem cells. These stem cells can either undergo self-renewal or through lineage-specific commitment generate erythroid progenitor cells. These progenitor cells undergo terminal erythroid differentiation through a series of maturation stages to produce enucleated reticulocytes, which subsequently mature first in the bone marrow and then in circulation into red cells. Dramatic changes in cellular and membrane features are noted during terminal erythroid differentiation of proerythroblasts, the first morphologically recognizable erythroid cells, into reticulocytes. These include decreases in cell size, increased chromatin condensation, progressive hemoglobinization, and finally enucleation. While it has long been recognized that marked changes occur in membrane organization during erythroid differentiation, our understanding of these changes is limited. This review summarizes our current knowledge on the changes in membrane protein expression and assembly during erythropoiesis and discusses how this information can be used for developing strategies for studying normal and disordered erythropoiesis.

Process of erythropoiesis

Erythropoiesis is a process during which multipotential hematopoietic stem cells proliferate, differentiate, and eventually form mature erythrocytes. Eight distinct developmental stages have been identified during erythropoiesis: burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), proerythroblast, basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, reticulocyte, and mature erythrocyte. The BFU-E and CFU-E are two distinct erythroid progenitor cell populations that have been functionally defined in colony assays [1]. The earliest morphologically recognizable erythroblast is the proerythroblast. Proerythroblast differentiates to generate basophilic, polychromatic, and orthochromatic erythroblasts

and the orthochromatic erythroblasts expel their nuclei to produce reticulocytes. This ordered differentiation process involving 3- to 4-cell mitosis is referred to as terminal erythroid differentiation and is accompanied by decreases in cell size, enhanced chromatin condensation, progressive hemoglobinization, and marked changes in membrane organization. Transition of reticulocytes into mature red cells occurs first in bone marrow and then in the bloodstream, a process referred to as reticulocyte maturation. Reticulocyte maturation is accompanied by loss of intracellular organelles such as mitochondria [2–4] and ribosomes as well as extensive membrane remodeling [5–7].

Composition and organization of mature red cell membrane

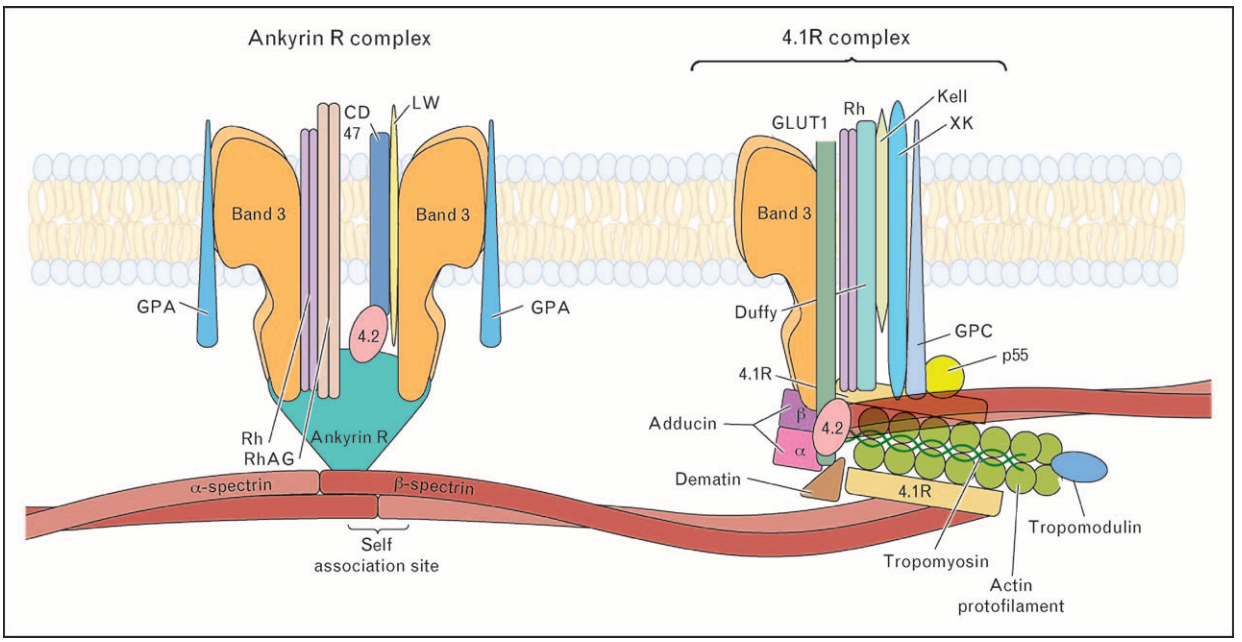
Studies during the last three decades have led to a detailed characterization of the protein composition and structural organization of the mature red cell membrane [8–10,11•,12]. The red cell membrane is currently viewed as a composite structure composed of a plasma membrane anchored to a two-dimensional membrane skeletal network through linkage of transmembrane proteins to the skeletal network. The major skeletal proteins include α-spectrin, β-spectrin, actin, protein 4.1R, ankyrin R, protein 4.2, p55, adducin, dematin, tropomyosin, and tropomodulin. More than 50 transmembrane proteins have been identified in the red cell, more than half of which carry blood group antigens [13]. The well-studied blood-group-carrying proteins include band 3, glycophorin (GP)A, GPC, Rh, RhAG, XK, Kell, Duffy, CD44, CD47, aquaporin 1 (AQP1), Lutheran (Lu), and intercellular adhesion molecule-4 (ICAM-4). These proteins exhibit distinct functions. Band 3 serves as an anion exchanger, while Rh/RhAG are probably gas transporters [14] and Duffy functions as a chemokine receptor [15,16]. Kell possesses endothelin-3 converting enzyme activity [17]. CD47 prevents premature removal from the circulation by functioning as a marker of 'self' on the outer surface where it binds to the inhibitory receptor SIRPα [18,19]. Lu, CD44, ICAM-4, and integrins α4β1 and α5β1 mediate cell–cell and cell–extracellular matrix interactions. Certain transmembrane proteins function as transporters or pumps. These include the water transporter AQP1, the glucose transporters GLUT1 and GLUT4, the sodium/hydrogen exchanger NHE1, and Na/K-ATPase.

α-Spectrin and β-spectrin in the form of $\alpha_2\beta_2$ tetramers comprise the structural core of the skeletal network and this structure is strengthened by interaction of spectrin with other skeletal proteins including actin, 4.1R, adducin, dematin, tropomyosin, and tropomodulin. The spectrin-based skeleton is attached to the lipid bilayer through protein–protein interactions between transmembrane and skeletal proteins at two major sites. The first involves ankyrin R, which interacts with cytoplasmic domains of band 3 and RhAG and forms the 'ankyrin R-complex' in close proximity to the spectrin tetramerization site [20]. This macromolecular complex also contains 4.2, GPA, RhAG, Rh, CD47, and ICAM-4. The second involves protein 4.1R, which associates with GPC, protein p55, Duffy, XK, and Rh proteins to form the '4.1R-complex' at the spectrin–actin junctional complex [21•]. Recent studies have shown that 4.2, band 3 and GLUT1 are also localized to the junctional complex, 4.2 through its binding to the carboxyl-terminal EF-hands of α-spectrin [22], band 3 through its interaction with adducin [23], and GLUT1 by interaction with adducin and dematin [24]. A schematic model depicting our current understanding of the composition and organization of red cell membrane proteins is shown in Fig. 1.

Membrane remodeling during reticulocyte maturation

The last step in terminal erythroid differentiation is the maturation of the enucleate multilobular reticulocyte into the discoid mature red cell. The maturation process, which lasts 2–3 days, first in the bone marrow and then in circulation, is accompanied by loss of organelles such as mitochondria and ribosomes [2–4] as well as extensive remodeling of the plasma membrane [7]. Membrane remodeling includes a ~20% loss of surface area due to membrane vesiculation [25,26], a decrease in cell volume, an increase in membrane mechanical stability [5,6], and acquisition of biconcave shape, implying a major reorganization of membrane and skeletal components. However, little is known about the specific molecular changes that accompany reticulocyte maturation. Only the loss of the transferrin receptor has been extensively studied [27] and the loss of glucose transporter, aquaporin-1, and Na/K-ATPase has been reported [28,29]. To comprehensively analyze the various molecular changes that accompany murine reticulocyte maturation, Liu *et al.* [30••] has recently examined the differences in the expression of 30 distinct membrane proteins between reticulocytes and mature red cells. They showed that during reticulocyte maturation, tubulin, cytosolic actin, transferrin receptor 1 (CD71), and ICAM-4 are lost, while the membrane content of myosin, tropomyosin, adducin, GLUT4, Na/K-ATPase, NHE1, GPA, CD47, Duffy, and Kell is reduced. In contrast, band 3, Rh, RhAG, GPC, and XK appear to increase after maturation, probably due to loss of membrane surface area. The cell content of major cytoskeletal proteins α-spectrin, β-spectrin ankyrin R, 4.1R, 4.2, p55, tropomodulin, and 4.9 is not changed. In addition, the release of AQP1 from reticulocytes into exosomes during reticulocyte maturation was also reported [31]. The degradation of tubulin and actin was, at least in part, mediated

Figure 1 Schematic model of red cell membrane organization



Ankyrin R complex: tetrameric band 3, Rh, RhAG, and 4.2 directly bind to ankyrin R. GPA and LW are associated with the complex through their interaction with band 3, CD47 through its interaction with 4.2. This complex is attached to spectrin near the center of the tetramer (dimer–dimer interaction site) by the binding of ankyrin R to spectrin. 4.1R complex: this complex contains the ternary complex of spectrin, F-actin, and 4.1R, as well as the actin-binding proteins, tropomyosin, tropomodulin, adducin, and dematin. The 4.1R directly interacts with p55 and several transmembrane proteins, GPC, Duffy, XK, Kell, and Rh. The dimeric band 3 binds to 4.1R and adducin while GLUT1 binds to adducin and dematin. 4.2 binds to the C-terminal EF hands of α-spectrin at the junction.

by the ubiquitin–proteasome degradation pathway. Studies of various membrane protein–protein interactions showed that while there was no difference in spectrin tetramer formation between reticulocytes and mature red cells, the spectrin–actin–4.1R junctional complex was weaker in reticulocytes. Moreover, the linkage of transmembrane proteins GPC, XK, Kell, and Duffy (all components of 4.1R-complex) to the membrane skeleton was weaker in reticulocytes. Interestingly, these weaker interactions appear in part due to phosphorylation of 4.1R in reticulocytes. These findings have begun to provide new insights into the mechanistic basis of membrane structural changes that accompany reticulocyte maturation. The various molecular changes seen during murine reticulocyte maturation are summarized in Table 1.

Membrane assembly during terminal erythroid differentiation

A number of earlier studies in the 1980s and 1990s investigated the synthesis and assembly of membrane proteins during erythropoiesis and demonstrated the asynchronous nature of synthesis and assembly of α and β-spectrin, ankyrin R, and band 3 during avian or murine erythroid differentiation [32–36]. However, as these studies only focused on the expression of a very limited number of membrane proteins, our knowledge of the erythroblast membrane protein composition and organization at distinctly different development stages is limited compared to our current detailed understanding of the structure and function of the membrane of the mature red cell.

To systematically study membrane assembly during murine terminal erythroid differentiation, a recent study derived erythroblasts at distinct developmental stages by culturing highly purified proerythroblasts from mice injected with Friend virus [37], and determined by Western blotting and by flow cytometry the expression levels of 23 major red cell proteins at different stages of

Table 1 Molecular changes during murine reticulocyte maturation

Lost	Decreased	Unchanged	Increased
Tubulin	Myosin	α-Spectrin	Band 3
Cytosolic actin	Tropomyosin	β-Spectrin	Rh
TfR1 (CD71)	Adducin	Membrane-associated actin	RhAG
ICAM-4	GLUT4	Ankyrin R	GPA (TER119)
	Na-K-ATPase	4.1R	GPC
	NHE1	4.2	XK
	GPA	p55	
	CD47	Tropomodulin	
	Duffy	4.9	
	Kell		

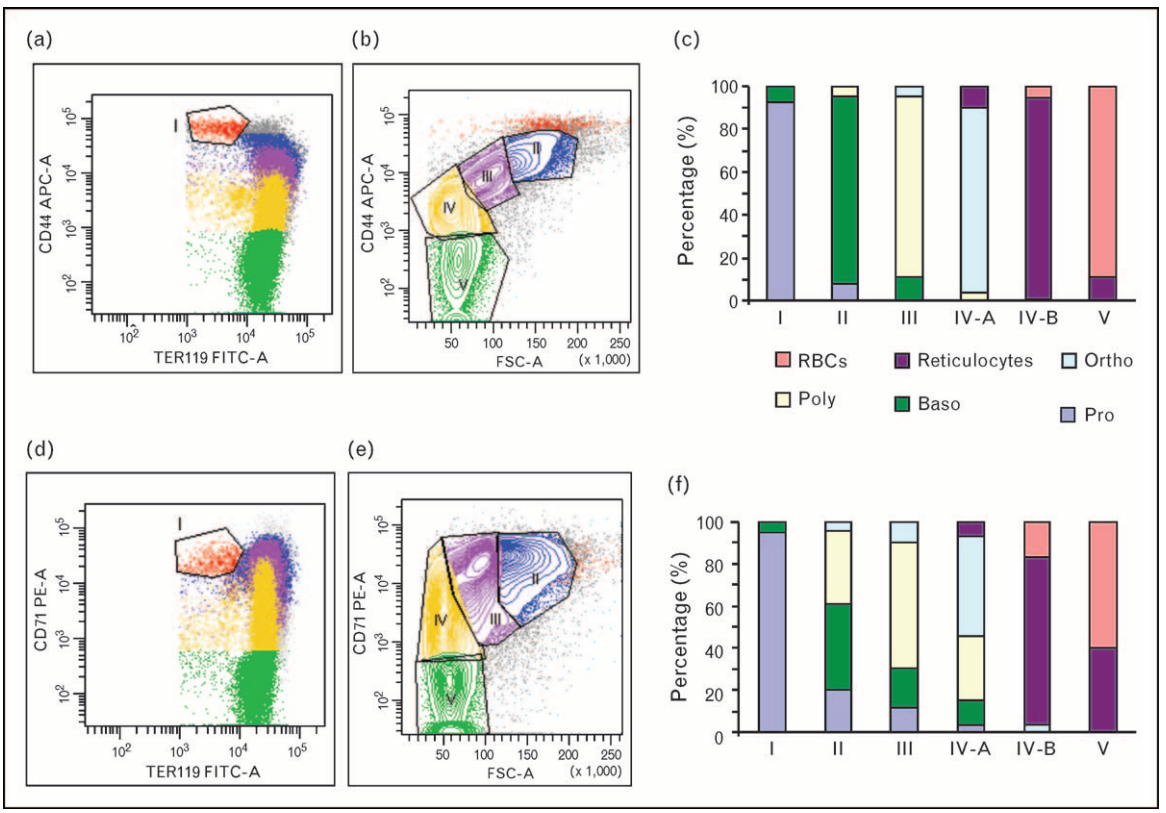
development [38••]. In this in-vitro culture system, proerythroblasts progressively differentiate into basophilic erythroblasts, polychromatic erythroblasts and orthochromatic erythroblasts, and reticulocytes during 48 h. The expression levels of all skeletal proteins (α-spectrin, β-spectrin, ankyrin R, 4.1R, p55, adducin, dematin, tropomodulin, and 4.2) increased progressively during terminal differentiation, except for actin, whose expression level decreased in late-stage erythroblasts compared to proerythroblasts. In contrast, three distinct patterns of protein expression were noted for transmembrane proteins. The first group of proteins comprising band 3, GPA, Rh, RhAG, CD47, and Duffy were expressed at low levels in proerythroblasts but their expression levels progressively increased during terminal differentiation with highest levels in orthochromatic erythroblasts. The second group, composed of adhesion molecules CD44, Lu, ICAM-4, and β1 integrin, exhibited a reverse type of expression pattern with highest level of expression in proerythroblasts and a progressive reduction as differentiation proceeded. Two isoforms of glycosylated β1 integrin were expressed during erythroid differentiation. It was noted that in this group of adhesive proteins, the most dramatic change occurred in surface expression of CD44, with a more than 30-fold decrease from proerythroblasts to orthochromatic erythroblasts. The expression levels of the third group proteins that included transferrin receptor (CD71), Kell, and XK protein changed little during the entire course of erythroid differentiation. It was also noted that the glycosylation status of Kell changed during differentiation, with the unglycosylated form expressed in proerythroblasts and the glycosylated form expressed in late-stage erythroblasts.

Table 2 Molecular changes during murine terminal erythroid differentiation

Decreased	Unchanged	Increased	
Actin	TfR1(CD71)	α-Spectrin	4.2
CD44	Kell	β-Spectrin	p55
Lu	XK	Ankyrin R	Band 3
ICAM-4		4.1R	Rh, RhAG
β1 Integrin		Adducin	CD47
		Tropomodulin	Duffy
		4.9	

Thus, during terminal erythroid differentiation there is a distinct temporal regulation of synthesis and assembly of

Figure 2 Flow cytometric analysis and isolation of erythroblasts from bone marrow cells



(a–c) Murine bone marrow cells labeled with antibodies against TER119 and CD44. (a) Plot of CD44 versus TER119. (b) Plot of CD44 versus FSC of all TER-positive cells. (c) Quantitation of the purity of erythroblasts populations sorted on the basis of CD44 surface expression levels. (d–f) Murine bone marrow cells labeled with antibodies against TER119 and CD71. (d) Plot of CD71 versus TER119; (e) plot of CD71 versus FSC of all TER-positive cells. (c) Quantitation of the purity of erythroblasts populations sorted on the basis of CD71 surface expression levels. Baso, basophilic erythroblasts; Ortho, orthochromatic erythroblasts; Poly, polychromatic erythroblasts; Pro, proerythroblasts; RBCs, red blood cells. Adapted from our published work in [38••].

various membrane proteins. The gradual loss of adhesive proteins during terminal erythroid differentiation implies that cell–cell adhesion and cell–extracellular matrix interactions involved in the formation of erythroblastic islands [39•], specialized niches in which all erythroid precursors differentiate and enucleate, undergo dynamic alterations. The observed patterns of synthesis and assembly of skeletal proteins and transmembrane proteins involved in linking the bilayer to membrane skeleton implies a dynamic sequence of events in the assembly of the red cell membrane during erythropoiesis. The molecular changes during murine terminal erythroid differentiation are summarized in Table 2.

Membrane assembly during human terminal erythroid differentiation

An effective two-phase culture system has been developed and optimized to study erythroid differentiation of human $CD34^+$ hematopoietic stem cells [40–42]. Using this system, Southcott *et al.* [43] studied the order of appearance of a number of membrane proteins that encode human blood group antigens. To extend these findings, a recent study has comprehensively examined the expression of 25 red cell membrane and skeletal proteins during human erythropoiesis (our unpublished data). A number of similarities and a few differences in the expression pattern of various membrane proteins were noted between human and mouse erythropoiesis. Similar to murine terminal erythroid differentiation, the expression of all skeletal proteins was increased during human erythropoiesis. However, in contrast to the finding of decreased expression of LU and ICAM-4 during terminal erythroid differentiation of murine cells, the expression of these two adhesive proteins increased during human erythropoiesis. Furthermore, the decrease in surface expression of CD44 during terminal erythroid differentiation of human erythroid cells was only one-third of that seen for murine cells. The biologic significance of these differences needs to be further explored.

Use of cell surface markers to distinguish distinct stages of erythroblasts

The findings that surface expression of CD44 progressively decreased by more than 30-fold while the transferrin receptor (CD71) expression changed little from proerythroblasts to orthochromatic erythroblasts suggested that CD44 might be a much more robust surface marker for distinguishing between different stages of erythroid differentiation than CD71, which is widely used to study murine erythropoiesis [44,45]. Indeed, staining of murine bone marrow cells with both CD44 antibody and an erythroid-specific GPA antibody, TER119, in conjunction with forward scatter enabled the separation of distinct stages of erythroblasts from proerythroblasts to orthochromatic erythroblasts with high purity (Fig. 2a–c) [38••]. In contrast, the use of CD71 resulted in the generation of a heterogenous mixture of erythroblasts at various stages of development except for a pure population of proerythroblasts (Fig. 2d–f).

Conclusion

Recent comprehensive characterization of molecular changes during erythropoiesis provided new insights into the genesis of red cell membrane function during erythroblast differentiation and during reticulocyte maturation. It also enabled the identification of optimal surface markers for distinguishing erythroblasts at distinct stages of maturation. These insights will enable the isolation of highly purified populations of erythroblasts at distinct stages of maturation for future functional studies and also offer a means of defining stage-specific defects in erythroid maturation in inherited and acquired red cell disorders and in bone marrow failure syndromes.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 197).

1 Gregory CJ, Eaves AC. Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. Blood 1978; 51:527–537.

2 Gronowicz G, Swift H, Steck TL. Maturation of the reticulocyte in vitro. J Cell Sci 1984; 71:177–197.

3 Zhang J, Randall MS, Loyd MR, *et al.* Mitochondrial clearance is regulated by Atg7-dependent and -independent mechanisms during reticulocyte maturation. Blood 2009; 114:157–164.

4 Kundu M, Lindsten T, Yang CY, *et al.* Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. Blood 2008; 112:1493–1502.

5 Chasis JA, Prenant M, Leung A, Mohandas N. Membrane assembly and remodeling during reticulocyte maturation. Blood 1989; 74:1112–1120.

6 Waugh RE, Mantalaris A, Bauserman RG, *et al.* Membrane instability in late-stage erythropoiesis. Blood 2001; 97:1869–1875.

7 Mel HC, Prenant M, Mohandas N. Reticulocyte motility and form: studies on maturation and classification. Blood 1977; 49:1001–1009.

8 Bennett V. The spectrin–actin junction of erythrocyte membrane skeletons. Biochim Biophys Acta 1989; 988:107–121.

9 Bennett V, Baines AJ. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev 2001; 81:1353–1392.

10 Discher DE. New insights into erythrocyte membrane organization and microelasticity. Curr Opin Hematol 2000; 7:117–122.

11 • Mohandas N, Gallagher PG. Red cell membrane: past, present, and future. Blood 2008; 112:3939–3948.
This comprehensive review describes our current understanding of the structural organization of the mature red cell membrane and describes the mechanistic basis for altered membrane function of various red cell membrane disorders.

12 Mohandas N, Evans E. Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects. Annu Rev Biophys Biomol Struct 1994; 23:787–818.

13 Reid ME, Mohandas N. Red blood cell blood group antigens: structure and function. Semin Hematol 2004; 41:93–117.

14 Soupene E, Lee H, Kustu S. Ammonium/methylammonium transport (Amt) proteins facilitate diffusion of NH3 bidirectionally. Proc Natl Acad Sci USA 2002; 99:3926–3931.

15 Chaudhuri A, Zbrzezna V, Polyakova J, *et al.* Expression of the Duffy antigen in K562 cells. Evidence that it is the human erythrocyte chemokine receptor. J Biol Chem 1994; 269:7835–7838.

16 Chaudhuri A, Zbrzezna V, Johnson C, *et al.* Purification and characterization of an erythrocyte membrane protein complex carrying Duffy blood group antigenicity. Possible receptor for *Plasmodium vivax* and *Plasmodium knowlesi* malaria parasite. J Biol Chem 1989; 264:13770–13774.

17 Lee S, Lin M, Mele A, *et al.* Proteolytic processing of big endothelin-3 by the kell blood group protein. Blood 1999; 94:1440–1450.

18 Oldenborg PA, Zheleznyak A, Fang YF, *et al.* Role of CD47 as a marker of self on red blood cells. Science 2000; 288:2051–2054.

19 Tsai RK, Discher DE. Inhibition of 'self' engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. J Cell Biol 2008; 180:989–1003.

20 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.

21 • Salomao M, Zhang X, Yang Y, *et al.* Protein 4.1R-dependent multiprotein complex: new insights into the structural organization of the red blood cell membrane. Proc Natl Acad Sci USA 2008; 105:8026–8031.
This article established the concept of 4.1R-based macromolecular complex and provided new insights into the structural organization of red blood cell membrane.

22 Korsgren C, Peters LL, Lux SE. Protein 4.2 binds to the carboxyl-terminal EF-hands of erythroid alpha-spectrin in a calcium- and calmodulin-dependent manner. J Biol Chem 2010; 285:4757–4770.

23 Anong WA, Franco T, Chu H, *et al.* Adducin forms a bridge between the erythrocyte membrane and its cytoskeleton and regulates membrane cohesion. Blood 2009; 114:1904–1912.

24 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.

25 Waugh RE, McKenney JB, Bauserman RG, *et al.* Surface area and volume changes during maturation of reticulocytes in the circulation of the baboon. J Lab Clin Med 1997; 129:527–535.

26 Come SE, Shohet SB, Robinson SH. Surface remodelling of reticulocytes produced in response to erythroid stress. Nat New Biol 1972; 236:157–158.

27 Pan BT, Blostein R, Johnstone RM. Loss of the transferrin receptor during the maturation of sheep reticulocytes in vitro. An immunological approach. Biochem J 1983; 210:37–47.

28 Johnstone RM, Mathew A, Setchenska MS, *et al.* Loss of glucose transport in developing avian red cells. Eur J Cell Biol 1998; 75:66–77.

29 Mairbaurl H, Schulz S, Hoffman JF. Cation transport and cell volume changes in maturing rat reticulocytes. Am J Physiol Cell Physiol 2000; 279:C1621–C1630.

30 •• Liu J, Guo X, Mohandas N, *et al.* Membrane remodeling during reticulocyte maturation. Blood 2010; 115:2021–2027.
This article systematically examined the molecular changes during murine reticulocyte maturation and provides mechanistic insights into membrane and cellular changes that accompany reticulocyte maturation.

31 Blanc L, Liu J, Vidal M, *et al.* The water channel aquaporin-1 partitions into exosomes during reticulocyte maturation: implication for the regulation of cell volume. Blood 2009; 114:3928–3934.

32 Chang H, Langer PJ, Lodish HF. Asynchronous synthesis of erythrocyte membrane proteins. Proc Natl Acad Sci USA 1976; 73:3206–3210.

33 Blikstad I, Nelson WJ, Moon RT, Lazarides E. Synthesis and assembly of spectrin during avian erythropoiesis: stoichiometric assembly but unequal synthesis of alpha and beta spectrin. Cell 1983; 32:1081–1091.

34 Hanspal M, Hanspal JS, Kalraiya R, *et al.* Asynchronous synthesis of membrane skeletal proteins during terminal maturation of murine erythroblasts. Blood 1992; 80:530–539.

35 Hanspal M, Hanspal JS, Kalraiya R, Palek J. The expression and synthesis of the band 3 protein initiates the formation of a stable membrane skeleton in murine Rauscher-transformed erythroid cells. Eur J Cell Biol 1992; 58:313–318.

36 Peters LL, White RA, Birkenmeier CS, *et al.* Changing patterns in cytoskeletal mRNA expression and protein synthesis during murine erythropoiesis in vivo. Proc Natl Acad Sci USA 1992; 89:5749–5753.

37 Koury MJ, Sawyer ST, Bondurant MC. Splenic erythroblasts in anemia-inducing Friend disease: a source of cells for studies of erythropoietin-mediated differentiation. J Cell Physiol 1984; 121:526–532.

38 •• Chen K, Liu J, Heck S, *et al.* Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. Proc Natl Acad Sci USA 2009; 106:17413–17418.
This article systematically examined the dynamic changes of red cell membrane protein expression during murine erythropoiesis. A new experimental strategy for distinguishing unambiguously between erythroblasts at successive developmental stages was described.

39 • Chasis JA, Mohandas N. Erythroblastic islands: niches for erythropoiesis. Blood 2008; 112:470–478.
This review outlines our current understanding of erythroblastic islands. It provides comprehensive discussion of the role of erythroblastic islands in regulating normal and disordered erythropoiesis.

40 Fibach E, Rachmilewitz EA. A two-step liquid culture – a novel culture procedure for studying erythroid cell development. Haematologia (Budap) 1991; 24:211–220.

41 Malik P, Fisher TC, Barsky LL, *et al.* An in vitro model of human red blood cell production from hematopoietic progenitor cells. Blood 1998; 91:2664–2671.

42 Giarratana MC, Kobari L, Lapillonne H, *et al.* Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol 2005; 23:69–74.

43 Southcott MJ, Tanner MJ, Anstee DJ. The expression of human blood group antigens during erythropoiesis in a cell culture system. Blood 1999; 93: 4425–4435.

44 Socolovsky M, Nam H, Fleming MD, *et al.* Ineffective erythropoiesis in Stat5a(−/−)5b(−/−) mice due to decreased survival of early erythroblasts. Blood 2001; 98:3261–3273.

45 Liu Y, Pop R, Sadegh C, *et al.* Suppression of Fas–FasL coexpression by erythropoietin mediates erythroblast expansion during the erythropoietic stress response in vivo. Blood 2006; 108:123–133.