Cytoskeletal dynamics: A view from the membrane

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Many aspects of cytoskeletal assembly and dynamics can be recapitulated in vitro; yet, how the cytoskeleton integrates signals in vivo across cellular membranes is far less understood. Recent work has demonstrated that the membrane alone, or through membrane-associated proteins, can effect dynamic changes to the cytoskeleton, thereby impacting cell physiology. Having identified mechanistic links between membranes and the actin, microtubule, and septin cytoskeletons, these studies highlight the membrane's central role in coordinating these cytoskeletal systems to carry out essential processes, such as endocytosis, spindle positioning, and cellular compartmentalization.

The cytoskeleton underlies many aspects of cell physiology, including mitosis, cell division, volume control, cell stiffness, cell polarity, and extracellular matrix patterning. These events in turn impact development and tissue differentiation. The cytoskeleton receives, integrates, and transmits both intracellular and extracellular signaling cues. Most of these cues have to signal through a lipid bilayer before reaching the cytoskeleton. Thus, membrane–cytoskeleton interactions are central to deciphering how cytoskeletal remodeling is integrated throughout cells and tissues. Although signaling occurs across both the plasma and intracellular membranes, in this review we focus on the interplay between the cytoskeleton and the plasma membrane, which is predominantly composed of phospholipids (for a detailed review of plasma membrane lipid composition and localization, see Suetsugu et al., 2014).

Common to eukaryotic cytoskeletal networks is the fact that they are formed from proteins with the inherent ability to self-assemble into long polymers. These polymers exist in a dynamic equilibrium with a monomeric pool, resulting in constant turnover in the cell. The ensemble of regulatory proteins, which regulates these dynamics, acts as the interface between cellular signaling and cytoskeletal remodeling. Not surprisingly then,

many regulators of the cytoskeleton interact with membranes. However, it is still mostly unclear how these interactions work to regulate cytoskeletal dynamics and pattern specific subcellular networks in vivo. The cytoskeletal networks composed of actin, microtubules, and septins integrate various signals received at the membrane, and facilitate distinct functions in response. Actin has long been known to be intimately associated with membranes, and two major forms of actin regulation have been linked to the plasma membrane: (1) modulation of the actin monomer pool by phosphoinositides; and (2) modulation of actin assembly factors by membrane-associated small GTPases, by membrane-associated proteins, and by direct binding of assembly factors to the membrane. Also at the membrane, the actin-rich cortex interfaces with the microtubule cytoskeleton to coordinate intracellular events. Recent work has revealed mechanistic insights into this coordination with respect to spindle orientation, a critical event in development. To organize intracellular events, the membrane is compartmentalized, and this appears to be partially mediated by septins. We discuss recent studies that are beginning to mechanistically probe these membrane-associated cytoskeletal networks.
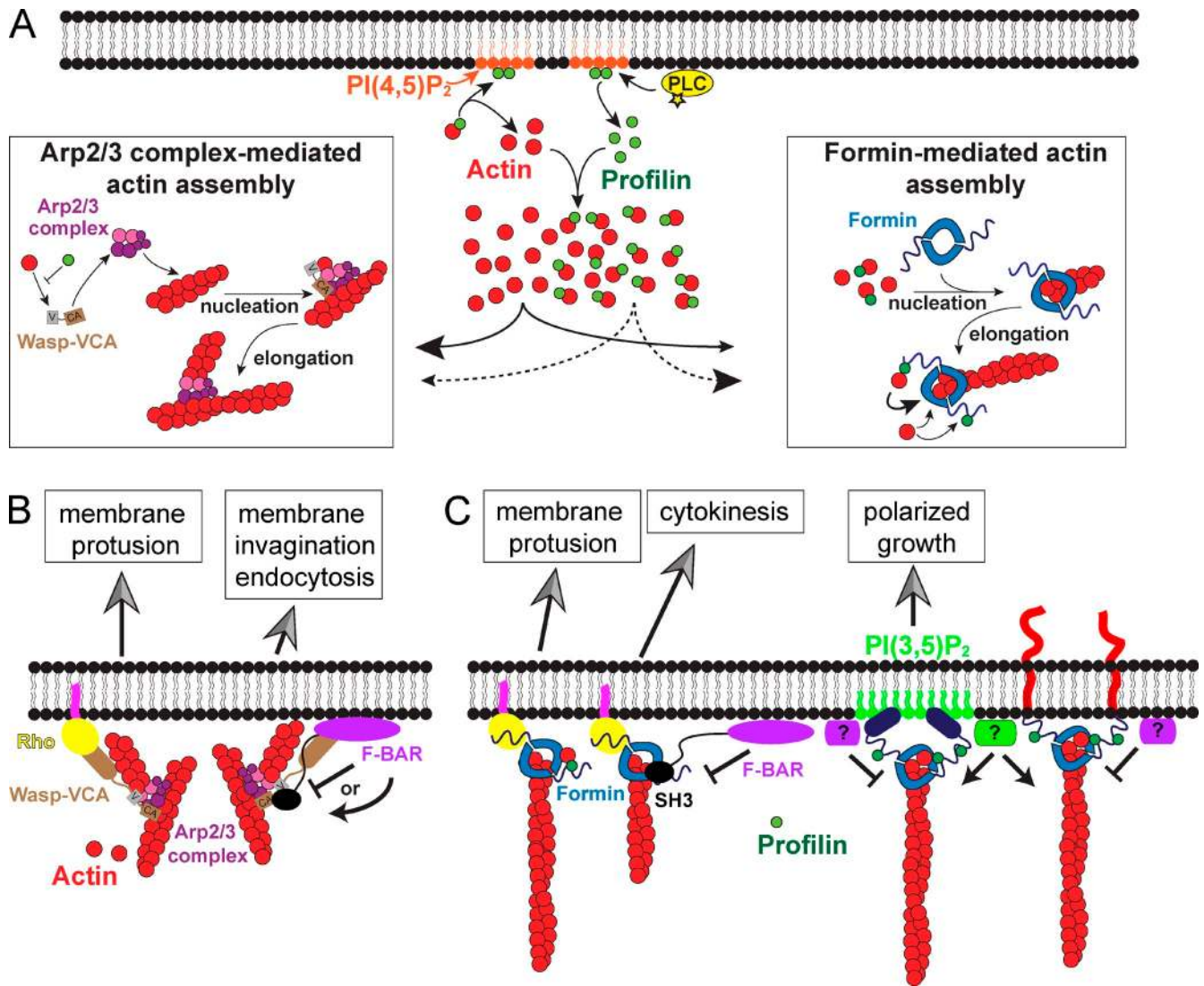
Membrane regulation of actin dynamics

Cells simultaneously assemble, maintain, and disassemble different F-actin networks within a common cytoplasm; each are tailored to facilitate a particular fundamental process such as motility, polarization, division, or endocytosis (Chhabra and Higgs, 2007; Blanchoin et al., 2014). F-actin networks with specified organization and dynamics are produced through the coordinated action of different overlapping sets of diverse actin-binding proteins with an array of complementary properties that include actin monomer (G-actin) binding, assembly, end capping, bundling, and severing/disassembling (Blanchoin et al., 2014). F-actin network assembly, organization, and dynamics are therefore controlled by the spatial and temporal regulation of the activity of actin-binding proteins. The association of these actin-binding proteins with the membrane is multifaceted. In some cases, actin-binding proteins are modulated by binding directly to phosphoinositide lipids. In other cases, membrane-associated proteins modify the activity of actin-binding

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Figure 1. **Regulation of actin assembly by membrane lipids.** (A) Membrane phosphoinositides such as $PI(4,5)P_2$ and $PI(3,4,5)P_3$ might control the spatial and temporal assembly of diverse actin filament networks by regulating profilin activity. Profilin bound to $PI(4,5)P_2$ cannot associate with actin, which potentially could establish a pool of free actin monomers that might favor the nucleation of branched actin filaments by the Arp2/3 complex, which is activated by binding to the WASP V-CA domain (left). Alternatively, phosphorylated phospholipase C (PLC) releases profilin by hydrolyzing $PI(4,5)P_2$, which could facilitate a pool of actin bound to profilin that might favor the elongation of unbranched actin filaments by formin (right) or Ena/VASP (not depicted). (B) Small activated GTPases of the Rho superfamily insert into the membrane via a covalent lipid modification. These GTPases recruit and activate a nucleation-promoting factor such as WASP/WAVE that further modulates Arp2/3 complex activity. F-BAR proteins interact with WASP and either activate or inhibit actin polymerization activity. These activities lead to diverse functions, as indicated in the text boxes. (C) Small activated GTPases of the Rho superfamily directly bind to and recruit formins to the membrane, where they activate actin polymerization. F-BAR proteins can further modulate actin dynamics by either activating or inhibiting formin activity at the membrane to drive processes such as membrane protrusion and cytokinesis. In eukaryotes, such as plants, that lack formins with obvious Rho-binding domains, many formins bind directly to the membrane via an N-terminal PTEN domain (dark blue) that binds to $PI(3,5)P_2$, driving polarized growth, or via an N-terminal transmembrane domain (red). Question marks designate hypothetical membrane-associated proteins that negatively or positively regulate formin-mediated actin polymerization.

proteins. Subsets of actin-binding proteins are even integral membrane proteins.

Phosphoinositide lipids associate with diverse types of actin-binding proteins, and either inhibit or stimulate their activity (for review see Saarikangas et al., 2010). The actin nucleation promotion factors, WAVE and WASP, facilitate actin polymerization via the Arp2/3 complex upon binding $PI(4,5)P_2$. In contrast, actin-capping protein, the F-actin–severing protein ADF/Cofilin, and the G-actin–binding protein profilin are all inhibited by binding $PI(4,5)P_2$ (Saarikangas et al., 2010).

Regulation of actin-binding proteins by association with and/or release from phosphoinositide lipids is an exciting possibility that could help explain the self-organization of diverse F-actin networks. However, the importance of phosphoinositide lipid regulation of most actin binding proteins has not been validated in vivo.

Membrane regulation of profilin

Cells maintain a reserve of up to hundreds of micromolar of unassembled G-actin monomers, which is available for rapid

polymerization upon activation of assembly factors and/or production of free actin filament ends (Pollard et al., 2000). Despite the effective critical concentration for actin assembly being only 0.1 μM, a higher concentration of unassembled actin is maintained in part by G-actin–binding proteins that prevent its de novo assembly. Profilin is the primary evolutionarily conserved small G-actin–binding protein (Carlsson et al., 1977), which prevents actin filament assembly by inhibiting the formation of actin dimer and/or trimer nuclei (Jockusch et al., 2007). Actin monomers bound by profilin can only be added to actin filaments that are assembled by actin assembly factors such as Arp2/3 complex, formin, and Ena/VASP (Dominguez, 2009). Profilin-bound actin was assumed to be equally incorporated into F-actin networks assembled by different nucleation factors. However, by simultaneously binding to G-actin and continuous stretches of proline residues that are found on specific actin assembly factors such as formin and Ena/VASP (Ferron et al., 2007), profilin significantly increases the elongation rate of formin-assembled filaments (Romero et al., 2004; Kovar et al., 2006). Conversely, profilin inhibits Arp2/3 complex–nucleated branch formation by competing with the nucleation-promoting factor WASP for G-actin (Suarez et al., 2015). As a result, profilin facilitates formin- and Ena/VASP-mediated actin assembly over assembly by the Arp2/3 complex (Rotty et al., 2015; Suarez et al., 2015). It is therefore likely that the spatial and temporal regulation of profilin helps govern the type of F-actin network assembled, as profilin activity determines whether G-actin is incorporated into networks generated by one actin assembly factor over another (Fig. 1 A).

Diverse profilins also bind to membrane phosphoinositides such as $PI(3,4,5)P_3$ and $PI(4,5)P_2$, which inhibits profilin's interactions with G-actin and proline-rich stretches (Lassing and Lindberg, 1985, 1988; Lu et al., 1996; Lambrechts et al., 2002; Moens and Bagatolli, 2007). Multiple hydrophobic regions of profilin, including the actin- and proline-rich–binding regions, have been implicated in binding to phosphoinositides (Jockusch et al., 2007). Association of profilin with membrane phosphoinositides has been proposed to regulate the temporal and spatial levels of profilin-actin by two possible mechanisms (Fig. 1 A). One possibility is that external signal-mediated phosphorylation of phospholipase C hydrolyzes $PI(4,5)P_2$, releasing membrane-bound profilin to presumably facilitate actin assembly by formin and Ena/VASP (Goldschmidt-Clermont et al., 1991). Second, sequestration of profilin to membrane regions with high concentrations of $PI(4,5)P_2$ could increase the level of free G-actin, unbound to profilin, that might preferentially incorporate into branched actin filament networks generated by the Arp2/3 complex. Despite the proposal of these general hypotheses nearly 25 years ago (Goldschmidt-Clermont et al., 1991), there is unfortunately little in vivo evidence that phosphoinositide regulation of profilin occurs (Saarikangas et al., 2010). However, most higher eukaryotes express multiple profilin isoforms that associate with the particular ligands with significantly different affinities, such as actin- or proline-rich ligands like formin, which could tailor them for different cellular roles (Jockusch et al., 2007). Therefore, regulation by phosphoinositides would theoretically be a convenient way for individual profilin isoforms to facilitate

self-organization of diverse actin filament networks by favoring particular actin assembly factors at discrete cellular locations (Neidt et al., 2009; Mouneimne et al., 2012; Ding and Roy, 2013). Further work is required to explore this exciting possibility.

Membrane regulation of actin assembly factors

Mechanistic insights for the role of the membrane are emerging in the case of the regulation of actin assembly factors. The most well-documented example of this is modulation of actin polymerization by small GTPases of the Rho superfamily. Most actin assembly factors are inherently inactive, but can be activated at the right time and place by small GTPase signaling cascades (Chesarone and Goode, 2009; Campellone and Welch, 2010). When activated, these small GTPases dock on the membrane due to exposure of a covalent lipid modification that intercalates into the membrane. Many actin assembly factors have GTPase-binding domains; binding to the active GTPase induces a conformational change, usually relieving an auto-inhibited state (Fig. 1, B and C). In the case of Arp2/3 complex, the SCAR/WAVE complex interacts with active GTPases and in turn activates the Arp2/3 complex, which generates filaments. Recently, new insights have emerged with respect to control of actin assembly at specific membrane sites. The WAVE complex was found to interact with a sequence motif found on a large number of diverse membrane proteins, ranging from channels to cell adhesion molecules. Binding occurs on a conserved face of the WAVE complex, which when mutated in flies leads to defects in the organization of the actin cytoskeleton (Chen et al., 2014). Future work is needed to sort out the signaling networks connected to this diverse set of membrane proteins and the specific physiological signals leading to activation of Arp2/3 complex-mediated actin polymerization.

While the details of specific membrane recruitment are still being sorted out, it is clear that small GTPases bind to and activate the SCAR/WAVE complex, which in turn activates the Arp2/3 complex. However, another actin assembly factor, the formins, are not always fully activated by binding small GTPases (Seth et al., 2006; Maiti et al., 2012). In fact, many formins have other mechanisms to bind to the membrane (for review see Cvrčková, 2013). For instance, in plants, formins do not have obvious GTPase-binding domains, and in fact, class I formins are integral membrane proteins themselves. Thus, regulation of these molecules at the membrane is likely mediated by interactions with proteins or specific lipids at the membrane (Fig. 1 C). In support of this, moss class II formins contain a PTEN domain that mediates binding to $PI(3,5)P_2$ (van Gisbergen et al., 2012). Recruitment to $PI(3,5)P_2$-rich membrane domains and the ability to rapidly elongate actin filaments is essential for formin function during polarized growth (Vidali et al., 2009; van Gisbergen et al., 2012). However, examination of formin molecules at the cell cortex demonstrated that only a fraction of these molecules generate actin filaments (van Gisbergen et al., 2012). Thus, additional molecules associated with $PI(3,5)P_2$ at the membrane likely modulate the activity of this formin (Fig. 1 C).

Whether there is a common family of molecules in eukaryotes that regulates membrane activity of actin assembly factors is unclear. However, a possible candidate class of

membrane-associated molecules is the Bin-Amphiphysin-Rvs (BAR) domain–containing proteins (Aspenström, 2009; Suetsugu et al., 2010; Cvrčková, 2013). The positively charged BAR domains, which are found on many different proteins (Suetsugu et al., 2010), form α-helical coiled-coils that fold up into a crescent shape. These domains do not have high specificity for a particular lipid, but rather through their structure can sense or participate in membrane bending (Suetsugu et al., 2010, 2014).

In yeast and animals, a family of proteins with an extended BAR domain, known as F-BAR proteins, are essential scaffolds upon which cytoskeletal proteins can assemble in order to generate specific subcellular structures and functions (Roberts-Galbraith and Gould, 2010). During endocytosis, nucleation-promoting factors for the Arp2/3 complex are recruited to the membrane by interacting with F-BAR proteins. F-BAR proteins not only recruit nucleation-promoting factors, but also modify their activity (Kamioka et al., 2004; Itoh et al., 2005; Tsujita et al., 2006; Takano et al., 2008; Henne et al., 2010; Roberts-Galbraith and Gould, 2010; Wu et al., 2010). In budding yeast, two F-BAR proteins oppositely regulate Las17, a homologue of the WASP actin nucleation–promoting factor (Fig. 1 B). Early in endocytosis, Syp1 recruits WASP but maintains it in an inactive state (Rodal et al., 2003; Sun et al., 2006; Boettner et al., 2009; Feliciano and Di Pietro, 2012). Upon vesicle maturation, Bzz1 activates WASP activity (Sun et al., 2006), thereby inducing a burst of actin polymerization mediated by the ARP2/3 complex that promotes internalization of endocytic vesicles. Further physiological support for this model has come from studies in neurons (Dharmalingam et al., 2009) and animal cells (Tsujita et al., 2006).

F-BAR proteins also recruit formins to membranes. In fission yeast, the F-BAR proteins Cdc15 and Imp2 help recruit the essential cytokinesis formin Cdc12 to the division site (Chang et al., 1997; Carnahan and Gould, 2003; Ren et al., 2015). Similarly, the budding yeast Cdc15 homologue Hof1p acts redundantly with Rvs167 (a BAR domain–containing protein also containing a C-terminal SH3) to promote formation of the contractile actin ring (Nkosi et al., 2013). Although F-BAR proteins have clearly defined roles in recruiting formins, several recent studies have revealed how F-BAR proteins directly modulate formin activity. In mammals, the F-BAR protein srGAP2 binds to and directly inhibits the actin-severing activity of the formin FMNL1, which is mediated by its formin homology (FH) 1 domain (Mason et al., 2011). During *Drosophila melanogaster* embryogenesis, the F-BAR protein Cip4 binds to the formin Dia's FH1 domain and inhibits the ability of Dia to promote actin assembly. Cip4 is a known activator of the WASP–WAVE–Arp2/3 complex pathway. Thus, while Cip4 activates Arp2/3 complex activity, it can simultaneously inhibit Dia activity (Yan et al., 2013). More recently, it was demonstrated in budding yeast that the SH3 domain of the F-BAR protein Hof1p dampens the actin nucleation activity of the formin Bnr1p without displacing Bnr1p from the actin filament end (Fig. 1 C; Graziano et al., 2014). These studies suggest that F-BAR proteins may have a conserved role in regulating diverse sets of actin nucleation factors at the membrane. Thus, understanding how BAR domain–containing proteins interact with and regulate specific subsets of actin regulators may help to decipher the distinct F-actin domains at the cell cortex. Additionally, since BAR domain–containing proteins are found widely throughout eukaryotes (Ren et al., 2006), it is possible that these molecules may have been an early link between membranes and actin modulation that, with various elaborations, evolved differently in distinct lineages.

Connecting actin and microtubules to the membrane enables cortical force generation

The cell cortex in animal cells plays a fundamental role in cell division, migration, and polarization (Kunda et al., 2008; Pollard and Cooper, 2009; Stewart et al., 2011; Abu Shah and Keren, 2014). The cortex integrates external stimuli—from extracellular matrix and neighboring cells—and transmits them into the cell to effect cytoskeletal changes crucial for development. A key component of the cortex is the thin F-actin shell underneath the cell membrane that is crucial for providing cortical stiffness and is a key determinant of cell shape (Pollard and Cooper, 2009; Guo et al., 2013). Perturbations in cortical F-actin architecture can alter the physical properties of the cortex, thereby affecting cell stiffness and strength. A recent study demonstrates that the bulk of the actin cortex is nucleated by the formin mDia1 and Arp2/3 complex (Bovellan et al., 2014), which suggests that fine-tuning of F-actin cortical structure and mechanics may be mediated by adjusting the relative contribution of each actin assembly factor.

Several studies (for reviews see Basu and Chang, 2007; Akhshi et al., 2014) show that changes in microtubule stability also positively and negatively regulate cortical F-actin structures, including formation of lamellipodia and stress fibers. Here we focus on the converse: regulation of microtubule function by the actin-rich cortex. An excellent example of this regulation is how these two elements set the orientation of the mitotic spindle, which determines the plane of cell division, thereby impacting cell fate and tissue organization. It has been known for quite some time that, during cell division, an intact cortical F-actin meshwork and an intact astral microtubule array are required for spindle orientation (O'Connell and Wang, 2000; Théry et al., 2005; Toyoshima and Nishida, 2007; Fink et al., 2011; Luxenburg et al., 2011; Castanon et al., 2013). However, how the F-actin cortex is involved in this process, and how the membrane supports the underlying cytoskeletal organization to bring about spindle alignment toward a specialized cortical domain, remains unclear in many cellular systems.

The prevailing notion is that the F-actin network provides a platform for a cortical anchor, or a complex of anchoring proteins, that could either mediate attachment (i.e., tethering) of astral microtubules or recruit force generators such as motor proteins that exert pulling forces on the microtubules emanating from the spindle. In this notion, the plus ends of astral microtubules would engage with these cortical platforms through so-called +TIPs (plus tip tracking proteins), including adenomatous polyposis coli protein (APC), CLASP, CLIP170, LIS1, dynactin, and dynein (Coquelle et al., 2002; Rogers et al., 2002; Reilein and Nelson, 2005; Siller and Doe, 2008; Ruiz-Saenz et al., 2013). Data to support this idea has been found in

several organisms, including *Caenorhabditis elegans* zygotes (Couwenbergs et al., 2007; Nguyen-Ngoc et al., 2007), *Drosophila* neuroblasts (Siller et al., 2006), and cultured human cells (Kiyomitsu and Cheeseman, 2012). These studies have identified an evolutionarily conserved ternary complex composed of Gαi, the α subunit of heterotrimeric G-protein; LGN, a leucine-glycine-asparagine repeat protein; and NuMA, a nuclear mitotic apparatus protein; as the cortical anchoring complex that recruits dynein as the force generator for spindle orientation. NuMA interacts with LGN (Du and Macara, 2004; Bowman et al., 2006; Siller et al., 2006), which in turn binds to the myristoylated Gαi that is directly attached to the membrane. NuMA can also bind the membrane directly through a C-terminal PIP-binding domain in a manner independent of LGN and Gαi (Zheng et al., 2014). Intriguingly, when the F-actin meshwork was disrupted, NuMA and Gαi dissociate from the cell cortex (Luxenburg et al., 2011; Machicoane et al., 2014; Zheng et al., 2014), signifying that their membrane association is weak. These observations raise interesting questions about the physical nature of the anchoring platform, and suggest that additional mechanisms may be required to attach anchoring proteins to the F-actin meshwork or to stabilize them at the cortex.

Recent work has shown that the actin-binding proteins ezrin/radixin/moesin (ERM) are probably the missing puzzle pieces at the cell cortex mediating spindle orientation (Solinet et al., 2013; Machicoane et al., 2014). ERMs help organize the F-actin meshwork, bridging it to the cell membrane, and this may be necessary for establishing and maintaining the Gαi-LGN-NuMA cortical platform. ERMs, when activated by Ste20-like (SLK) kinase (Machicoane et al., 2014), adopt an open conformation that binds F-actin and the plasma membrane. An N-terminal FERM domain, which binds $PI(4,5)P_2$ directly (Fievet et al., 2004; Roch et al., 2010; Roubinet et al., 2011), mediates interaction with the membrane. Interestingly, the FERM domain also binds to and stabilizes microtubules (Solinet et al., 2013), possibly via interaction with CLASP family of +TIPs (Ruiz-Saenz et al., 2013), which suggests that ERMs may function as microtubule-tethering factors. However, evidence suggests that they do more than just tethering microtubules. Depletion of ERMs or inhibition of ERM activation leads to loss of cortical rigidity, mislocalization of LGN and NuMA, and abnormal spindle rocking behavior (Carreno et al., 2008; Machicoane et al., 2014). It is interesting to speculate that ERMs may be required to increase membrane rigidity by pinning the F-actin meshwork to the plasma membrane. As proposed (Zheng et al., 2014), this rigidity may enable the cortical platform to counteract astral microtubule–mediated and dynein-generated pulling forces on the cortical anchors. It is noteworthy that the budding yeast version of the dynein cortical anchor, Num1, interacts with the plasma membrane directly via a BAR-like domain and a PH domain (Farkasovsky and Küntzel, 1995; Tang et al., 2009, 2012; Klecker et al., 2013; Lackner et al., 2013). In budding yeast, actin is dispensable for maintenance of the Num1 cortical platform (Heil-Chapdelaine et al., 2000a) or to support dynein-dependent spindle movements (Heil-Chapdelaine et al., 2000b), as membrane rigidity is provided by turgor pressure and the cell wall. During animal development, it is
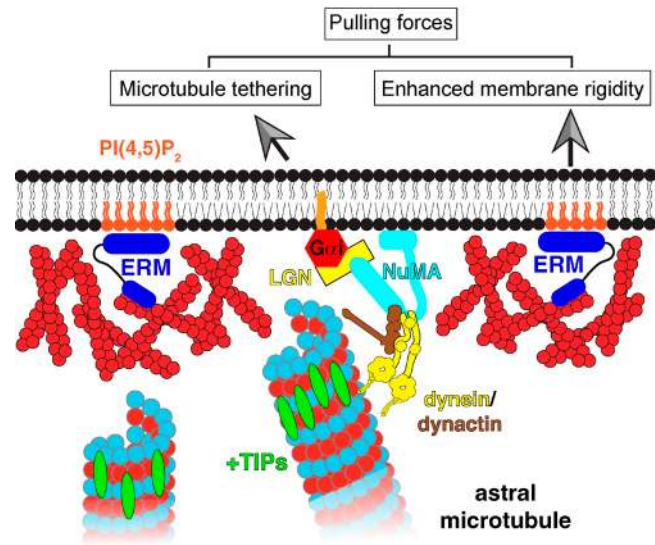

Figure 2. **Regulation of microtubule tethering by actin-dependent membrane rigidity.** ERM increases membrane rigidity to support Gαi-LGN-NuMA–dependent anchoring and pulling of astral microtubules by cytoplasmic dynein. Activated ERMs in an open conformation may link F-actin to the cell membrane. Membrane association of the Gαi-LGN-NuMA complex mediated by the lipid anchor on Gαi and the PIP-binding domain on NuMA are presumably weak. Stiffening of the membrane (indicated by straight phospholipid tails) or yet unidentified interactions with F-actin or ERMs may further stabilize the Gαi-LGN-NuMA platform to prevent anchorage detachment.

conceivable that stabilization of membrane rigidity, as exemplified by ERMs, may represent a general mechanism for modulating pulling forces on astral microtubules (Fig. 2). It is therefore tempting to speculate whether the recently characterized human cortical actin–associated protein, MISP, which has a role in astral microtubule stability and spindle orientation (Zhu et al., 2013), would orchestrate actin cytoskeleton communication with the cell membrane and the astral microtubules in a similar manner. Deciphering how actin-dependent membrane rigidity is controlled locally at specific regions of the cell cortex will surely constitute a major challenge to unraveling the mechanisms governing spatial and temporal regulation of oriented cell division.

Septins: links between polymer assembly and membrane function

An additional layer of membrane compartmentalization is provided by septins. Septins are a component of the cytoskeleton that directly bind to membranes in order to polymerize and in turn help organize cell membranes. Knowing how membranes specify septin assemblies at a particular place and time is essential to understand the mechanistic role of septins in cytokinesis and beyond.

Septins were first observed at the plasma membrane in budding yeast (Byers and Goetsch, 1976; Rodal et al., 2005; Ong et al., 2014). Early work found that human septins exhibit a preference for $PI(4,5)P_2$ and proposed that a conserved polybasic sequence in septins links them to phospholipids (Zhang et al., 1999). More recently, recombinant budding yeast septins were assembled on lipid monolayers containing high levels (10–50%) of $PI(4,5)P_2$ (Bertin et al., 2010). Interestingly, the presence of the lipids could promote filament formation even
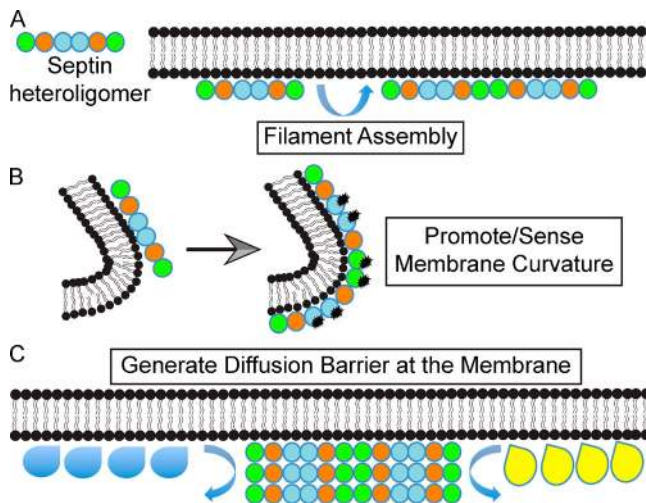
Figure 3. **Septins assemble on and organize membranes.** (A) Membrane association of septin heteroligomers promotes assembly of septin filaments. (B) Septins may play a role in promoting or sensing membrane curvature. Posttranslational modifications (indicated by black stars) are implicated in this function. (C) By providing a diffusion barrier, septins play a role in compartmentalizing the membrane.

with septin proteins that were otherwise defective for polymerization, which suggests that membranes can facilitate filament assembly. The first dynamic look at septin assembly with reconstituted septin proteins supported lipid bilayers with low levels of PI(4,5)P_2, and single-molecule total internal reflection fluorescence (TIRF) imaging found that septin filaments elongate through diffusion in two dimensions and annealing (Bridges et al., 2014). The notion that polymerization occurs at the membrane is supported by the finding that cytosolic pools of septins in diverse fungi and mammals consist of minimal heteromeric rods (or heteroligomers) but not filaments (Sellin et al., 2011; Bridges et al., 2014). Thus, membranes are intimately involved in septin filament formation (Fig. 3 A).

Septins are frequently found in areas where membranes are highly curved, such as the mother-bud neck in yeast and the bases of dendritic spines and primary cilia (Fares et al., 1995; Xie et al., 2007; Hu et al., 2010). This raises the possibility that septins sense and/or generate curvature, which is supported by the finding that septin filaments can tubulate phospholipid liposomes in vitro (Tanaka-Takiguchi et al., 2009; Fig. 3 B). In vivo, septins are recruited to curved blebs of membrane that are pulled back toward the cell center, which suggests that there may be a capacity for them to recognize specific curvatures (Tanaka-Takiguchi et al., 2009; Gilden and Krummel, 2010). Recent work has shown that septins can promote the formation of curved and ordered bundles of F-actin at the highly curved membranes of furrow canals during embryo cellularization, which suggests that septins and actin may collaborate for curvature sensing (Mavrakis et al., 2014). In mycelia of filamentous fungal systems, there are septin regulatory kinases that are only required for straight septin filaments without impacting septins that assemble at the curved surfaces, which suggests that posttranslational modifications could influence curvature preference or sensing (DeMay et al., 2009; Fig. 3 B).

Finally, there has been substantial interest in the role of septins as diffusional barriers, and work from yeast to human cilia has suggested the possibility that septins can functionally compartmentalize membranes (Takizawa et al., 2000; Barral et al., 2000; Hu et al., 2010; Fig. 3 C). Despite the first observations of a barrier function over a decade ago, the mechanism by which septin compartmentalizes membranes has proven to be highly elusive. The first clues as to a molecular basis for the ER-based barrier have come from several recent studies. Yeast genetics uncovered a link between sphingolipid domains and septin-based ER barriers, and a second study identified a role for one specific septin, Shs1, in these barriers (Chao et al., 2014; Clay et al., 2014). Finally, a critical functional role for septins in membrane compartmentalization came from a screen looking at regulators of calcium influx in cultured mammalian cells (Sharma et al., 2013). This study showed that septins are required for establishing PIP$_2$-rich microdomains at sites of ER–plasma membrane contacts. These functional studies, along with the development of reconstitution methods for probing the barrier properties in artificial lipid membranes, should pave the way for understanding how septins influence membrane diffusion. But it is clear that a reciprocal relationship between certain membrane domains and septins underlies their organization and function.

Conclusions

As more mechanistic connections emerge between the membrane and the cytoskeleton, it is becoming clear that a new generation of tools is needed. In particular, being able to track the dynamics and localization of specific lipid species, as well as physical methods to measure membrane rigidity in living cells, is critical. Additionally, most studies have been performed in individual cells, but not in the context of developing tissues or varied extracellular environments. Thus, how mechanical strains on the membrane translate into cytoskeletal reorganization ultimately effecting cell physiology and development constitutes the next generation of questions in cytoskeletal dynamics.

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