assembly and maintenance in budding yeast (Figure 1B).

In conclusion, these studies have revealed the existence of conserved AHs in related yeast and vertebrate basket Nups, thereby extending to peripheral Nups the repertoire of membrane-binding proteins required for de novo NPC assembly. However, the extent to which these AHs have evolved to cope with species-specific requirements remains to be determined. In particular, their implication in Y-complex recruitment to NPC assembly sites should be validated in live vertebrate cells and assessed in yeast. In addition, future studies will be required to establish whether and how their conserved membrane-bending properties contribute, along with other NE-shaping proteins, to membrane remodeling and/or curvature stabilization required for NPC assembly and maintenance.

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Translating Membrane Tension into Cytoskeletal Action by FBP17

Mirjam M. Zegers[1,*] and Peter Friedl[1,2,3,*]
[1]Department of Cell Biology, Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands
[2]David H. Koch Center for Applied Research of Genitourinary Cancers, Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77230, USA
[3]Cancer Genomics Centre Netherlands (CGC.nl), 3584 CG Utrecht, the Netherlands
*Correspondence: mirjam.zegers@radboudumc.nl (M.M.Z.), peter.friedl@radboudumc.nl (P.F.)
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A recent article by Tsujita et al. (2015) in *Nature Cell Biology* provides insight into how cells sense and translate plasma membrane tension toward polarized actin polymerization and migration. They identify FBP17 as a multifunctional adaptor that senses membrane curvature and delivers feedback to actin dynamics and directed cell migration.

Directed cell migration requires cells to polarize their actin cytoskeleton into a single front to protrude, elongate, and generate interactions with the substrate. Cell polarization results from local membrane-proximal signaling, followed by cytoskeletal remodeling and forward stretching of the leading edge, which locally and globally alters cell shape and increases tension of the plasma membrane (Friedl and Wolf, 2010; Houk et al., 2012; Lieber et al., 2013). Besides extracellular chemical and mechanical signals, which locally engage cytoskeletal activity through receptor-mediated mechanosensing, cells further sense plasma membrane tension and integrate these joint inputs into dynamics of the actin cytoskeleton, cell polarity, and migration. With increasing membrane tension, moving cells retract small protrusions but stabilize an already-existing leading edge, thereby retaining polarization and persistence of migration (Houk et al., 2012). Thus, membrane tension and cytoskeletal activity are tightly connected and thereby impact mechanotransduction and cell function in complex physicochemical environments.

The molecular mechanisms of sensing membrane tension are incompletely understood. High membrane tension flattens the membrane by reducing folds and curves. Therefore, lipid-binding proteins that interact with curved but not flat membranes are candidate proteins for tension sensing. Important curvature sensors include FCH-Bin-Amphiphysin-Rvs (F-BAR)-domain containing proteins, which bind, stabilize, and promote membrane curvature through the F-BAR domain (Zhao et al., 2013). F-BAR domain proteins further contain binding domains

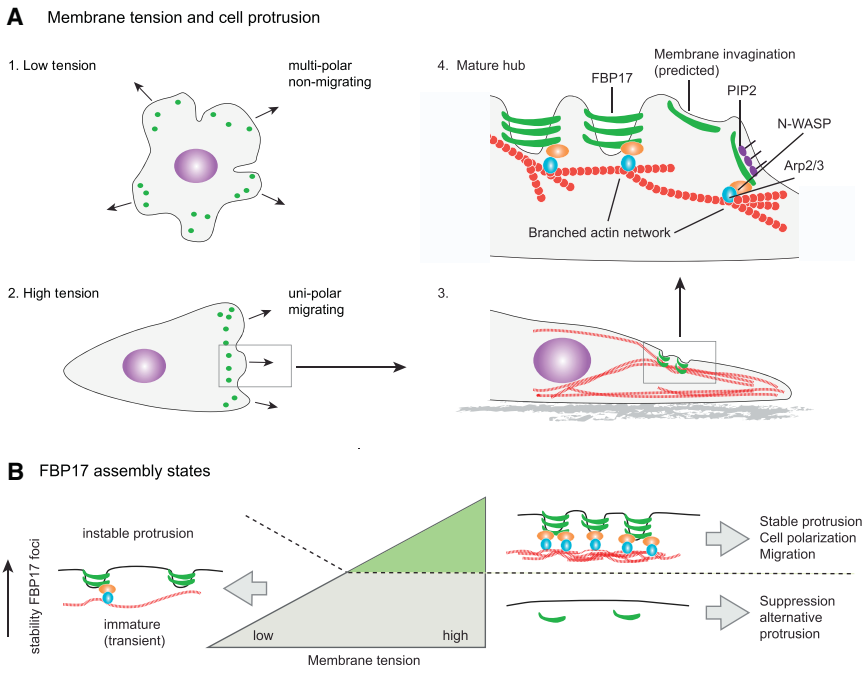

Figure 1. Impact of Membrane Tension on FBP17 Localization and Function in Protrusion Formation and Cell Migration

(A) (1 and 2) Function of FBP17 foci in actin network formation and development of a leading edge at reduced (1) or increased (2) membrane tension. FBP17-positive foci emerge near outward protrusions. (3) Predicted localization of FBP17 foci adjacent to actin networks at the leading edge. (4) Model of focalized membrane invagination stabilized by FBP17 through its F-BAR domain and functioning as hub toward N-WASP, Arp2/3, and branched actin networks. (B) Differential response of FBP17 foci to membrane tension and predicted maturation state. Low membrane tension enables immature FBP17 foci throughout the cell, which disassemble with increasing tension. Mature FBP17 foci, possibly stabilized through multi-protein assembly, are favored with increasing membrane tension and define the leading edge. Besides FBP17, other F-BAR-domain positive Toca family members Cdc42-interacting protein 4 and Toca1 may exert similar functions.

for the cytoskeletal regulators N-WASP and dynamin, as well as phosphoinositides in the plasma membrane, and therefore potentially act as a bridge between membrane and cytoskeletal dynamics (Diz-Muñoz et al., 2013). In addition to occurring through BAR-domain proteins, tension sensing may occur through mechanosensitive channels; however, how membrane tension is connected to cytoskeletal dynamics during cell migration remained unclear (Gauthier et al., 2012).

By combining advanced live-cell imaging with molecular interference and modulation of membrane tension, Tsujita and co-workers, in a recent publication in *Nature Cell Biology*, identify the F-BAR-domain protein FBP17 as an important membrane tension-sensitive hub that discriminates between low and high membrane tension and, by controlling labile, transient, and stable protrusions, defines front-rear polarity in moving cells (Tsujita et al., 2015). In cells spreading on substrate, nascent lamellipodia undergo rapid oscillatory cycles of protrusion and retraction, based on actin flow followed by collapse of the network. The regulation of such fast actin kinetics is not fully resolved but involves the function of Arp2/3, which is responsible for branching of actin filaments and forward flow. FBP17 regulates Arp2/3-dependent actin nucleation by activating N-WASP/WASP (Tsujita et al., 2006) by a mechanism that depends on its binding to curved membranes (Diz-Muñoz et al., 2013). Assuming that FBP17 localizes to small invaginations of the plasma membrane as submicron-sized patches and thereby triggers actin network formation, Tsujita et al. altered plasma membrane tension to test the impact on FBP17 engagement and downstream actin dynamics. Reducing plasma membrane tension globally by hypertonic shrinking resulted in rapid recruitment of patch-like FBP17 to the membrane and enhanced lamellipod formation at multiple sites (Figure 1A1). As result, vigorous but non-polar cytoskeletal dynamics were coupled to a non-migrating state ("running on the spot"). Conversely, increasing tension by hypotonic cell swelling resulted in prompt collapse of small oscillatory lamellipodia with FBP17 disappeared, whereas a single prominent leading edge with persisting FBP17 patches was retained or newly formed (Figure 1A2). These patches were distinct from clathrin-positive endocytic vesicles, suggesting a non-endocytic type of membrane domain. Because FBP17 patches required an intact F-BAR domain to bind to curved membrane, FBP17 foci may represent relatively stable invaginations of the plasma membrane from where actin-dependent protrusions are promoted (Figures 1A3 and 1A4). Consistently, downregulation of FBP17 by RNAi was sufficient to inhibit polarized lamellipod protrusion. This establishes FBP17 as an essential link between the membrane and actin network assembly.

The connection between FBP17 and its downstream effectors N-WASP and Arp2/3 was directly shown by expressing FPB17-W588K/P602L, an SH3-domain mutant defective for N-WASP activation, which failed to rescue polarized protrusion when endogenous FBP17 was downregulated. Likewise, direct interference with N-WASP or Arp2/3 by RNAi or with pharmacological inhibitors impaired polarized protrusion. Thus, FBP17 connects via N-WASP and Arp2/3 to actin network formation (Figure 1A4). Because interference with N-WASP and Arp2/3 further disabled the stability of polarized FBP17 patches, a feedforward mechanism was uncovered, suggesting that actin-dependent lamellipod protrusion stabilizes its driving FBP17 hubs nearby but disassembles hubs in secondary, smaller, or more-transient protrusions. FBP17 engages with membrane phosphoinositides through its PH domain (Zhao et al., 2013), and experimentally increased PIP2 levels supported FBP17 focalization and actin-rich protrusions (Figure 1A4). Thus, FBP17 may act as a dual effector that senses both membrane tension and PIP2 levels.

The differential response to increased membrane tension suggests distinct subtypes of FBP17-positive hubs with distinct outcome. A less-stable, transient

subset is associated with short-lived, highly oscillatory but less dedicated protrusions that are enabled at low but dissolve upon high membrane tension (Figure 1B, left). A second, more-stable hub withstands high membrane tension and supports a robust leading lamellipodium (Figure 1B, right). An additional subset of small lamellipodia, which form in complete absence of FBP17 puncta, was identified by kinetic cell analysis.

By forming a membrane-anchored hub, FBP17 emerges as a central mechanical and signaling integrator between the plasma membrane and the actin cytoskeleton. The lifetime of FBP17 foci ranged from seconds to minutes, with high positional stability and occasional rearward movement with the actin flow. FBP17 foci may stabilize through both maintenance of membrane curvature and PIP2 microdomains (Zhao et al., 2013), and, beyond N-WASP and Arp2/3, they may anchor further actin regulators (Saarikangas et al., 2010).

With this identification as tension-sensitive bridge between the plasma membrane and protruding actin networks, important questions arise regarding the identity of this FBP17 compartment, its ultrastructure and molecular composition, and its links to FBP17 functions in endocytosis. Membrane invaginations associated with BAR-domain proteins include clathrin-dependent and clathrin-independent compartments, which control vesicle trafficking and membrane turnover and regulate overall membrane availability and tension (Gauthier et al., 2012). It thus remains to be determined, by ultrastructural and superresolution analysis, whether these actin-associated FBP17 foci represent a yet-unappreciated stable type of membrane invagination or a particular state within a continuum between nascent invagination and extensive tubulation (Tsujita et al., 2006). It will also be relevant to dissect the relative importance of FBP17 hubs in further types of cell migration, including amoeboid, mesenchymal, and collective movements (Friedl and Wolf, 2010). Thus, the differential tension response of FBP17 hubs may contribute to cytoskeletal switches by tuning protrusive actin branching in diverse contexts of cell migration. In conclusion, the sensing of membrane curvature and hence tension by F-BAR-domain proteins, including FBP17, has the potential to be a universal tension-sensing mechanism to regulate physical integration of cell dynamics in complex processes in morphogenesis, repair, and cancer.

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