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REVIEW ARTICLE

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Bacterial toxins modifying the actin cytoskeleton

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Summary Numerous bacterial toxins recognize the actin cytoskeleton as a target. The clostridial binary toxins (Iota and C2 families) ADP-ribosylate the actin monomers causing the dissociation of the actin filaments. The large clostridial toxins from *Clostridium difficile*, *Clostridium sordellii* and *Clostridium novyi* inactivate, by glucosylation, proteins from the Rho family that regulate actin polymerization. In contrast, the cytotoxic necrotic factor from *Escherichia coli* activates Rho by deamidation and increases the formation of actin filaments. The enterotoxin of *Bacteroides fragilis* is a protease specific for E-cadherin and it promotes the reorganization of the actin cytoskeleton. The bacterial toxins that modify the actin cytoskeleton induce various cell disfunctions including changes in cell barrier permeability and disruption of intercellular junctions.

Key words Toxins · Actin · Rho proteins · Ras proteins · Intercellular junction

Introduction

Some bacteria exert their pathogenic effects by producing toxins. Some toxins are responsible for all the symptoms observed in the natural disease, such as paralytic syndrom caused by *Clostridium tetani* and *Clostridium botulinum* neurotoxins or diarrhea induced by *Clostridium difficile* toxins.

Bacterial toxins interact with cells in various ways. Some toxins bind to a cell surface receptor and induce a signal through the membrane, some form pores in the membrane and others are internalized by endocytic vesicles and modify a cytosolic target. A large number of internalized toxins recognize the actin cytoskeleton. Some toxins interact directly with actin monomers, and others modify a regulatory protein from the Rho family. Most of these toxins are produced by anaerobic bacteria from the *Clostridium* genus, which mostly cause gastrointestinal diseases and gangrene. Perturbation of the actin cytoskeleton induces changes in cell morphology and various functions such as motility, cytokinesis, exocytosis, endocytosis and increases epithelium and endothelium permeability.

Toxins active against actin monomers

The toxins of this group disturb the actin cytoskeleton by directly modifying actin monomers. They include *C. botulinum* C2 toxin, *Clostridium perfringens* Iota toxin, *Clostridium spiroforme* toxin and *C. difficile* transferase (CDT) (Table 1). These toxins are involved in gastrointestinal diseases in animals and, less frequently, in humans.

C2 toxin is produced by *C. botulinum* types C and D, which also synthesize the neurotoxins C1 and D respectively, responsible for animal botulism. C2 toxin is an enterotoxin and causes hemorrhagic and necrotic lesions, which are often observed in the intestinal wall of birds and cattle that have died from botulism. In addition, in experiments, C2 toxin has been shown to induce hemorrhagic enteritis in mice.

C. perfringens E synthesizes Iota toxin and is associated with enterotoxemia in calves. Toxigenic *C. spiroforme* are the etiological agents of enteritis in rabbit and some cases have been described in humans. Certain *C. difficile* strains produce, in addition to toxins A (ToxA) and B (ToxB), an

Table 1 Toxins acting on the actin cytoskeleton and enzymatic activities. Amino acid sequence identity was determined by the Gap program from the Genetics Computer Group package

Toxin and microorganisms	Amino acid sequence identity	Enzymatic activity	Cofactor	Substrate[1]
Binary toxins				
C2 family		ADP ribosylation	NAD	
C. botulinum C and D				β/γ G-actin (Arg177)
Iota family	29–41%			
C. perfringens Iota				αβγ G-actin (Arg177)
C. spiriforme toxin				
C. difficile CDT	72–82%			
C3 enzymes		ADP ribosylation	NAD	Rho (Asn41)
C. botulinum D (1873)	66%			
C. botulinum C (006)	63%			
C. limosum	36%			
S. aureus				
Bacillus cereus				
Large clostridial toxins				
C. sordellii LT82	76%		UDP-glucose	Ras (Thr35), Rac, Rap, Ral
C. sordellii LT9048			UDP-glucose	Ras, Rac, CDC42
C. sordellii HT	48%	Glucosylation	UDP-glucose	Rho (Thr37), Rac, CDC42 (Thr35)
C. difficile ToxB			UDP-glucose	Rho (Thr37), Rac, CDC42 (Thr35)
C. difficile F ToxB	32%		UDP-glucose	Rac
C. difficile ToxA			UDP-glucose	Rho (Thr37), Rac, CDC42 (Thr35)
C. novyi Toxin α			UDP-*N*-acetyl-glucosamine	Rho (Thr37), Rac, CDC42 (Thr35)
Rho-activating toxins				
E. coli CNF1	85%	Deamidation		Rho (Gln63), Rac, CDC42
CNF2				
Bordetella bronchiseptica DNT	32%			Rho (Gln63)
Proteolytic enterotoxin				
Bacteroides fragilis BFT		Proteolysis		E-cadherin

[1]Amino acids in brackets indicates the site of modification.

ADP-ribosyltransferase called CDT whose role in disease is unknown.

The actin modifying toxins are binary toxins (Fig. 1). They consist of two independent proteins not linked by covalent or disulfide bonds, and are encoded by two genes. The binding components (C2-II for C2 toxin, Ib for Iota toxin, Sb for *C. spiroforme* toxin, and CDTb for CDT) are synthesized as a precursor protein (90–100 kDa) that requires the proteolytic cleavage of a 20 kDa N-terminal peptide to be active. The active binding components recognize a cell surface receptor that has not yet been identified, and mediate the internalization of the enzymatic components (C2-I for C2 toxin, Ia for Iota toxin, Sa for *C. spiroforme* toxin, and CDTa for CDT) into the cells. The whole toxin is internalized into endocytic vesicles and the enzymatic component is then delivered to the cytosol.

The enzymatic components catalyze the ADP-ribosylation of G-actin monomers, but not F-actin, at Arg-177. The actin in cells is in dynamic equilibrium between G-actin monomers and actin filaments. The filaments polymerize at one end (fast growing end or barbed end) and depolymerize at the other (slow growing end or pointed end). The ADP-ribosylated actin monomers bind to the fast growing ends of actin filaments and inhibit actin polymerization by preventing the incorporation of further actin monomers, in a manner similar to that of capping proteins. The acceptor amino acid (Arg-177) is located in domain III of actin, which is involved in actin monomer recognition. In contrast, activity at the slow growing ends is not modified, and depolymerization leads to a complete dissociation of the actin filaments. In addition, ADP-ribosylation inhibits actin ATPase activity. The loss of actin microfilaments induces morphological changes (cell rounding and detachment from the support) and alterations in cellular functions dependent on the actin cytoskeleton [1, 43].

The clostridial actin-ADP-ribosylating toxins have a common structure, but differ immunologically and in biological activity. They can be divided into two families, one corresponding to the C2 toxin of *C. botulinum* (C2 family), and the other to the *C. perfringens* Iota toxin, *C. spiroforme* toxin and *C. difficile* transferase (Iota family) [37, 38].

The toxins of the Iota family are immunologically related and display 77–82% amino acid sequence identity (Table 1). Moreover, the binding and enzymatic components can be interchanged to form a toxin that is fully active in cell rounding and is lethal to mice. For example, the binding components Ib and Sb can mediate the internalization and cytotoxicity of CDTa.

In contrast, the toxins from the two families are unrelated, or only weakly related, and no functional complementation between the components of each family has been demonstrated. Therefore, the binding components of the Iota family cannot translocate the enzymatic components of C2 toxin or vice versa.

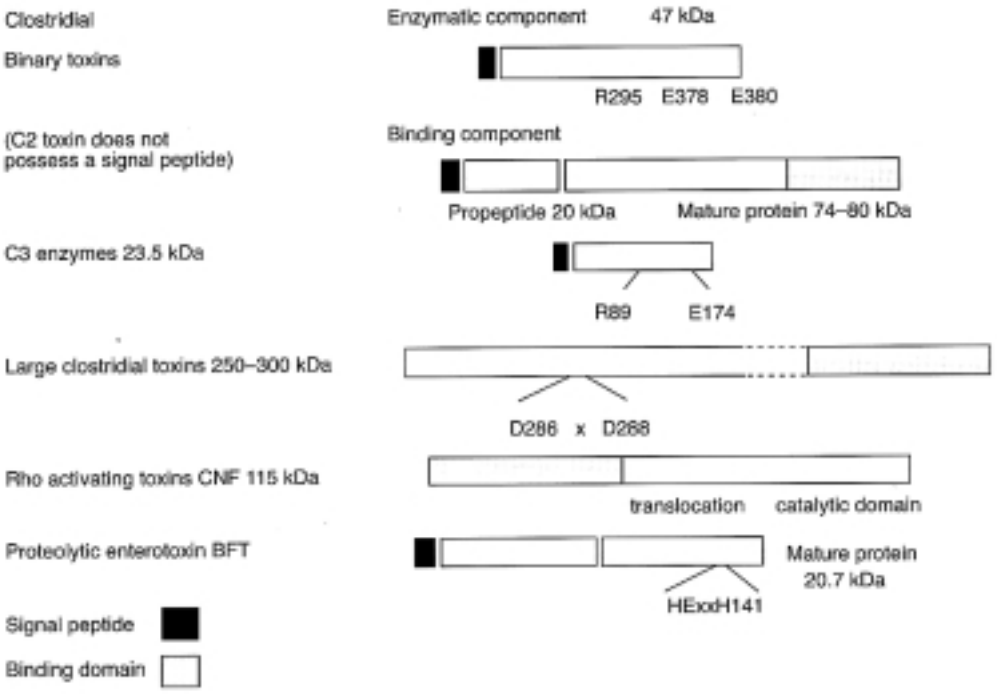

Fig. 1 Structure of toxins modifying the actin cytoskeleton. The residues indicated correspond to the amino acids involved in the enzymatic site

The binary toxins are internalized into cells by receptor-mediated endocytosis. However, the binding components of C2 and Iota toxins recognize different receptors which have not yet been identified. C2 toxin enters via acidic endocytic vesicles, whereas toxins of the Iota family enter via non-acidic endosomes [39]. The intracellular targets of the enzymatic components are also different. Iota toxin is able to modify all actin isoforms (muscular and non-muscular actin), whereas C2 toxin only ADP-ribosylates β/γ cytoplasmic and γ smooth muscle actin [1].

The binding components of the Iota family are significantly similar (34–38% sequence identity) to the protective antigen (PA) of the anthrax toxins. The anthrax toxins are also binary toxins consisting of 3 independent proteins. PA is the binding component and can mediate the internalization into cells of the edema factor (EF) or the lethal factor (LF), the enzymatic components active within cells. These binding components are proteolytically split into 3 fragments, signal peptide (29 N-terminal residues), propeptide (20 kDa) and mature protein (74–80 kDa). The PA crystal structure comprises 4 domains. Activation by furin releases the 167 N-terminal residues (propeptide) of domain 1, and residues 168–258 (domain 1´) form a hydrophobic surface that is thought to be the binding site for EF and LF. Domain 2 contains a β-barrel core and a large flexible loop involved in membrane insertion. The function of the small domain 3 is unknown. Domain 4, which is located in the C-terminal part and contains a β-sandwich, is involved in the recognition of the cell surface receptor [40]. The corresponding amino acid sequences of the binding components of the clostridial binary toxins are significantly similar (30–43% identity) to domains 1 to 3 of PA, indicating that these components have a conserved structure and similar folding. The low homology (less than 10% identity) between domain 4 of PA and the corresponding sequences of the binding components of clostridial binary toxins reflects the fact that different receptors recognized by each component.

The active site has been mapped to the C-terminal part of the enzymatic components, and is similar to that in other ADP-ribosylating toxins. The active site consists of a NAD-binding cavity, composed of a β-strand and an α-helix flanked by two residues important for catalytic activity (Arg or His, and Glu) [6]. The residues (STS(I/L)) forming the β-strand are highly conserved in the enzymatic components and also in C3 enzymes, cholera toxin, *Escherichia coli* heat labile toxin and pertussis toxin, whereas those involved in the α-helix are less conserved [15]. The flanking residues (Arg-295, Glu-378 and Glu-380) of Ia, which are conserved in all the enzymatic components of the actin ADP-ribosylating toxins, have been identified by site directed mutagenesis as essential for enzymatic activity [36]. The mutated proteins, when introduced into Vero cells in the presence of Ib, were not cytotoxic, confirming that the intracellular ADP-ribosylation of actin is responsible for the cytotoxic effects of Iota toxin. Actin ADP-ribosylating toxins have a biglutamic motif (Glu-378-X-Glu-380) that is probably involved in NAD binding and in the catalytic reaction, whereas in other ADP-ribosylating toxins, the equivalent residues are Gln-X-Glu [36]. Similar results have been reported for C2-I [2].

Toxins modifying proteins regulating actin assembly

In response to environmental signals, cells regulate their shape, adhesion and motility by modification of the actin cytoskeleton. External signals that trigger actin assembly and disassembly act through various plasma membrane receptors and signal transduction pathways including Rho-family proteins from the Ras GTPase superfamily. Rho proteins act as molecular switches; they are active in GTP-bound forms and inactive if linked to GDP.

The inactive state is stabilized by complex formation with nucleotide dissociation inhibitor (GDI). GTPases are activated by GDP-GTP exchange, which is promoted by guanine nucleotide exchange factors (GEF) and ERM proteins (ezrin, radixin, and moesin). In the activated state, GTPases interact with many effectors to induce a coordinated cellular response. GTPases return to the inactive state by hydrolysis of GTP, mediated by GTPase activating protein (GAP) (Fig. 2).

Rho proteins control various aspects of actin organization. Rho induces the assembly of actin-myosin filaments (stress fibers) and of associated focal adhesion complexes. Rac promotes actin polymerization at the cell periphery to form lamellipodia and ruffles, whereas CDC42 is responsible for the formation of filopodia or microspikes.

More than eight target protein kinases have been identified for each of the GTPases of the Rho family, but the function of only a few is known. Rho-kinase (RhoK) is activated by binding to RhoGTP and increases the myosin light chain phosphorylation by inhibiting myosin light chain phosphatase, leading to myosin filament assembly and F-actin bundling. The lipid kinase, phosphatidylinositol-4-phosphate-5-kinase (PI4P5K), is likely to be a central target of Rac and to a lesser extent, of Rho. PI4P5K increases the synthesis of phosphatidylinositol 4,5-biphosphate (PIP2) which is known to increase actin polymerization by release of capping proteins from the barbed ends of actin filaments. N-WASP has been identified as an effector of CDC42, involved in filopodium formation [17, 28, 29, 54, 58].

Rho proteins are also involved in gene transcription and cell cycle progression. Rac and CDC42 can activate the c-Jun and P38 mitogen-activated-protein kinase pathway, but the mechanism is unclear [17, 29, 54].

C3 and C3-like enzymes C3 enzyme was originally described in *C. botulinum* C and D. Two types of C3 gene have been characterized, both encoding basic proteins (pI around 10) of 23.5 kDa with 66% amino acid sequence identity. The C3 genes are part of a transposon structure (21.5 kb) located on phage DNA, which also contains the neurotoxin genes in *C. botulinum* C and D [18, 32, 43]. C3-like enzymes have also been identified in *Clostridium limosum, Bacillus cereus* and *Staphylococcus aureus* (Table 1) [1].

C3 enzyme is an ADP-ribosyltransferase smaller than the enzymatic component of the actin-ADP-ribosylating toxins (Fig. 1). No binding component has been found for C3 enzyme, which therefore does not efficiently enter cells. This enzyme seems to correspond to the enzymatic component of ADP-ribosylating toxins (23% amino acid sequence identity) lacking the machinery for cell internalization. The active site is located in the C-terminal part of the molecule and is related to that of actin ADP-ribosylating toxins and other ADP-ribosylating toxins. The conserved β-strand and α-helix forming the NAD cavity are flanked by the catalytic residues Arg-89 and Glu-174.

C3 enzyme specifically modifies Rho proteins at Asn-41. This residue is located on switch I of small GTPases, the conformation of which changes depends on the bound

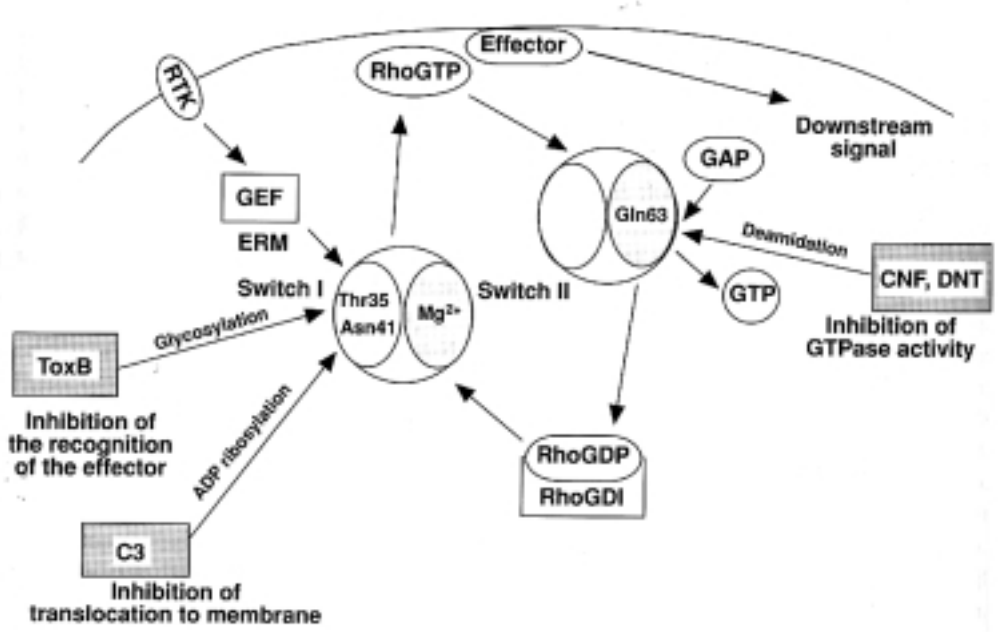

Fig. 2 Rho cycle and interaction with toxins. Rho is inactive in the GDP-bound form, and active in the GTP-bound form. The flexible parts of the molecule according to the bound nucleotide are called switch I, which is involved in the recognition of the effector, and switch II, which supports GTPase activity. C3 and ToxB modify residues of switch I and inactivate Rho, whereas CNF and DNT act on Gln-63 in switch II, inhibiting GTPase activity and resulting in permanently active Rho. GDI, guanine nucleotide inhibitor; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; ERM, ezrin, radixin and moesin; RTK, tyrosine kinase receptor

nucleotide, and which is the domain interacting with the effector. Rho is only ADP-ribosylated by C3 if it is dissociated from the guanine nucleotide dissociating factor (GDI). ADP-ribosylation of Rho does not modify GTPase activity and does not affect binding with effectors such as protein kinase N [52]. The inactivation of Rho by C3 involves the inhibition of translocation of Rho to the membrane, which seems to be mediated by the association of Rho, via the insert helix, with a membrane-bound effector and the insertion of the prenylated C-terminus into the lipid bilayer [11].

Rho ADP-ribosylated by C3 is inactivated causing the disorganization of actin filaments (Fig. 2). The actin filaments are depolymerized and only patches corresponding to short actin filaments are detectable by immunofluorescence (Fig. 3A).

Large clostridial toxins The large clostridial toxins group includes the largest bacterial toxins (250–300 kDa). They are produced by *Clostridium* involved in gastrointestinal diseases and gangrene (Table 1).

C. difficile is responsible for pseudomembranous colitis and about 30% of the postantibiotic treatment diarrhea common in hospitalized patients. This microorganism produces two toxins: an enterotoxin or toxin A (ToxA) and a very potent cytotoxin or toxin B (ToxB).

C. sordellii is involved in gangrene in humans and animals, hemorrhagic enteritis in cattle and enterotoxemia in sheep. This microorganism also produces two toxins, a lethal toxin (LT) which is closely related to ToxB (88% amino acid sequence identity and immunological cross reactions between the two toxins) and a hemorrhagic toxin (HT).

C. novyi is an etiological agent of gangrene with major edema. The main toxin produced is the alpha toxin.

The large clostridial toxins have a common structure (Fig. 1). The C-terminal part, about 25–30% of the molecule, contains repeats of 20–50 amino acids which are related to those in glucosyltransferases produced by other bacteria such as *Streptococcus* [57]. This domain is involved in the binding to cell surface receptor. Therefore, polyclonal and monoclonal antibodies specific for this domain neutralize the cytopathic effects. It has also been found that recombinant amino acid repeats block the binding of ToxA to the cell surface. A trisaccharide motif has been found to bind to ToxA, and cells bearing this structure are highly sensitive to ToxA [55]. In rabbit ileum, the cell surface receptor of ToxA has been reported to be the glycoprotein sucrase-isomaltase [44]. The receptors for the other large toxins are not known.

The central part contains a hydrophobic region with a short predicted transmembrane segment in all of the large clostridial toxins. Comparison with other toxins, such as diphtheria toxin, suggests that this central domain is probably involved in the translocation of the active domain into the cytosol.

The binding site for the small GTPases and the enzymatic site are located in the N-terminal part. Residues 1–546 in ToxB (2366 amino acids) and LT (2364 amino acids) retain enzymatic activity in vitro and are cytotoxic on microinjection [4, 21]. Region 364–516 determines substrate specificity. It seems that residues 364–468 are important for the recognition of Rho family proteins, and residues 468–516 are involved in the recognition of Ras proteins [20]. As reported for various glucosyltransferases, the large clostridial toxins have a common DXD motif that is essential for enzymatic activity. Replacement of Asn-286 and Asn-288 by Ala in LT induces a dramatic decrease in glucosyltransferase activity and binding of UDP-glucose. The DXD motif may be involved in the coordination of divalent cations, which is required for enzymatic activity, or in the binding of UDP-glucose [4]

The large clostridial toxins modify various small GTPases by monoglucosylation. ToxA and ToxB from *C. difficile* and alpha-toxin from *C. novyi* glucosylate proteins from the Rho family (Table 1). ToxA and ToxB use UDP-glucose to transfer the glucose moiety to Thr-37 in Rho and to the equivalent position (Thr-35) in Rac and CDC42. Alpha-toxin recognizes the same target proteins but catalyzes a slightly different reaction consisting of the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to Rho proteins. Thr-37 is part of switch I of Rho proteins and binds the nucleotide through the coordination of Mg^{2+} [52].

ToxA, ToxB and alpha-toxin induce actin cytoskeleton changes involving the loss of stress fibers and reorganization of cortical actin. The cells become rounded and retraction filaments are apparent (Fig. 3A).

LT is highly related to ToxB and is also a mono-glucosyltransferase using UDP-glucose as cosubstrate. LT modifies Rac from the Rho family, CDC42 is also modified by LT in certain strains. In addition, LT glucosylates GTPases from the Ras family including Ras, Rap and Ral (Table 1).

LT induces actin cytoskeleton changes different from those obtained with *C. difficile* toxins. The cells have an irregular outline, lose of stress fibers, and undergo reorganization of cortical actin, with numerous microspikes rich in actin and fimbrin [42].

In 3T3 cells, the glucosylation of Ras by LT at Thr-35 inhibits the stimulation of the MAP kinase cascade induced by growth factors [42]. LT modifies Ras in the GDP-bound form. Intrinsic and stimulated GTPase activity is decreased and the interaction of modified Ras with its effector Raf is completely inhibited, leading to blockade of the MAP kinase cascade [19]. In contrast, the MAP kinase pathway is not blocked by LT in *Xenopus* oocytes. In these cells, LT induces cdc2 kinase activation and germinal vesicle breakdown [45].

Necrotizing toxins Certain *E. coli* strains isolated from humans and animals with diarrhea or extraintestinal infections, produce a cytotoxic necrotizing factor (CNF) that causes multinucleation in cultured cells and necrosis in rabbit skin. CNF1 is produced by strains isolated from humans and animals, and CNF2 is produced by *E. coli* strains isolated from cow and sheep. The two toxins are similar in size (115 kDa), are immunologically related and share 85% amino acid sequence identity [35]. CNF1 is chromosomally encoded and CNF2 is plasmid encoded.

CNFs are synthesized without a signal peptide. The catalytic domain has been identified in the C-terminal part (amino acids 720–1007). The central part which contains a putative membrane-spanning segment, is probably involved in the translocation of the toxin into the cytosol, and the 299 N-terminal amino acids are responsible for binding to a cell surface receptor (Fig. 1) [26].

CNFs induce a large increase in cell volume and actin filaments. The cells become spread on the substrate and flattened, with numerous ruffles on the surface and thickened stress fibers. The increase in polymerized actin has been found to be mediated by Rho [8].

The enzymatic activity of CNFs has recently been elucidated. CNFs catalyze the deamidation of Rho Gln-63 to give glutamic acid. This reduces the electrophoretic mobility of Rho on gels. Gln-63 is located in switch II of Rho, which is involved in GTPase activity. Therefore, Rho modified by CNFs is permanently active due to the loss of intrinsic and Rho-GAP-stimulated GTPase activity. This prevents the transition of Rho-GTP into the GDP steady-state form (Fig. 2) [9, 48].

Bordetella bronchiseptica, responsible for swine atrophitic rhinitis, produces a toxin called dermonecrotizing toxin (DNT), which is related to CNFs. DNT also acts by deamidation of Gln-63 in Rho [22].

A toxin acting on cadherin

Bacteroides fragilis strains associated with diarrheal diseases in young animals and children have been found to produce an enterotoxin called *B. fragilis* toxin (BFT). Two types of *bft* gene have been characterized and encode proteins with an overall amino acid sequence identity of 92% [10, 23]. The *bft* genes are part of a 6 kb pathogenicity island in *B. fragilis* [30].

BFT is synthesized as an inactive protoxin secreted by means of a signal peptide (18 N-terminal amino acids). The precursor is processed proteolytically, to release the 211 N-terminal residues. The resulting C-terminal 186 residues (20.7 kDa) constitute the active form (Fig. 1) [10, 23].

BFT is cytotoxic for cultured cells such as HT29 and induces cell rounding, swelling, and redistribution of F actin with the loss of stress fibers and condensation of actin filaments in a peripheral ring around the cell [24, 47]. BFT seems to be active outside cells without requiring internalization because the known inhibitors of endosomal and Golgi trafficking do not prevent the effects of the toxin [47]. The C-terminal part contains a predicted amphipathic region of 20 residues which may be involved in the insertion of BFT multimers into the cell membrane. Possible pore formation has not been demonstrated experimentally [10].

The BFT sequence shows the presence of a zinc-binding motif (HExxH) characteristic of zinc metalloproteases. Although BFT is able to cleave G-actin in vitro, neither the proteolysis of actin components nor a decrease in F actin content have been observed in cells [46]. Recently, it has been found that BFT cleaves E-cadherin specifically and may induce a rearrangement of β-catenins leading to reorganization of the actin cytoskeleton [57].

Effects of toxins altering the actin cytoskeleton on cell functions

The inactivation of small G proteins and the disassembly of the actin cytoskeleton impair various cell functions and tissue organization.

Increase in cell barrier permeability One of the main functions of epithelial and endothelial tissues is to control the permeability between the two biological compartments that regulate their composition. Of the four classes of intercellular junction (tight junctions, adherence junctions, desmosomes and gap junctions), tight junctions are the most critical in limiting paracellular fluxes. Tight junctions consist of transmembrane proteins (occludin and claudin) connecting adjacent cells. Occludin is linked at the cytosolic sites of tight junctions to zonula occludens proteins (ZO1, ZO2 and ZO3) which are linked to actin filaments [12].

The integrity of the actin cytoskeleton has a crucial role in the assembly of the tight junctions and the regulation of barrier permeability. Cytochalasin D, which impairs actin filament polymerization, has been found to increase paracellular permeability in CaCo-2 cell monolayers [27]. Toxins that directly act on actin monomers and disassemble actin filaments induce similar effects. Therefore, Iota toxin markedly alters monolayer permeability and ZO1 organization (Fig. 3B).

Rho and Rac are required for the establishment of tight junctions and adherence junctions mediated by cadherin [3, 16, 53]. Toxins that modify Rho and/or Rac increase cell barrier permeability and alter the organization of the tight junctions detected by the disruption of ZO1 immunostaining (Fig. 3B). C3 enzyme preferentially alters actin filament organization in the apical pole and the associated tight junction of polarized intestinal epithelial cells [33]. ToxB and ToxA are also very potent toxins that alter cell barrier permeability [41]. In contrast, LT does not significantly disorganize ZO1 organization in CaCo-2 cells (Fig. 3B). The effects of toxins modifying the actin cytoskeleton on the intermediate junctions involve the reduction of the intensity of labeling with anti-cadherin antibodies (Fig. 3C).

In polarized T84 and MDCK cells, BFT induces a marked decrease in cell barrier permeability if applied to the basolateral side. The effects are less pronounced if the toxin is applied to the apical side. Upon basolateral application, BFT induces a loss of microvilli and F actin filaments at the apical side and an increase in actin filaments at the basal pole, accompanied by the disruption of tight junctions [5, 34]. BFT also seems to stimulate Cl^- secretion in T84 monolayers [5].

CNF, in contrast, increases the polymerization of F-actin at focal contacts of adjacent cells, induces the formation of

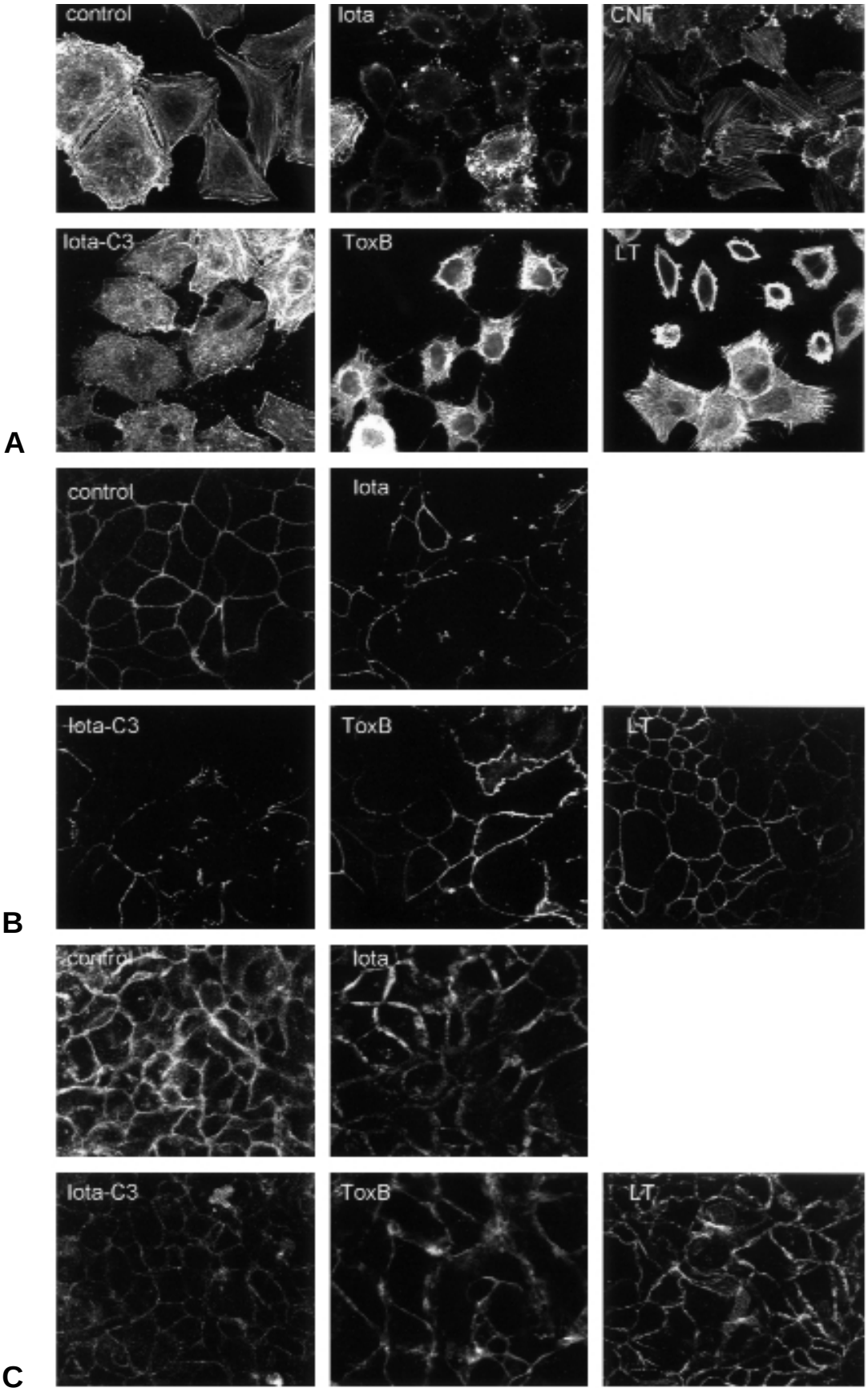

Fig. 3 Effects on actin filaments and intercellular junctions of toxins modifying the actin cytoskeleton. (A) HeLa cells untreated (control) and treated with *Clostridium perfringens* Iota toxin, *Escherichia coli* CNF, C3 enzyme (a fusion Iota-C3 toxin that only exhibits C3 enzymatic activity was used to internalize the C3 enzyme into cells), *Clostridium difficile* ToxB and *Clostridium sordellii* LT (10^{-7} M), were stained with Texas red-phalloidin and observed by confocal microscopy. Polarized CaCo-2 cells grown on filters, untreated and toxin-treated, were stained with anti-ZO1 antibodies (B), and with anti-E-cadherin antibodies (C) to detect tight and intermediate junctions respectively

actin stress fibers and significantly increases cell monolayer permeability. Depolymerization and the polymerization of actin and the subsequent reorganization of the actin cytoskeleton alter intestinal barrier function [14].

Other effects Rho proteins are involved in cell morphology, adhesion, motility, cytokinesis, contractile response, cell growth, and apoptosis and participate in many functions in the body such as tissue organization, inflammatory cell migration and tumor cell invasion [31]. These functions may be altered by toxins modifying actin and Rho proteins.

Rho is important in integrin-clustering and controls cell adhesion mediated by integrin. Aggregation and adhesion of lymphocytes to extracellular matrix can be blocked by C3 [28].

The signaling of various hormones, neurotransmitters and growth factors involves the stimulation of phospholipase D (PLD), hydrolyzing the major membrane phospholipid, phosphatidyl choline, to produce phosphatidic acid and choline, which are involved in various physiological processes such as cell growth, differentiation, and membrane trafficking. Ral has been found to have a pivotal role in the stimulation of PLD by phorbol ester, and large clostridial toxins that glucosylate Ral block PLD activation and subsequent signaling [51]. Activation of PLD may also result from the stimulation of the muscarinic acetylcholine receptor. The signaling pathway involves Rho and an increase in of PIP2 and may be blocked by ToxB and C3 [49, 50].

Compelling data have shown that the actin cytoskeleton and small GTPases from the Rho family are involved in endocytosis and exocytosis. Therefore, cortical actin, trimeric G proteins and Rho are involved in exocytosis in chromaffin cells. Iota toxin and C3 have been used as tools to explore exocytosis regulation in these cells [13]. It has been shown that Rho and Rac inhibit transferrin-receptor-mediated endocytosis [25].

Lamellipodia and membrane ruffling controlled by Rac and Rho are involved in endocytosis and bacterial entry into cells. CNF induces membrane ruffles and increases bacterial uptake by endocytosis [7]. *Shigella* invasion is mediated by Rho-dependent membrane ruffling and can be blocked by C3 and ToxB [1].

In addition, Rac regulates NADPH oxidase complex formation and the subsequent production of superoxide in neutrophils and monocytes. This function can be altered by toxin inactivation of small G-proteins [29].

Concluding remarks The production of toxins is a powerful bacteria-host interaction. The actin cytoskeleton and Rho proteins, which precisely control actin polymerization, are involved in many cell functions and are crucial targets for toxins.

Bacterial toxins acting on the cytoskeleton dramatically disturb cell morphology, intercellular junctions, the permeability of epithelia and endothelia and functions dependent on actin. They are involved in severe gastrointestinal diseases and gangrene. Toxins are also valuable tools for cell biology and treatment. Toxins that recognize specific regulatory molecules of the Rho-Ras family are powerful reagents for investigating the signaling pathways dependent on these proteins. As Ras and Rho have been implicated in cancer, toxins that specifically inactivate these targets could be useful as antitumor agents. Toxins can efficiently enter cells and could be useful to internalize heterologous proteins or therapeutic compounds. In this respect, binary toxins, the functional domains of which are on separate proteins, are an attractive model ([2], and manuscript submitted).

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