

Formation of Nanotube-Like Protrusions, Regulation of Septin Organization and Re-guidance of Vesicle Traffic by Depolymerization of the Actin Cytoskeleton Induced by Binary Bacterial Protein Toxins

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Abstract A large group of bacterial protein toxins, including binary ADP-ribosylating toxins, modify actin at arginine-177, thereby actin polymerization is blocked and the actin cytoskeleton is redistributed. Modulation of actin functions largely affects other components of the cytoskeleton, especially microtubules and septins. Here, recent findings about the functional interconnections of the actin cytoskeleton with microtubules and septins, affected by bacterial toxins, are reviewed.

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1 Introduction

Actin is an essential protein that plays a central role in numerous cellular processes. It is an abundant, highly conserved protein that is expressed in most cell types in a very large amount (Blanchoin et al. 2014; Dominguez and Holmes 2011). Actin dynamics are regulated by numerous proteins, which play pivotal roles in actin-based functions such as migration, phagocytosis, secretion, adhesion, cell morphology, cell contact, intracellular traffic, cytokinesis and gene regulation (Pollard and Cooper 2009; Dominguez and Holmes 2011; Pollard et al. 1994; Lanzetti 2007; Posern and Treisman 2006; Castellano et al. 2001). Actin is also substrate of numerous posttranslational modifications including methylation, phosphorylation, glycosylation, ubiquitination and ADP-ribosylation (Terman and Kashina 2013). While many of the aforementioned modifications occur catalyzed by endogenous enzymes, modification of actin is also typical for some groups of bacterial protein toxins. Especially ADP-ribosylation of actin is observed with various bacterial protein toxins (Aktories et al. 2011). Modification of arginine-177 was the first toxin-induced covalent modification of actin identified (Aktories et al. 1986b). This modification causes depolymerization of F-actin and blocks actin polymerization. Actin is also ADP-ribosylated at threonine-148, a modification, which causes the opposite effect (Lang et al. 2010). It induces polymerization of actin. This modification by *Photorhabdus* toxins will be discussed in detail in other chapters of this volume. In addition, actin is modified by toxin-induced cross-linking (Satchell 2009; Sheahan et al. 2004). Here, we will briefly review the action of ADP-ribosylation of actin at arginine-177 and will focus on recent findings on the functional consequences of the depolymerization of F-actin on the reorganization of microtubules and septins and the formation of microtubule-based cell protrusions. Moreover, we will discuss recent findings about the consequences of F-actin depolymerization on polarized traffic in epithelial cells.

2 Actin-Depolymerizing Toxins

ADP-ribosylation of actin was first identified with *Clostridium botulinum* C2 toxin (Aktories et al. 1986a, b; Ohishi and Tsuyama 1986). This toxin is normally produced by *C. botulinum* type C and D strains. Both strains are now classified as *C. botulinum* group III type strains. C2 toxin is often produced in parallel with botulinum neurotoxins C (BoNt/C) or D (BoNt/D). Moreover, *C. botulinum* group III type strains often produce in addition to neurotoxins and C2 toxin, the ADP-ribosylating toxin C3, which modifies Rho proteins (Aktories et al. 1987; Aktories and Frevert 1987). Highly related to C2 toxin are toxins from the iota toxin family, which modify actin like C2 toxin (Stiles and Wilkens 1986; Schering et al. 1988; Barth et al. 2004; Stiles et al. 2014; Sakurai et al. 2009). Members of this group are *C. perfringens* iota toxin, *C. spiroforme* toxin and *C. difficile* transferase

CDT (Aktories and Barth 2004; Aktories et al. 2011). Moreover, a novel *C. perfringens* toxin has been described, which shares high similarity with the iota toxin family (Irikura et al. 2015). Also *Bacillus cereus* produces an actin-ADP-ribosylating toxin, termed vegetative insecticidal protein (VIP), belonging to this family (Han et al. 1999). A common thing in all these toxins is that they consist of a binary structure, which means that the binding components and the enzyme components of the toxins are separated proteins (Ohishi et al. 1980; Stiles and Wilkins 1986; Barth et al. 2004; Aktories et al. 1992). This structure of the toxins will be described briefly below. However, actin-modifying toxins are also produced in form of single chain effectors, including *Salmonella Typhimurium* effector SpvB (Otto et al. 2000; Lesnick et al. 2001), *Aeromonas salmonicida/hydrophila* toxin AexT (Fehr et al. 2007; Vilches et al. 2008), *Photobacterium luminescens* toxin Photox (Visschedyk et al. 2010) and *Aeromonas hydrophila* effector VgrG1 (Suarez et al. 2010). While the binary toxins are able to enter cells by an inbuilt machinery, the single chain toxins are introduced into target cells by type III (e.g., SpvB and AexT) or type VI secretion systems (e.g., VgrG1) and, thus, depend on the direct contact of bacteria with target cells (Gotoh et al. 2003).

2.1 Structure of Binary Toxins

A large group of actin-ADP-ribosylating toxins are binary in structure. This means, they consist of two separate toxin components. One component possesses the biological activity, that modifies actin. The other component is involved in the binding of the toxins to the target cells membrane and the translocation of the enzyme component into the cytosol of host cells. The binding components of all binary toxins are highly similar to the binding component of anthrax toxin, the protective antigen (PA) (Young and Collier 2007). Similar to PA, they form heptamers (whether they can also form octamers like PA is not clear) (Blöcker et al. 2003). In each case, they consist of four domains involved in activation (domain I), oligomerization (domain II), channel formation (domain III) and receptor binding (domain IV) (Barth et al. 2004; Stiles et al. 2014). While domains I–III are highly related between all binary toxins, the binding domain IV is largely different between iota-like toxins and the other toxins. Activation of the binding component occurs by proteolytic cleavage of the N-terminal domain and release of a ~20 kDa fragment. This cleavage allows oligomerization and formation of heptamers. This process might occur in solution or on the surface of target cells after receptor binding.

The enzyme component reveals typical features of an ADP-ribosyltransferase. However, all these enzyme components consist of a doublet of an ADP-ribosyltransferase structure, which has evolved by gene duplication (Han et al. 1999; Schleberger et al. 2006; Margarit et al. 2006; Sakurai et al. 2009; Tsuge et al. 2003). Actually, the N-terminal part of this toxin domain represents an

ADP-ribosyltransferase folding without enzyme activity. This part is mainly involved in interaction with the binding component. In contrast, the C-terminal part represents the catalytic domain, which is able to modify actin.

2.2 Receptors and Uptake

The interaction of the binding components of the binary toxins with their cell receptors is a prerequisite for cytotoxicity (Fig. 1). Quite early it was shown that C2II binds to hybrid and complex carbohydrates on the surface of target cells (Eckhardt et al. 2000). In addition, specific binding proteins might be involved. The receptor for *C. difficile* toxin CDT, iota toxin, and *C. spiroforme* toxin CST has been identified as the lipolysis-stimulated lipoprotein receptor LSR (Papatheodorou et al. 2011, 2012). LSR is a single path membrane protein with a long extracellular part, consisting of an Ig-like V-type domain in the N-terminal part and a cysteine-rich intracellular part (Schmidt et al. 2015). As the name suggest, the LSR receptor protein appears to be involved in lipid metabolism and may play a role in uptake of chylomicrons (Bihain and Yen 1998; Mann et al. 1995; Yen et al. 1994). Moreover, LSR is essential for the organization of tricellular tight junctions that are involved in epithelial barrier function (Furuse et al. 2012; Masuda et al. 2011) and appears to play a major role in tumor development and metastasis (Shimada et al. 2016; Herbsleb et al. 2008).

After binding of the heptameric binding component to its specific receptor, the enzyme component can attach and, thereafter, the whole complex is endocytosed. In low-pH endosomes, the binding component inserts into the vesicle membrane by forming a beta barrel pore, through which the enzyme component is translocated into the cytosol of target cells (Barth et al. 2000; Blöcker et al. 2003; Nagahama et al. 2012, 2014). Translocation depends on chaperones, including HSP90 (Haug et al. 2003), cyclophilins (Kaiser et al. 2009) and others (Kaiser et al. 2012).

3 Modification of Actin by ADP-Ribosylating Toxins

Using NAD^+ as a second substrate, all above-mentioned binary actin-ADP-ribosylating toxins attach ADP-ribose onto actin at arginine-177 (Vandekerckhove et al. 1987, 1988; Gülke et al. 2001). In addition, also single chain toxins (effectors) like *Salmonella* ssp. protein SpvB modify actin at arginine-177 (Hochmann et al. 2006). Although the overall structure of actin is only minimally affected by ADP-ribosylation (Margarit et al. 2006), the toxin-induced attachment of ADP-ribose has major consequences for the polymerization of actin. ADP-ribosylated actin is no longer able to polymerize (Aktories et al. 1986b; Schering et al. 1988; Ohishi and Tsuyama 1986), because the bulky ADP-ribose at this position is not compatible with the structure of the double helix formed by

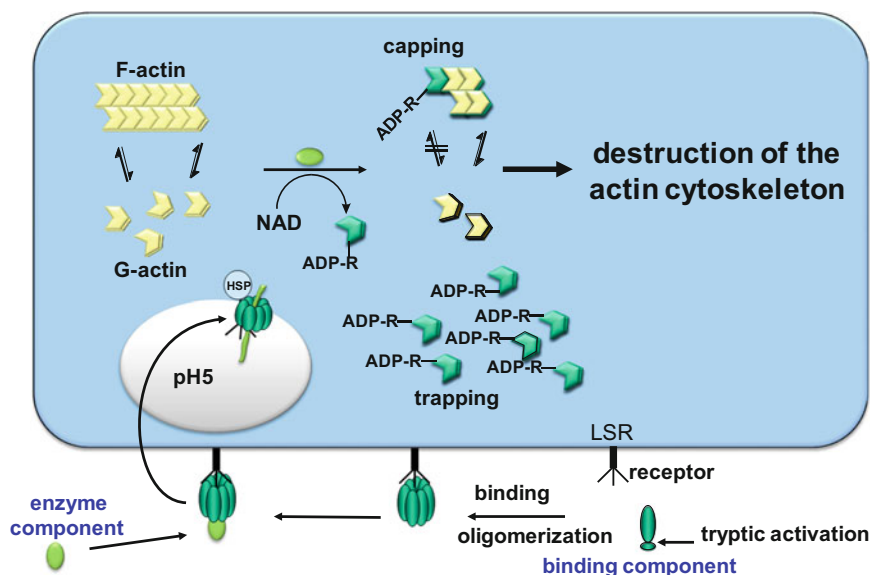


Fig. 1 Actions of binary actin-ADP-ribosylating toxins. The binary actin-ADP-ribosylating toxins (e.g., *C. difficile* toxin CDT or *C. botulinum* C2 toxin) consist of an enzyme component and a binding component, which is activated by proteolytic cleavage. The activated binding components form heptamers and interact with their respective cell membrane receptors [e.g., CDT interacts with lipolysis-stimulated lipoprotein receptor (LSR)]. The toxin–receptor complexes are endocytosed. In low pH endosomes, the binding components undergo conformational changes and insert into membranes to form pores. Through the pores, the enzyme components are translocated into the cytosol. This occurs with the help of chaperones (e.g., HSP90). In the cytosol, the enzyme components of the binary toxins ADP-ribosylate monomeric G-actin. ADP-ribosylated actin is not able to polymerize and trapped in its monomeric form. ADP-ribosylated actin can act as a barbed-end capping protein to inhibit polymerization of non-modified actin. Thereby, the actin cytoskeleton is relocalized and, eventually, depolymerizes

polymerized F-actin (Holmes et al. 1990; Margarit et al. 2006). The only position, where this group can be accommodated, is the barbed end of actin filaments (Wegner and Aktories 1988; Aktories and Wegner 1989). Here, the attachment of ADP-ribose turns the modified actin molecule into a capping protein, which inhibits further binding at this side. In contrast, the pointed end, which favors actin depolymerization, is not affected. Thus, the capping protein-like function inhibits polymerization of still unmodified actin molecules. Moreover, ADP-ribosylated actin changes further properties of the microfilament protein. For example, the modification blocks the ATP hydrolysis by actin, which is especially well observed in the presence of cytochalasin (Geipel et al. 1989, 1990; Margarit et al. 2006). Moreover, the release rate of phosphate from actin is decreased after ADP-ribosylation (Margarit et al. 2006). Also, the interaction of ADP-ribosylated actin with actin-binding proteins is affected. For example, ADP-ribosylation has consequences for the nucleation activity of the gelsolin–actin complex. However, this is only observed when the weakly bound actin of the gelsolin–actin–actin

complex (G-A-A_R) is modified (Wille et al. 1992). Eventually, inhibition of polymerization (e.g., trapping of monomeric ADP-ribosylated actin and capping of F-actin) causes destruction of the actin cytoskeleton and rounding up of cells.

4 Cellular Consequences of the ADP-Ribosylation of Actin in Arginine-177

The functional consequences of the ADP-ribosylation of arginine-177 of actin have been studied in various cells types. Mainly *C. botulinum* C2 toxin was employed for these experiments. By using rather high toxin concentrations, a typical effect is the rounding up of cells under cell culture conditions (Wiegers et al. 1991). Motility, migration and contraction is largely decreased by C2 toxin (Norgauer et al. 1988; Verschueren et al. 1995). The actin-depolymerizing toxins have been used as pharmacological tools to study the role of the actin cytoskeleton in secretory processes, showing that C2 toxin has a bidirectional effect on the release of catecholamines, and time-dependently increases or inhibits the release of noradrenaline by affecting the actin cytoskeleton (Matter et al. 1989). In adrenal Y-1 cells, C2 toxin increases steroid release in a cAMP-independent manner (Considine et al. 1992), while ADP-ribosylation of actin inhibits stimulated release of insulin from HIT-T15 cells or pancreatic islands (Li et al. 1994). These differences most likely depend on the type of secretion, and how and whether vesicles have excess to exocytotic sites (Trifaro et al. 2008). ADP-ribosylation of actin increases the superoxide production (Norgauer et al. 1988). However, recently it was shown that the toxin-induced depolymerization of actin has major functional consequences on other components of the cytoskeleton namely on microtubules and on septins.

4.1 Effects of Actin-Depolymerizing Toxins on Microtubules

Microtubules are filaments, which consist of α - and β -tubulin heterodimers. As known for actin filaments, microtubules are dynamic structures undergoing tread-milling (Waterman-Storer and Salmon 1997). Accordingly, similar to the barbed (plus) and pointed (minus) ends of actin filaments, microtubules are polarized structures, possessing a rapidly growing plus end and a slowly growing minus end (Desai and Mitchison 1997; Waterman-Storer and Salmon 1997; Nogales 2000). The minus ends of microtubules are generally oriented to and stabilized by the microtubule organization center, which is in most cells the centrosome. The plus ends are often directed to the cell membrane, exhibiting high functional dynamics called dynamic instability. Various microtubule-binding proteins are involved in regulation of the dynamic instability. The plus end binding proteins 1 (EB1) or the cytoplasmic linker protein 170 (CLIP170) are so-called *TIPs* that regulate microtubule growth at the plus ends. Interaction of microtubules with the cortical actin

lattice causes growth inhibition and stabilization of microtubules. Here, capture proteins like CLIP-associated protein (CLASP2) and actin-cross-linking-family 7 protein (ACF7) are involved, which bind both to actin and to microtubules (Kodama et al. 2003; Mimori-Kiyosue et al. 2005). When actin is ADP-ribosylated in arginine-177 and F-actin depolymerization is induced, the regulation of microtubule dynamics is significantly affected.

It has been shown that C2 toxin, iota toxin and CDT are able to induce an increase in microtubule growth (Uematsu et al. 2007; Schwan et al. 2009). Moreover, growing microtubules that contact the cell membrane form protrusions, which are filled with microtubules but not with F-actin (Fig. 2). These cell protrusions usually have a diameter of 0.5–1 μm and can reach a length of more than 100 μm . The structures are dynamic and can grow and retract (Schwan et al. 2009).

Long cellular protrusions are a typical feature of filopodia (Blanchoin et al. 2014; Ahmed et al. 2010; Leijnse et al. 2015). Also cytonemes and the various types of *tunneling nanotubes* (TNT) are characterized by long extensions, which appear to be involved in various cellular functions, including cell–cell contact, cargo traffic between cells and cell–cell signaling (Buszczak et al. 2016; Onfelt et al. 2004; Austefjord et al. 2014; Sisakhtnezhad and Khosravi 2015; Fairchild and Barna 2014). However, filopodia and also TNT depend essentially on actin microfilaments and depolymerization of F-actin inhibits formation of filopodia and of TNT. By contrast, the protrusions formed by toxins that cause ADP-ribosylation of actin in arginine-177 depend on the depolymerization of F-actin. Moreover, also latrunculin B and cytochalasin can induce long cell protrusions that exclusively are based on microtubules. Interestingly, in tumor metastasis, cells have been described that possess *cell tentacles* (Matrone et al. 2010; Vitolo et al. 2013), which appear to match the structural features of toxin-induced microtubule-based protrusions.

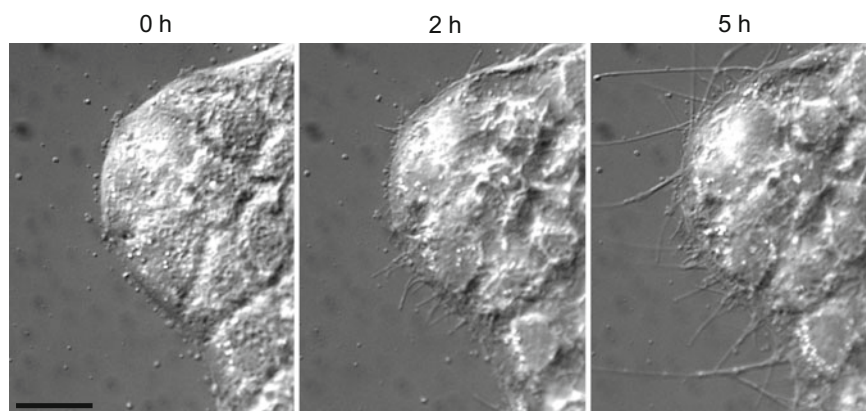


Fig. 2 Formation of microtubule-based cell protrusions induced by ADP-ribosylation of actin. Human adenocarcinoma Caco-2 cells were treated with *C. difficile* toxin CDT (CDTa 20 ng/ml and CDTb 40 ng/ml) for the indicated times. Differential interference contrast microscopy images show protrusion formation over time. Scale bar represents 20 μm

Notably, also motile and primary cilia, which are formed on each cell, are based on microtubules and do not contain F-actin (Pedersen et al. 2012; Malicki and Avidor-Reiss 2014). However, the toxin-induced protrusions are generally much longer than cilia and do not share the complex structure of cilia.

4.2 Mechanisms Involved in Protrusion Formation: A Role for Septins

Microtubule-based protrusions, which are formed by actin ADP-ribosylation of arginine-177, are well studied in epithelial cells especially human colon adenocarcinoma cells (CaCo-2) (Schwan et al. 2009, 2011, 2014). The protrusions are formed in highly polarized CaCo-2 cells and they are observed in primary mouse intestinal wild-type cells. Protrusions, formed by toxin-induced depolymerization of actin, depend on the growth of microtubules at plus ends. This can be followed by monitoring the dynamics and migration of GFP-labeled EB1 at the top of growing microtubules. Interestingly, Clasp-2 and ACF-7 proteins, which normally are involved in microtubule capture, appear to be redistributed from the actin cortex into the cytosolic fraction after partial depolymerization of actin (Schwan et al. 2009). Although the precise mechanism of protrusion formation is still enigmatic, recent data suggest that septin play a crucial role.

Septins are suggested to be the fourth structural component of the cytoskeleton besides microfilaments, microtubules and intermediate filaments (Mostowy and Cossart 2012). Septins are conserved from yeast to humans. The human genome contains 13 septin genes. Septin proteins can be grouped into four major families which are represented by SEPT1, SEPT3, SEPT6 and SEPT7 (Beise and Trimble 2011). Almost all septins possess a GTPase domain, which is related to other P-loop nucleotide triphosphatases, including Ras proteins (Leipe et al. 2002; Sirajuddin et al. 2007). In addition, they contain a specific domain, which is called SUE (septin unique element). Septin forms hetero-oligomers of 8 septin molecules, which, unlike actin and tubulin monomers, are associated in a unipolar manner, where the GTPase domains associate to form the G–G interface and the N/C terminal parts interact to compose a NC–NC interface. The octamers can form long filaments, bundles and rings. Coiled-coil structures at the C-terminus appear to have finger like projection, which are perpendicular to the septin filaments and might be involved in protein–protein interactions. Septins bind to actin and also to microtubules (Weirich et al. 2008). Formation of higher septin structures appears to be regulated by GTP-binding proteins of the Rho family especially Cdc42.

When cells are intoxicated by binary toxins, partial depolymerization of actin causes redistribution of septins, which are usually associated with cytosol-located actin filaments, to the membrane. There, septins form ring-, chevron- and funnel-like structures that appear to be contact points for microtubule plus ends (Nölke et al. 2016) (Figs. 3 and 4). As shown recently, the time course of septin accumulation and protrusion formation induced by actin-depolymerizing toxins

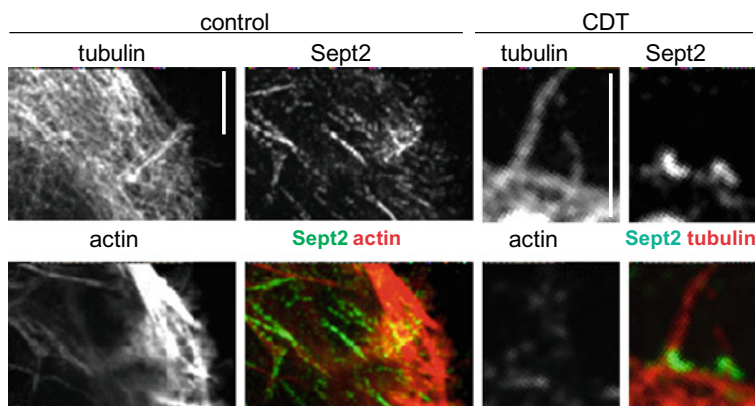


Fig. 3 Septins are involved in toxin-induced protrusions formation. Human adenocarcinoma Caco-2 cells were treated with CDT (CDTa 200 ng/ml and CDTb 400 ng/ml) for 1.5 h. Cells were stained by immunofluorescence for tubulin and septin2. Additionally, F-actin was stained by phalloidin-Atto 568. After CDT-treatment, F-actin is depolymerized and protrusions form at sites of reduced cortical actin. Protrusions have septin2 chevron structures at their base. Scale bar represents 5 μm

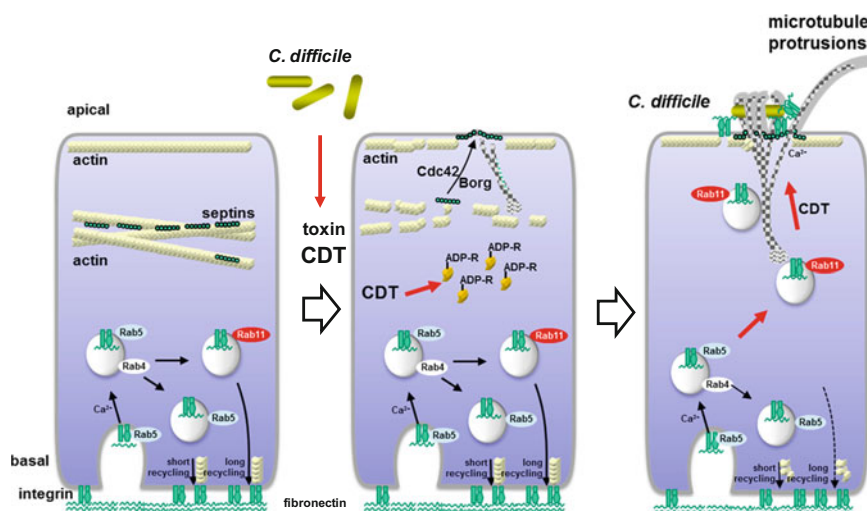


Fig. 4 Model of the formation and function of microtubule-based cell protrusions induced by actin-depolymerizing toxins. *Right panel* “Short” and “long” recycling processes at the basal side of an epithelial cell is shown. The recycling occurs with Rab5- (short recycling) and Rab11- (long recycling) associated vesicles. Septins are mainly localized in the cytosol associated with F-actin. *Middle panel* The binary *C. difficile* toxin CDT enters cells and ADP-ribosylates actin at arginine-177. Thereby, the actin cytoskeleton is partially depolymerized. Septins are released from F-actin and are translocated by means of the small GTPase Cdc42 and its effector Borg to membranes, where the cortical F-actin at the apical side is diminished. At the plasma membrane, septins accumulate in chevron-like structures, interact with microtubules and participate in the formation of microtubule-based protrusions. *Right panel* Depolymerization of F-actin by CDT causes re-guiding of vesicles from the basal membrane to the apical side. The microtubule-based protrusions are involved in interactions with bacteria and increase bacterial adherence

suggest that septins start the restructuring of the membrane. The chevron- or funnel-like structures formed by septin guide microtubules to the membrane and might be essential for protrusion formation, because knockdown of septins largely reduces protrusion formation (Noelke et al. 2016). Similarly, forchlorfenuron, an inhibitor of septin dynamics also blocks protrusion formation. Notably, similar chevron-like structures are observed in neuronal cells at locations where neurite- and axon-branching is observed (Hu et al. 2012). However, neurite-branching mainly depends on actin filaments, whereas (as already mentioned) F-actin is missing in toxin-induced protrusions. EB1 or EB3 proteins apparently regulate the initial interactions of microtubules with septins. These proteins, which control microtubule growth at the plus ends, interact with septins with affinities in the nanomolar range (Nölke et al. 2016). Important questions remain: What are the mechanisms that underlay septin accumulation at the cell membrane caused by depolymerization of F-actin? What defines membrane sites for septin accumulation? Recent studies provided some insights into the regulation of septins at the cell membrane. Septin accumulation at the cell membrane is apparently regulated by the small Rho GTPase Cdc42 and its effector Borg1-5 (Cdc42EP/CEP) (Joberty et al. 2001; Sheffield et al. 2003; Joberty et al. 1999). The current model, suggests that active GTP-bound Cdc42 is involved in trafficking septins to the membrane (Sadian et al. 2013). Fluorescence microscopic studies with a sensor for Cdc42 activation revealed active Cdc42 and Borg co-localization at sites of septin accumulation. Free cycling between the GTP and GDP forms of Cdc42 appears to be necessary for proper septin organization, because activation as well as inhibition of Cdc42 blocks septin accumulation and eventually protrusion formation (Nölke et al. 2016).

4.3 Role and Functions of Toxin-Induced Cell Protrusions

Depolymerization of the actin cytoskeleton by binary ADP-ribosylating toxins usually induces the formation of multiple long protrusions, which results in a web-like structure on the cell surface. What are the functions of these protrusions? From the point of host–pathogen interaction, it was shown that the protrusions might be involved in pathogen colonization, because the toxin-producing bacteria are embedded by this protrusion network, thereby increasing the adherence of the pathogen (Fig. 4). However, the function of the protrusions may be more diverse. Electron microscopic studies reveal that the membrane protrusions induced by toxins contain membranes of endoplasmic reticulum (ER) in addition to microtubule bundles. ER structures are also found in tunneling nanotubes (Zhang 2011; Wang et al. 2011). Apparently, Stim-1 proteins form the functional connection of ER membranes and microtubules. Stim-1 is a crucial regulator of calcium release-activated calcium channels (CRACs), which are based on the channel-forming Orai proteins (Fahrner et al. 2013; Frischauf et al. 2008). Thus, the protrusions appear to form functional Orai calcium channels. This depends on Orai organization in the cell membrane of the protrusions by Stim-1 (Galan et al. 2011; Fahrner et al. 2013).

4.3.1 Re-guidance of Vesicle Traffic

In addition to calcium signaling, the toxin-induced protrusions allow vesicle trafficking. Rab5- as well as Rab11-associated vesicles has been observed within microtubule-based protrusions, which move in antero- and retrograde directions (Schwan et al. 2014). Again vesicle traffic is typical for tunneling nanotubes (Buszczak et al. 2016) and also for cytonemes (Stanganello and Scholpp 2016). However, microfilaments are essential for vesicle transport in tunneling nanotubes, whereas trafficking in toxin-induced protrusions is only dependent on microtubules. In this respect, it is of interest that the depolymerization of actin results in major changes of cellular vesicle traffic. Depolymerization of actin affects the basolateral traffic of integrin receptors, which depends on F-actin. Mellman and coworkers reported that basolateral vesicle recycling was affected by actin-depolymerizing agents (Sheff et al. 2002). Using latrunculin B, several basolateral plasma membrane proteins were found to lose their typical polarized distribution. Depolymerization of F-actin by CDT causes redistribution of $\alpha 5/\beta 1$ -integrins associated with Rab-11-labeled vesicles from the basolateral side to the apical side of epithelial monolayers (Schwan et al. 2014) (Fig. 4). Moreover, extracellular matrix proteins like fibronectin and laminin are redistributed from basolateral $\alpha 5/\beta 1$ -integrin traffic to the apical surface of toxin-treated cells. Here, the reorganized microtubules serve as roads for vesicle traffic, resulting in membrane fusion of the vesicles at the base of microtubule-formed protrusions. In addition, vesicles from the basolateral side even enter the protrusion at the apical side, resulting in major re-guidance of vesicles. Because fibronectin is a binding protein for the toxin-producing pathogen, also these guiding effects eventually result in increase of bacterial adhesion, because the adherence proteins occur at the apical surface of cells (Schwan et al. 2014).

5 Conclusions

The actin cytoskeleton is a frequent target of bacterial protein toxins and effectors. While a huge group of toxins and effectors manipulate the actin cytoskeleton via Rho proteins (Aktories 2011; Popoff and Geny 2009; Lemichez and Aktories 2013; Lang et al. 2011; Gruenheid and Finlay 2003), some toxins directly modify actin in a bidirectional manner either inducing polymerization by *Photorhabdus* toxin-induced ADP-ribosylation of threonine-148 or depolymerization by ADP-ribosylation of arginine-177 of actin. Studies from recent years suggest that the toxin-induced depolymerization of the actin cytoskeleton has much more functional consequences as suggested previously. The toxin-induced depolymerization of the actin cytoskeleton unmasks regulatory mechanisms, which largely depend on other components of the cytoskeleton, e.g., microtubules and septins, showing the tight interaction of the whole cytoskeleton in numerous cellular functions. Using toxins as tools, specific functional pathways are selectively

suppressed and, so far unknown, regulatory principles uncovered. The recent findings that microtubule-based protrusions are formed by actin-depolymerizing toxins, are paradigmatic for this functional interconnection, which may be related to some extent to cytonemes, tunneling nanotubes, microtentacles or cilia formation. However, the toxin-induced protrusions clearly exhibit unique features, which are not observed in the other forms of cell protrusions. It is tempting to speculate that these novel types of cell structures not only resemble pathophysiological reactions but is also a physiologically important cell response.

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