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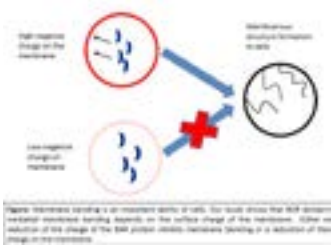
When structure leads to function: Protein complexes at the membrane in endocytosis

Statement of the Problem: The cell bends membranes to generate membrane structures, like the t-tubules in muscles. Bin1/Amphiphysin/Rvs domain proteins are part of the membrane bending machinery and are found in widespread phenomena like endocytosis or cell motility. BAR domain proteins can assemble spontaneously *in vitro* as well as *in vivo*. Which factors regulate the assembly, the membrane tension is a well-studied regulator. In contrast, the role of the membrane composition, as an initiator of membrane bending, is poorly understood.

Methodology & Theoretical Orientation: For this study, we collected electron micrographs to document the membrane bending activity of the BAR protein Bin1. We probed the electrostatic interactions between Bin1 and the membrane by changing the surface charge of the membrane, the ionic strength of the assay and using disease relevant mutants, where the positive charge (K35N) and the negative charge (D151N) are eliminated. The electrostatic interactions between Bin1 and artificial membranes were evaluated by liposome sedimentation. To test how the findings, translate into living cells, we assayed the phenotype of membrane bending-deficient Bin1 mutants in cells that have elevated or reduced levels of negatively charged lipids.

Findings: Our simple, artificial system could reproduce the complex membrane topology present in muscle cells. We focused on the two mutants. We found that in stringent conditions for membrane bending (high ionic strength, low membrane charge) the mutants showed disproportional lower bending activity. These finding were confirmed *in vivo*. We could rescue to mutant phenotype by increasing the membrane surface charge. Conversely, we induced a mutant phenotype in wt Bin1 by lowering the membrane surface charge.

Conclusion & Significance: We established the membrane charge as a novel regulator of membrane tubulation. We speculate that rapid phosphorylation and dephosphorylation of phosphoinositols can act as a switch for induction of membrane bending.



Biography

Carsten Mim has a longstanding interest in membrane and membrane-associated proteins throughout his career. As an experienced Electrophysiologist, he characterized the glutamate transporter EAAT3 and EAAT4. The kinetics of EAAT4 differ from other glutamate transporters, by a voltage sensitive step that slows the turnover rate at hyperpolarized membrane potentials. Further, recorded transient and steady state currents at different temperatures showed that the binding of glutamate is enthalpy-driven unlike the binding of Na+. To visualize membrane: protein complexes, he turned to electron microscopy. His work on the Bin/Amphyphysin/Rvs domain (BAR) protein endophilin in complex with the bilayer resulted in the unexpected discovery that the stability and dynamics of endophilin scaffolds entirely depend on non-specific interactions between amphipathic helices in the bilayer. His findings also provided a first structurally motivated hypothesis how BAR-scaffolds selectively recruit downstream interaction partners through a steric selection mechanism.

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