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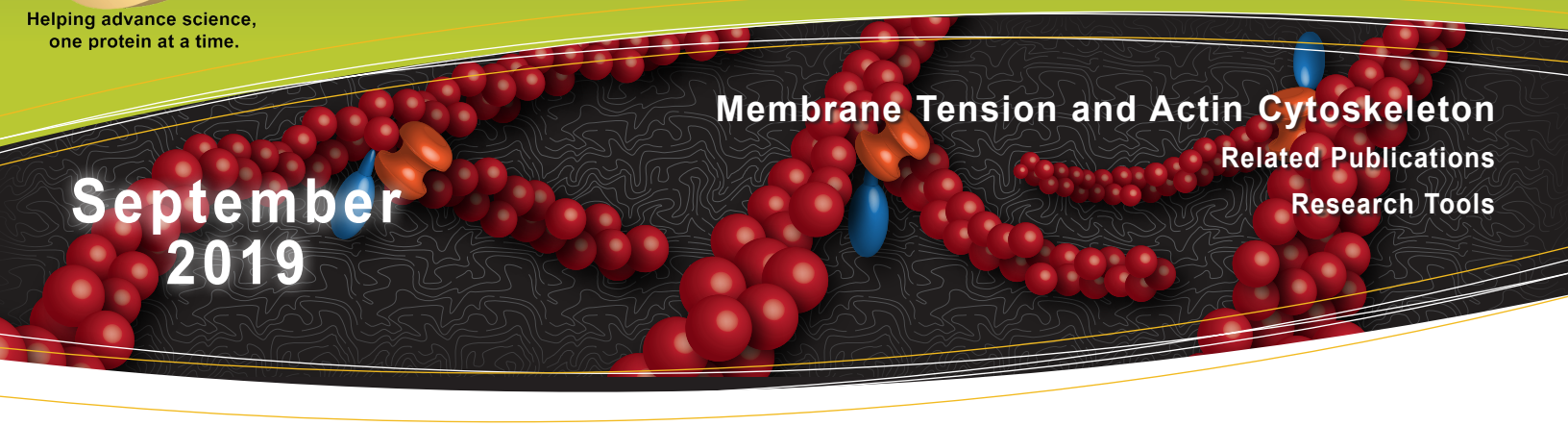

Membrane Tension and Actin Cytoskeleton

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Membrane Tension and Actin Cytoskeleton Interact to Regulate Cell Motility

Plasma membrane tension regulates many cellular functions, including exocytosis, endocytosis, division, and motility[1-6], all of which require a re-shaping of cellular morphology, which in turn relies upon an interplay between plasma membrane deformation and remodeling of the actin cytoskeleton[1,3,5-9]. This newsletter discusses coordinated interactions between the actin cytoskeleton and membrane tension during motility-associated morphogenesis.

Directed cell motility requires dynamic re-organization of the actin cytoskeleton at the front and rear of the cell (leading edge and trailing edge, respectively) with actin-based lamellipodial protrusions extending and pulling the cell forward in parallel with retraction at the trailing edge via changes in adhesion sites and RhoA-mediated actomyosin contractility[8,10,11]. As actin-based protrusions push the leading edge of motile cells forward and there is contraction at the trailing edge, the plasma membrane is deformed, changing membrane tension. Directed motility relies upon a balance between actin cytoskeleton remodeling and changes in membrane tension due to deformation[8] (Fig. 1).

One model of how the dynamic re-organization of both membrane tension and actin cytoskeleton interact to enable cell morphogenesis and motility emerged from early 2D cell motility experiments with either lamellipodial fragments from fish keratocytes or living, intact keratocytes[8,12]. The model hypothesizes that the polymerizing actin network within the leading edge of a motile cell pushes on the cell membrane from within, generating membrane tension that physically opposes the actin-based protrusions, rapidly equilibrates, and exerts globally constant force, per unit filament length, on the polymerizing actin network[12] (Fig. 1). At the center of the leading edge, high actin filament density results in low membrane tension per filament, allowing actin filaments to polymerize rapidly and drive protrusion of the leading edge. As the filament density decreases towards the cell's sides, resistance per filament increases until the membrane tension-induced load stalls actin polymerization, thereby establishing the front corners of the cell[12]. Similarly, an earlier study in fibroblasts found that membrane tension and lamellipodial protrusion numbers are inversely related[1]. Concomitantly, the trailing edge of the migrating cells has a disassembling actin network whose depolymerization is hastened by increased membrane tension, resulting in trailing edge retraction[12-14] (Fig. 1). Live cell imaging studies of motile keratocytes reported that both in-plane tension (tension in the membrane itself) and membrane-cytoskeletal attachment (adhesions and actomyosin-mediated contractility) regulate membrane tension in motile cells[15]. Indeed, total membrane tension is determined by both factors[8]. Lieber et al.[15] also confirmed that as actin polymerization increases at the leading edge, the stronger forward propulsion of filaments evokes higher membrane tension and vice versa. At the trailing edge, membrane tension is inversely correlated with myosin contraction levels and directly correlates with adhesion strength[15].

Recent studies in fibroblasts and fibroblast-like cells further clarify the interplay between the actin cytoskeleton and membrane tension. A high resolution microscopy study[16] in fibroblasts reveals that at the earliest stage of lamellipodial protrusion when the membrane reservoir is sufficient, the length of the protrusion is significant and membrane tension is low. The lamellipodia push outward on the membrane, membrane tension is increased (in-plane tension), and the extending protrusions smooth out any folds and curves in the plasma membrane. As the membrane reservoir is exhausted, tension rapidly increases and concomitantly the actin cytoskeleton is reorganized, resulting in decreased

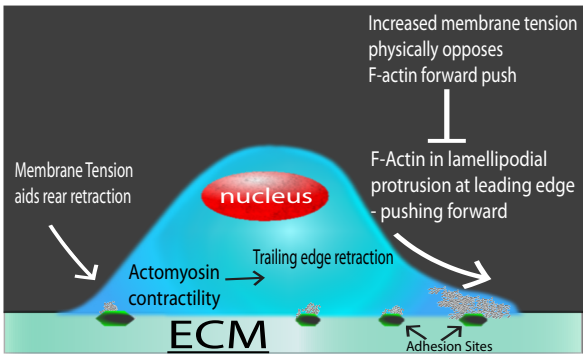

Figure 1: Schematic diagram of different forces in a migrating cell. Actin-based protrusions (i.e., lamellipodia) at the leading edge push the cell forward, while membrane tension physically opposes this motility. At the trailing edge, membrane tension aids in actomyosin-mediated contraction.

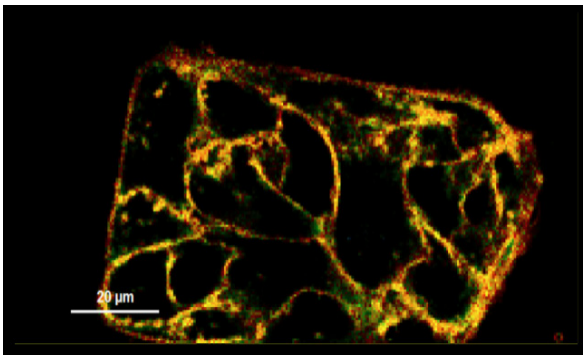

Figure 2: Fluorescent lifetime map of cell membranes stained with FLIPPER-TR membrane tension probe (Cat # CY-SC020). Green represents medium tension, yellow/orange/red is under higher tension, and blue is lower tension. Images courtesy of Colom et al. 2018.

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protrusion length[16]. In neutrophils, increased protrusions at the leading edge of a migrating cell coincide with increased membrane tension and decreased protrusion numbers at cellular locations other than the leading edge[7]. Another fibroblast study[17] reports cyclic oscillations in membrane tension inversely related to lamellipodial protrusion morphology. When tension increases, the protrusions decrease in size (reduced width) and shape (bend upwards) and as tension dissipates, protrusions return to their normal size, shape, and growth activity[17]. The actin-associated protein, FBP17 (formin-binding protein 17), is a membrane-bending protein and activator of WASP/N-WASP-dependent actin nucleation at the leading edge of migrating COS-1 (fibroblast-like) cells. Membrane tension inhibits FBP17 function by triggering its membrane detachment, providing a signaling pathway by which tension inhibits actin polymerization[18,19], suggesting the existence of a negative feedback loop where increased membrane tension eventually inhibits further protrusion development by inactivating pro-actin assembly molecules[8,18,19]. Other actin binding and capping proteins also regulate the dynamic re-modeling of the actin network that supports leading edge protrusions[20]. The phospholipase D2 and mammalian target of rapamycin complex 2 mechanosensory signaling cascade in neutrophils also provides a means by which tension inhibits actin nucleation[21].

Summary

The functional relevance of membrane tension extends beyond the biophysical realm as membrane tension (and other membrane attributes such as order and potential) impact nearly every vital biological process. Measuring the tension and dynamic changes in living cells has proven an exceedingly difficult undertaking. Challenges include validating the results obtained with 2D cell culture in 3D cell cultures and dissecting how regulation of membrane tension can vary not only between cell types but potentially between the state of the cell when tension is measured[8]. An exciting development is the development and commercial availability of fluorescent mechanosensitive probes specifically designed to study changes in membrane tension in real-time in living cells[9,22,23]. Cytoskeleton offers, Flipper-TR™, a new fluorescence probe to help cell biologists delve deeper into understanding how membrane tension interacts with the cytoskeleton to regulate basic cellular functions (Fig. 2). Additionally, Cytoskeleton offers live cell imaging probes for F-actin, microtubules, and DNA, as well as Biochem Kits for studying G-actin and F-actin levels in cells and interaction *in vitro*.

Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Actin™ Kit Includes SiR-Actin and Verapamil	630 / 680 nm	50 nmol	CY-SC001
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
SIR-DNA™ Kit Includes Verapamil	630 / 680 nm	50 nmol	CY-SC007
Flipper-TR™ Kit For fluorescence cell membrane microscopy	480 / 600 nm	50 nmol	CY-SC020

Biochem Kits

Product	Assays	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit Rabbit skeletal muscle actin	30-100	BK001
Actin Polymerization Biochem Kit (fluorescence format) Measure actin polymerization *in vitro*, contains rabbit skeletal muscle actin.	30-100	BK003
G-Actin/F-actin In Vivo Assay Biochem Kit Measure the distribution of monomer and polymer actin	30-100	BK037

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G-LISA Activation Assay Kits

Product	Assays	Cat. #
RhoA G-LISA™ Activation Assay (Luminescence format)	96	BK121
RhoA G-LISA™ Activation Assay Kit (Colorimetric format)	96	BK124
Rac1,2,3 G-LISA™ Activation Assay (Colorimetric format)	96	BK125
Rac1 G-LISA™ Activation Assay (Luminescence format)	96	BK126
Rac1 G-LISA™ Activation Assay Kit (Colorimetric Based)	96	BK128
Ras G-LISA™ Activation Assay Kit (Colorimetric Based)	96	BK131
Total RhoA ELISA	96	BK150

Acti-Stain™ Phalloidins

Product	Amount	Cat #
Acti-stain 488™ phalloidin	300 Slides	PHDG1-A
Acti-stain 555™ phalloidin	300 Slides	PHDH1-A
Acti-stain 670™ phalloidin	300 Slides	PHDN1-A
Rhodamine Phalloidin	1 x 500 µl	PHDR1