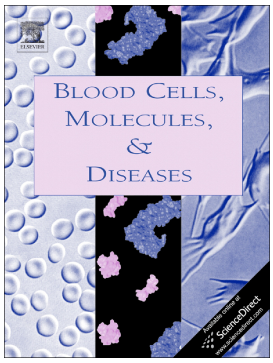

Cytoskeletal connectivity may guide erythrocyte membrane ex- and invagination – A discussion point how biophysical principles might be exploited by a parasite invading erythrocytes

Nir Gov, Ernst W. Müllner, Ulrich Salzer

PII: S1079-9796(17)30183-3
DOI: doi: 10.1016/j.bcmd.2017.05.002
Reference: YBCMD 2188

To appear in: *Blood Cells, Molecules and Diseases*

Received date: 21 April 2017
Revised date: ###REVISEDDATE###
Accepted date: 2 May 2017

Please cite this article as: Nir Gov, Ernst W. Müllner, Ulrich Salzer , Cytoskeletal connectivity may guide erythrocyte membrane ex- and invagination – A discussion point how biophysical principles might be exploited by a parasite invading erythrocytes, *Blood Cells, Molecules and Diseases* (2017), doi: 10.1016/j.bcmd.2017.05.002

Cytoskeletal connectivity may guide erythrocyte membrane ex- and invagination – a discussion point how biophysical principles might be exploited by a parasite invading erythrocytes

To the editor,

A series of biophysical and biochemical studies on erythrocyte membrane properties indicate that cytoskeletal connectivity influences membrane curvature. Increase in cytoskeleton/membrane interactions induce a bias towards membrane exvagination [1], whereas loss of connectivity and thus an increase in loose filament ends spontaneously endows the membrane with a concave curvature, eventually ending in the formation of endovesicles [2]. In light of recent findings on the host protein-composition of the parasitophorous vacuolar membrane (PVM) in erythrocytes infected by different apicomplexan species, we propose the hypothesis that the tick-borne intra-erythrocyte parasite Babesia divergens manipulates the host cytoskeleton to drive its invasion process.

The building blocks of the erythrocyte cytoskeleton consist of filamentous spectrin tetramers, six of which (on average) are being interconnected via short actin filaments in so-called junctional complexes. As prominent part of the junctional complexes, protein 4.1R regulates connectivity and membrane attachment by reversibly interacting with the integral membrane proteins glycophorin C, glycophorin D and/or the band 3 protein [3]. Additionally, membrane association of tetrameric spectrin filaments is also mediated by ankyrin which has two attachment sites more centrally located on the tetramer. The attachment of the hexagonal cytoskeletal lattice to the membrane is dynamically regulated by reversible phosphorylation events at the cytoskeleton-membrane interface.

The thermodynamic principle of entropy maximization applied to an ideal system of a planar lipid membrane supported by an extended cytoskeletal network indicates opposite effects on spontaneous curvature dependent on the degree of cytoskeletal connectivity. A highly connected and stretched network exhibits low entropy because of the restricted free moving space of the interconnecting filaments (tight springs). Narrowing the distance between membrane attachment points raises the entropy of the filaments because it increases the free moving space of the connecting filaments (relaxed springs). This entropic spring effect of a highly connected cytoskeletal network exerts a global contraction force on the membrane, imposing the tendency to spontaneous membrane exvagination and eventual exovesicle formation (Figure 1A). Accordingly, an increase in cytoskeleton/membrane attachment sites either by calcium influx or metabolic ATP-depletion indeed results in erythrocyte echinocytic shape transformation and release of exovesicles [4, 5].

In contrast, loss of cytoskeletal connectivity by destabilization of junctional complexes would increase the pool of spectrin filament tetramers either bound to the membrane with one end only or attached on their midpoint via the ankyrin-band 3 axis. Such loose spectrin filaments would increase their entropy by extending the free moving space around their membrane attachment points. In consequence, this entropic pressure would induce spontaneous membrane invagination and eventual endovesicle formation (Figure 1B). The curling phenomenon observed in isolated erythrocyte membranes (“ghosts”) was proposed to be caused by loose spectrin filaments [2]. Moreover, such an effect was also observed when polymers at low concentrations were grafted onto bilayers [6].

Partial destabilization and membrane detachment of junctional complexes can be triggered pharmacologically by treatment of erythrocytes with phorbol 12-myristate 13-acetate (PMA), inducing a specific phospho-modification of the 4.1R protein [7]. PMA treatment, which likely