Trends in Biochemical Sciences

CellPress

Review

Formins at the Junction

Katharina Grikscheit[1,*] and Robert Grosse[1,*]

The actin cytoskeleton and adhesion junctions are physically and functionally coupled at the cell–cell interface between epithelial cells. The actin regulatory complex Arp2/3 has an established role in the turnover of junctional actin; however, the role of formins, the largest group of actin regulators, is less clear. Formins dynamically shape the actin cytoskeleton and have various functions within cells. In this review we describe recent progress on how formins regulate actin dynamics at cell–cell contacts and highlight formin functions during polarized protein traffic necessary for epithelialization.

Trends

Formins are a large group of actin regulators that actively shape the epithelial actin cytoskeleton.

Formin-regulated actin polymerization is crucial during cell–cell contact and adherens junction formation.

Formin-dependent actin polymerizing activity is required for lumen formation in tissue culture and *in vivo*.

Polarized targeting of proteins to the apical membrane via vesicle-based transport mechanism depends on actin filament structures generated by formins.

Actin at Epithelial Cell–Cell Junctions

Within an epithelial tissue, the actin cytoskeleton must equilibrate between stability and contractility to maintain cell shape as well as cell polarity. Furthermore, it must remain flexible, especially during cell division or cell–cell contact formation. To do so, actin turnover is tightly regulated during filament nucleation and polymerization, which are controlled by a plethora of actin nucleation factors such as the Arp2/3 complex, formins, and more recently described factors including Spire, Cordon-bleu, and leiomodin 2 [1–4].

Interestingly, these groups of actin nucleators exhibit distinct mechanisms for their common tasks. For example, the Arp2/3 complex is a multisubunit complex that catalyzes actin polymerization from sites of preexisting filaments, resulting in a branched network. By contrast, formins generate unbranched filaments in a processive manner at the barbed end of the filament. Formins are a protein family comprising 15 members in mammals, including multiple isoforms and splice variants [5]. All formins share the ability to efficiently shape the cytoskeleton, making them master regulators of actin assembly within cells. In addition, formins are able to stabilize microtubules (Figure 1) [6–8].

The potent actin assembly of formins is translated into diverse cellular functions such as the control of cytokinesis, the activation of serum response factor (SRF)-regulated transcription, and also drive the pathological invasion of cancer cells [8–15]. In this review we highlight the emerging role of formins in regulating the dynamic turnover of actin at cell–cell contacts as well as the targeted trafficking processes that are crucial for epithelialization.

The four main types of cell–cell contacts (tight, gap and adhesion junctions, desmosomes) are all essential for tissue organization because they determine polarity, barrier function, stability, and cellular communication (Box 1). It has long been appreciated that adherens junctions (AJ) are both functionally and physically connected with the actin cytoskeleton. Sequestering of actin monomers using latrunculin B severely impairs cellular adhesion, indicating continuous actin polymerization at cell–cell contacts [16]. Conversely, E-cadherin ligation during cell–cell contact formation induces intensive actin remodeling, implicating a strong interdependency between AJs and the actin cytoskeleton [17–19]. However, the exact mechanism of how actin is linked to the AJ components and how it assembles during cell–cell attachment is not entirely understood [20,21]. Importantly, the AJ–actin interrelationship is not only relevant at newly-formed, nascent cell–cell contacts, but is equally important when cells are connected via stable contacts, such as within an epithelial tissue [22].

[1]Institute of Pharmacology, Biochemical–Pharmacological Center (BPC), University of Marburg, 35032 Marburg, Germany

*Correspondence: katharina.grikscheit@staff.uni-marburg.de (K. Grikscheit) and robert.grosse@staff.uni-marburg.de (R. Grosse).

 http://dx.doi.org/10.1016/j.tibs.2015.12.002

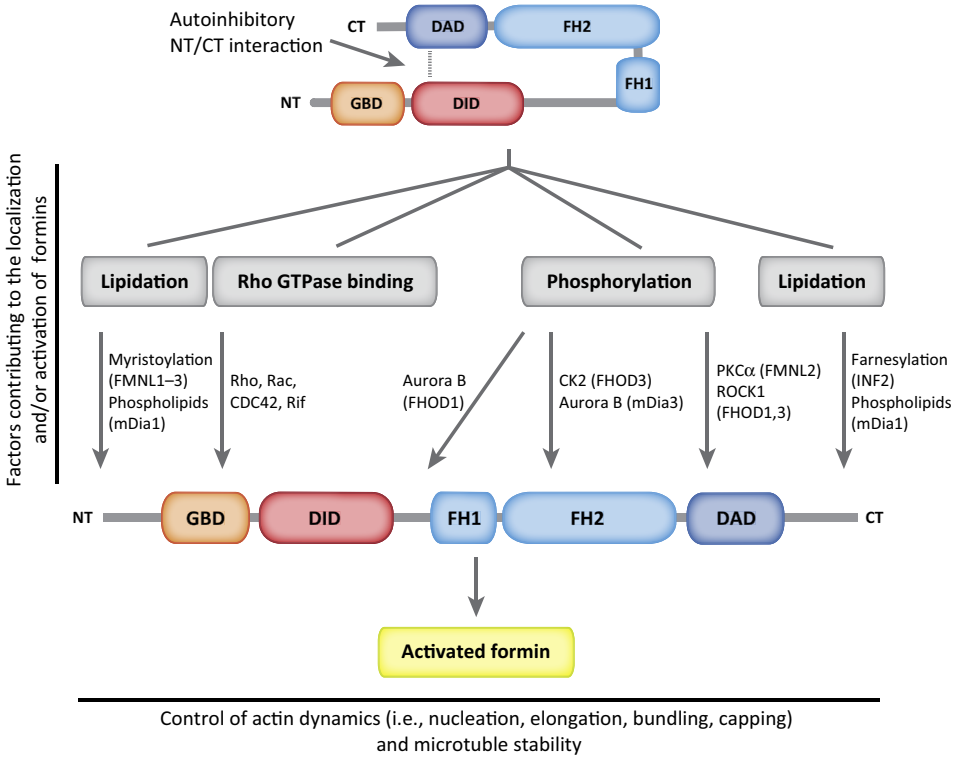

Figure 1. Formin Domain Organization and Regulation. Formins are multidomain proteins. The FH2 (formin homology 2) domain is highly conserved within the formin family and polymerizes F-actin. The FH1 domain is required for (monomeric) G-actin recruitment. The formin is rendered inactive through an intramolecular interaction between the DAD (diaphanous autoinhibitory domain) and the DID (diaphanous inhibitory domain). Active Rho GTPases such as Rho, Rac1, CDC42, or Rif trigger formin activity through release of autoinhibition [31]. Further signals such as lipidation, farnesylation, or phosphorylation have been shown to regulate activation and localization. Regulations specific for a formin or formin group are indicated in parentheses. Owing to a myristoylation signal at their N-termini (NT), formin-like proteins (FMNLs) localize to membranes [35,36,96]. INF2 (inverted formin 2) is farnesylated at the N-terminus, contributing to its association with the endoplasmic reticulum [97]. Binding of phospholipids to the Diaphanous 1 (Dia1) N-terminus as well as C-terminus (CT) affects its localization as well as activation [98]. Aurora B kinase phosphorylates residues in formin homology domain-containing 1 (FHOD1), which regulates actin cables after cell division [13]. The FH2 of Dia3 is phosphorylated by Aurora B kinase, which inhibits its ability to stabilize microtubules [8]. A muscle-specific isoform of FHOD3 contains two distinct phosphorylation sites: casein kinase 2 (CK2) phosphorylates a site in the FH2 domain, and Rho-associated kinase 1 (ROCK1) phosphorylates a second site in the DAD domain [37]. FHOD1 can be activated through phosphorylation by ROCK1 [38]. Protein kinase Cα (PKCα) phosphorylates FMNL2 in the DAD domain, thereby promoting its localization and activity [39]. Active formins regulate actin dynamics through filament nucleation, polymerization, severing, and capping. In addition, individual formins are involved in microtubule stabilization [6,7]. Abbreviation: GBD, GTPase binding domain.

Diverse actin-regulating factors have been uncovered at cell–cell contacts, such as the Arp2/3 complex, which interacts with E-cadherin and is crucial for junctional actin turnover *in vitro* and in living cells [16,23–27]. In the following sections we discuss the capacity of formins to polymerize actin and their relevance in tethering the actin cytoskeleton to the cell–cell contact site [28]. This is further exemplified by the increasing relevance of formins in physiological relevant 3D cell culture systems and *in vivo* developmental models of lumen formation (Figure 2).

Formin Regulation

Formins are large multidomain proteins that become activated and dimerized before they can elongate actin filaments (Figure 1). For details on the molecular mechanism of formin function we refer to previous excellent reviews [1,6,29,30]. In brief, formins consist of two functional entities: the N terminus comprises the regulatory domains, whereas the actin-polymerization domains