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Haematologica 2021 [Epub ahead of print]

Citation: Timothy J. Satchwell and Ashley M. Toye. Band 3, an essential red blood cell hub of activity. Haematologica. 2021; 106:xxx

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Band 3, an essential red blood cell hub of activity

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The red blood cell (RBC) is a marvel of cellular evolutionary specialisation. Whilst often considered simplistic owing to its absence of nuclei and other cellular organelles, this inability to respond transcriptionally and to replenish components through new protein synthesis necessitates complex post translational mechanisms through which the cell is able to control, adapt and regulate its key functions in the different environments it experiences traversing the circulation.

In this context, the membrane of the erythrocyte, perhaps more than any other cell, plays a crucial role. Erythrocyte membranes have a unique structure, comprising an array of integral membrane proteins with varied antigenic, transport and mechanical functions arranged in diverse multiprotein complexes (1). Underlying the membrane and connected to multicomplexes via adaptor proteins is the spectrin-based cytoskeletal meshwork. These protein interactions together with the membrane lipids themselves impart the characteristic properties of the biconcave red blood cell.

Central to the stability and functional regulation of the RBC membrane is band 3 (Anion exchanger 1, AE1), its most abundant protein at ~1.2 million copies per cell. Its unassuming name (a relic of its original identification as the third band on a Coomassie stained SDS PAGE separation of red blood cell membrane 'ghosts' (2)) belies the importance this protein plays not just through its direct contribution to gas exchange through the electroneutral exchange of Cl ions with the bicarbonate product (HCO₃⁻) of CO₂ metabolism, but as the major site of membrane cytoskeletal connectivity and a hub for haemoglobin and glycolytic enzyme binding.

A substantial body of evidence supports the binding of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to the cytosolic N-terminus of band 3 (3, 4) with competitive binding with the deoxygenated form of haemoglobin proposed first by the Low group, to direct the metabolism of glucose between glycolysis and the pentose phosphate pathway in response to cellular needs and the requirement for anti-oxidative measures. Put simplistically, band 3 and its interactions act as the RBC molecular switch to sense the oxygenated/deoxygenated state, imparting an appropriate metabolic response (see summary schematic in Figure 1A and 1B).

Crammed into a single paper within this issue of Haematologica, Issaian and colleagues have completed a heroic effort to deepen our understanding of the role that glycolytic enzyme binding to band 3 plays in erythrocyte metabolism (5). This is achieved using erythrocytes from well characterised mouse models or a rare natural human variant (B3 Neapolis) with truncations at the band 3 N terminus, in combination with detailed metabolic analyses. The authors demonstrate that loss of band 3 or mutation of the N terminal GAPDH binding site causes a failure of pentose phosphate pathway activation, with concurrent defects in glutathione recycling and evidence of oxidation products increasing arising during storage in the absence of the extreme N terminal 11 amino acids of band 3 (summarised in Figure 1C). Interestingly, the authors also show that reintroduction of the band 3 N terminus using cell penetrating peptides can rescue the observed metabolic defects. This metabolomics work is striking in the new level of detail it provides about the metabolism of normal and variant RBCs.

Another impressive feature of this manuscript is the depth of characterisation of the band 3 N terminal interactome provided by the authors. Varied proteomic approaches, including chemical crosslinking, characterised site-specific interaction interfaces between GAPDH and band 3, with additional prospective interacting proteins reported including enzymes involved in glutathione synthesis, recycling and lipid peroxidation pathways. However, dissecting the functional relevance of the band 3 interactome is beset with substantial challenges, with its own abundance, existence within at least 3 (and potentially many more sub-) populations each with genuine direct and additional indirect interactants (6), all complicating interpretation. It is unlikely and indeed in some
cases impossible (where binding sites for multiple proteins overlap) that all of the proteins reported bind to all of the copies of band 3, or even every band 3 containing complex. Efforts such as this, and others to uncover not just the identity but the plasticity and relevance of such interactions under physiologically relevant conditions, be they altered oxygenation (7), tonicity (8), mechanical deformation (9) or storage such as the work described here, are key to our evolving understanding of red blood cell structure function relationships.

Finally, given the essential role band 3 plays in RBC metabolism, the manuscript also touches on the potential of band 3 polymorphisms influencing the storage characteristics, using data from the REDSIII storage study (10). Here a note of caution needs to be applied as, besides a handful of already known polymorphisms in the band 3 N terminus (previously shown to be associated with HS), it remains unclear what precise impact these mutations have. Any alteration in band 3 abundance has knock on effects on RBC membrane stability which will impact on the expression of band 3 dependent and independent proteins alike. Nevertheless, more widely, further determination of the mechanistic basis by which polymorphisms (both pathogenic and non-pathogenic) can influence properties of the RBC beyond those most obviously apparent represents an important continuing endeavour.

Acknowledgements

TJS and AMT wrote the review together. TJS and the work in AMT’s lab is funded in part by a National Institute for Health Research Blood and Transfusion Research Unit (IS-BTU-1214-10032) in red blood cell products (University of Bristol) and also NHSBT R&D grant (WP15-04; WP15-05). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

References:

Figure 1.

Schematic adapted from Issaian et al.\textsuperscript{5} shows different conditions and the influence of band 3 on metabolism in 3 different scenarios (Panel A) In Deoxygenation/low oxidant stress conditions, deoxyhaemoglobin can bind to the N-term of band 3, while glycolytic enzymes become displaced from the same region and are then activated – resulting in increased fluxes through glycolysis and decreased fluxes through the pentose phosphate pathway. (Panel B) in oxygenated or high oxidative stress glycolytic enzymes can bind to the N-terminus of band 3, resulting in their partial inhibition, decreasing the metabolic flux through glycolysis and increasing flux through the pentose phosphate pathway to generate the reducing equivalent NADPH necessary to counteract oxidant stress. (C) In the absence of the extreme N-terminus of band 3 during storage, under high oxidative stress conditions, glycolytic enzymes cannot bind band 3 N-termini, so GAPDH flux continues, and pentose phosphate pathway is supressed leading to enhanced oxidative stress.