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General Aspects and Recent Advances on Bacterial Protein Toxins

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Bacterial pathogens produce protein toxins to influence host–pathogen interactions and tip the outcome of these encounters toward the benefit of the pathogen. Protein toxins modify host-specific targets through posttranslational modifications (PTMs) or noncovalent interactions that may inhibit or activate host cell physiology to benefit the pathogen. Recent advances have identified new PTMs and host targets for toxin action. Understanding the mechanisms of toxin action provides a basis to develop vaccines and therapies to combat bacterial pathogens and to develop new strategies to use toxin derivatives for the treatment of human disease.

During the dialogue established between bacterial pathogens and their host, secreted protein toxins play a key role in the specificity of the outcome of the infection. These toxins are fascinating objects if one considers that in some instances injection of small amounts of these purified proteins recapitulates the deadly symptoms of the infection, such as in the case of the spastic paralysis triggered by tetanus neurotoxin (TeNT). Here we will briefly introduce the diversity of bacterial protein toxins and then focus on new advances made on toxins acting on intracellular host targets.

Bacterial protein toxins can be classified into several functional groups despite their diverse structures and modes of action (Fig. 1) (for general reviews, see Alouf and Popoff 2006). These groups are:

1. A group of toxins that act directly on host cell plasma membrane receptors. By acting as host receptor agonists or antagonists, they corrupt signal transduction pathways. For instance, superantigens produced by staphylococci can bridge nonspecifically major histocompatibility complex class II at the surface of antigen-presenting cells with the T-cell receptor, leading to a nonclonal activation of immune cells and resulting in inflammatory storms (Alouf and Popoff 2006).
2. A group of toxins that disrupt membrane lipid bilayer integrity by forming pores of different size and molecular selectivity or because of their phospholipase activity (Bischofberger et al. 2009). For example, the pore-forming toxin listeriolysin O (LLO) produced by bacteria internalized into phagocytic com-

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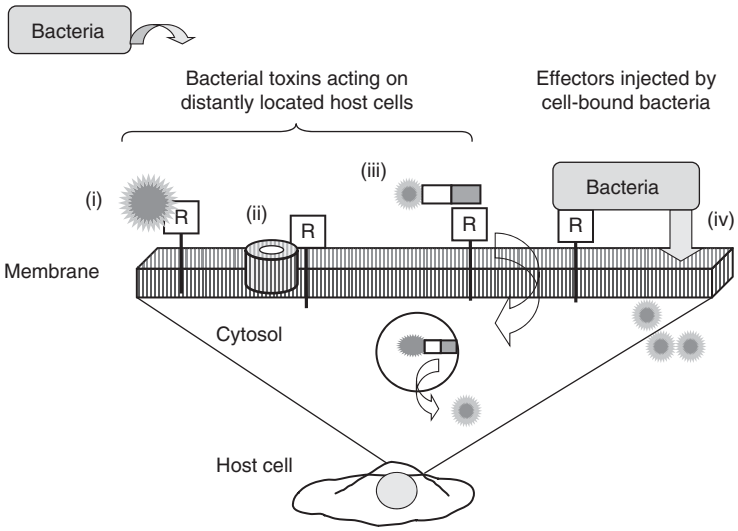

Figure 1. Classes of bacterial protein toxins. There are four major classes of bacterial toxins, including (i) toxins that bind to the surface of host cell plasma membrane receptors and modify host cell physiology by triggering intracellular signaling; (ii) toxins that bind to host cell plasma membranes and disrupt the membrane lipid bilayer through pore formation or expression of phospholipase activity; (iii) AB toxins with an A domain that possesses enzymatic activity and a B domain that binds and enters the host cell; and (iv) toxins with an enzymatic activity that is delivered into the host cell by an injection apparatus that is a component of the bacterial pathogens, which include the type III secreted cytotoxins. The first three groups of toxins often act at a site within the host that is distant from the bacterial pathogen, whereas type III secreted cytotoxins are delivered into the host cell directly by the bacterium, often paralyzing the host cell's ability to neutralize the bacterial pathogen.

partments disrupts the phagosomal membrane, releasing bacteria into the cytosol for dissemination (Cossart 2011). In addition, loss of plasma membrane integrity by pore-forming toxins corrupts cell signaling (Bischofberger et al. 2009; Cossart 2011).

3. A group of toxins that inject toxic enzymatic components into distant host cells once they have entered cells by receptor-mediated endocytosis. These sophisticated proteins can be functionally described as molecular syringes (Fig. 1). They are termed AB toxins because of the presence of a polypeptidic A domain that possesses enzymatic activity and one or more B domains that bind and enter the host cell. These toxins, such as TeNT and botulinum neurotoxins (BoNTs), are frequently endowed with a major virulent function. Therefore, these proteins are of major interest in the study of bacterial virulence mechanisms, such as transfer of virulence genes among bacteria, regulation of virulence factor expression, and the secretion apparatuses of these virulence factors. In addition, they represent valuable tools to study cell biological processes, that is: (1) receptor endocytosis and vesicular trafficking, such as the retrograde transport of Shiga toxin (Stx) from the surface to the endoplasmic reticulum (ER); (2) translocation of their enzymatic domain through intracellular membranes; and (3) the regulation and function of their cellular targets, as reviewed here (Fig. 2). Finally, it is of major interest to use these potent factors as medical tools, for instance, to kill cancer cells as well as to boost immune responses for vaccination.
4. A group of toxins that are synthesized within the bacterium and delivered directly into the host cell by an injection needle, including type III secreted cytotoxins of Gram-negative bacteria (Fig. 1) (Galan 2009). The type III delivery apparatus is derived by gene duplication from the flagellum apparatus.

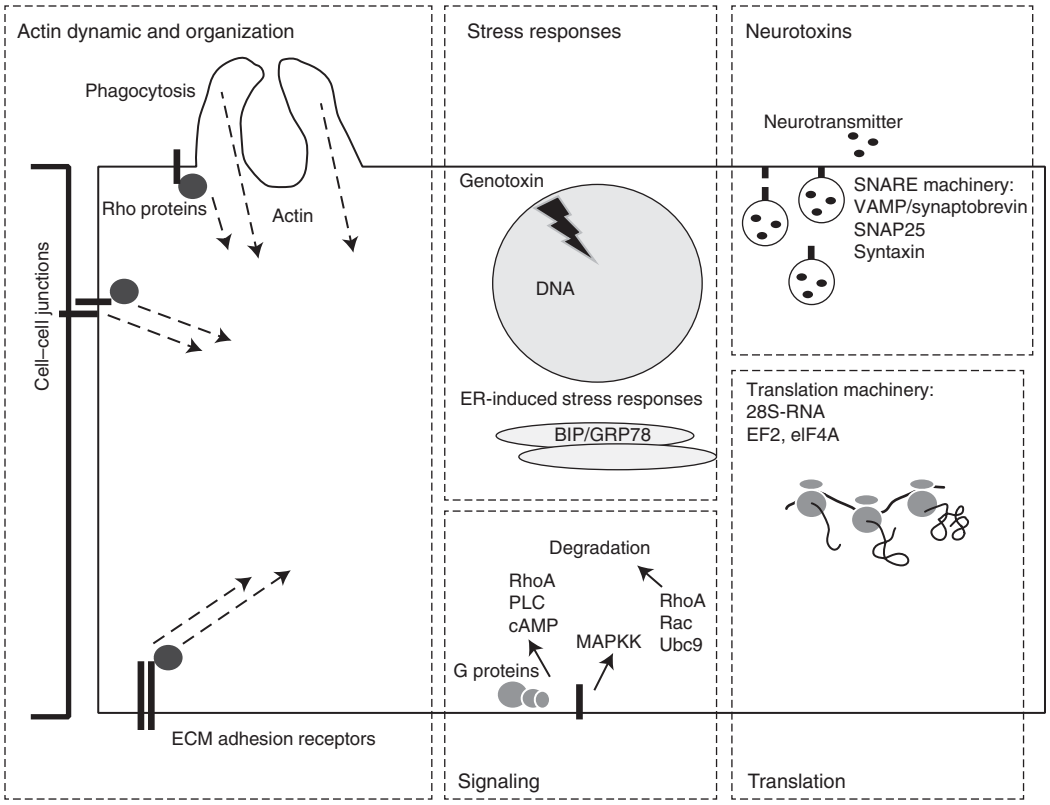

Figure 2. Cellular Achilles' heels targeted by bacterial toxins. Despite differences in provenance, structure, or enzymatic activities, potent bacterial toxins known to date target conserved key cellular factors and pathways (see Table 1 for details). (1) Several toxins directly target actin or Rho GTPases, upstream regulators of the actomyosin cytoskeleton organization and dynamic. These toxins have a major impact on cell–cell or cell–matrix adhesion, motility, and phagocytosis. (2) One group of toxins triggers stress responses, for example, by catalyzing DNA damage. (3) Neurotoxins directly cleave components of the SNARE machinery, thereby impairing exocytosis of neurotransmitters. (4) Several toxins impair cell translation by targeting ribosomal RNA or translation factors (eIF4A or EF2). (5) Other toxins corrupt major signaling pathways, notably resulting in high level of production of cAMP or a shutdown of MAP kinase signaling. They also exacerbate the destruction of signaling molecules, such as Ubc9, RhoA, and Rac1.

These cytotoxins (also called effectors) possess a catalytic activity that covalently modifies a host protein through a posttranslational modification (PTM) or through a noncovalent interaction. These cytotoxins are unique in that the bacterium delivers a large amount of molecules over a short period of time to overwhelm the physiology of the host cell. For example, *Salmonella* spp. inject SopE, a guanine nucleotide exchange factor that stimulates Rac GTPase activity to facilitate entry into pathogen-associated vacuoles in epithelial cells (Galan 2009).

TOXINS TARGETING ACTIN AND SMALL RHO GTPASES

A large number of potent bacterial toxins, notably those produced by several *Clostridium* spp., such as *Clostridium difficile*, and some pathogenic Gram-negative bacteria, such as uropathogenic strains of *Escherichia coli*, catalyze PTM of components of the cell actomyosin cytoskeleton (Table 1). The actomyosin cytoskeleton confers on host cells their shape and membrane dynamics. This function is required for cells to properly control their adhesion to

Table 1. Bacterial enzymatic toxins acting on host cell cytosolic factors

Toxin	Target	Activity	Effect	Bacteria	Reference
Toxins targeting actin cytoskeleton components					
C2	Actin R177	ADP-ribosylation	Depolymerization	*Clostridium botulinum*	Aktories et al. 1986
Iota	Actin R177	ADP-ribosylation	Depolymerization	*Clostridium perfringens*	Aktories et al. 2011
CDT	Actin R177	ADP-ribosylation	Depolymerization	*Clostridium difficile*	Aktories et al. 2011
CST	Actin R177	ADP-ribosylation	Depolymerization	*Clostridium spiroforme*	Aktories et al. 2011
VIP	Actin R177	ADP-ribosylation	Depolymerization	*Bacillus cereus*	Aktories et al. 2011
Tc (TccC3-subunit)	Actin T148	ADP-ribosylation	Actin clustering	*Photorhabdus luminescens*	Lang et al. 2010
MARTXvc (ACD domain)	Actin K50–actin E270	Actin cross-linking	Depolymerization	*Vibrio cholerae*	Aktories et al. 2011
CNF1, -2, -3	Rac1, RhoA, Cdc42 (Q61 or Q63)	Deamidase	Actin polymerization	*Escherichia coli*	Lemonnier et al. 2007
CNFy	RhoA (Q63)	Deamidase	Actin polymerization	*Yersinia pseudotuberculosis*	Hoffmann et al. 2004
DNT	Rac1, RhoA, Cdc42 (Q61 or Q63)	Transglutaminase/deamidase	Actin polymerization	*Bordetella pertussis*, *Bordetella parapertussis*	Lemonnier et al. 2007
C3 and C3-like	RhoA (N41)	ADP-ribosyltransferase	Actin cable depolymerization	*C. botulinum*, *Clostridium limosum*, *B. cereus*, *Staphylococcus aureus*	Aktories 2011
TcdA and TcdB	Rho GTPases (T35 or T37)	Glucosylation (UDP-glucose)	Actin depolymerization	*C. difficile*	Just et al. 1995
HT	Rho GTPases (T35 or T37)	Glucosylation (UDP-glucose)	Actin depolymerization	*Clostridium sordellii*	Aktories 2011
LT	Ras GTPases, Rac (T35)	Glucosylation (UDP-glucose)	Actin depolymerization	*C. sordellii*	Aktories 2011
α-Toxin	Rho GTPases (T35 or T37)	*N*-Acetyl-glucosamination (UDP-*N*-acetylglucosamine)	Actin depolymerization	*Clostridium novyi*	Aktories 2011
TpeL	Ras GTPases, Rac (T35)	*N*-Acetyl-glucosamination (UDP-*N*-acetylglucosamine)	Actin depolymerization	*C. perfrigens*	Aktories 2011
IbpA	Rho GTPases (Y32 or Y34)	Adenylylation (AMPylation)	Actin depolymerization	*Histophilus somni*	Worby et al. 2009
Tc (TccC5 subunit)	Rho GTPases (Q61 or Q63)	ADP-ribosylation	Actin polymerization	*P. luminescens*	Lang et al. 2010

Toxins corrupting ubiquitin and ubiquitin-like signaling					
LLO	Cellular membranes	Pore-forming toxin	Cell-induced degradation of Ubc9	*Listeria monocytogenes*	Ribet 2010
CNF1	RhoA	Deamidase	Smurf1-induced degradation of activated RhoA	*E. coli*	Visvikis 2010
CNF1	Rac1	Deamidase	HACE1 (XIAP, cIAP1/2)-induced degradation of activated Rac1	*E. coli*	Oberoi 2011; Torrino 2011
Toxins targeting cell translational machinery					
DT	EF2 (diphthamide-715)	ADP-ribosylation	Translation inhibition	*Corynebacterium diphtheriae*	Murphy 2011
ETA	EF2 (diphthamide-715)	ADP-ribosylation	Translation inhibition	*Pseudomonas aeruginosa*	Murphy 2011
Stx	28S ribosomal RNA (adenine base at position 4324)	*N*-Glycosidase	Translation inhibition	*Shigella dysenteriae*	Johannes and Romer 2010
Stx-like (1, 1c, 2, 2c, 2d, 2e, 2f)	28S ribosomal RNA	*N*-Glycosidase	Translation inhibition	Shigatoxigenic *E. coli* (STEC)	Johannes and Romer 2010
LT-1 (BPSL1549)	eIF4A (Q339)	Deamidase	Translation inhibition (uncouples ATPase and helicase activities)	*Burkholderia pseudomallei*	Cruz-Migoni et al. 2011
Toxins targeting SNARE machinery					
TeNT	VAMP/synaptobrevin	Zinc metalloprotease	Neurotransmission inhibition	*Clostridium tetani*	Hill et al. 2007
BoNT A, E	SNAP25	Zinc metalloprotease	Neurotransmission inhibition	*C. botulinum*	Hill et al. 2007
BoNT B, D, F, G	VAMP/synaptobrevin	Zinc metalloprotease	Neurotransmission inhibition	*C. botulinum*	Hill et al. 2007
BoNT C	SNAP25, syntaxin	Zinc metalloprotease	Neurotransmission inhibition	*C. botulinum*	Hill et al. 2007
Toxins targeting DNA and inducing endoplasmic reticulum stress responses					
EcCDT-I, II, III, IV	DNA	DNAse-I-like (B subunit)	Cell cycle arrest	*E. coli*	Guerra et al. 2011
SdCDT	DNA	DNAse-I-like (B subunit)	Cell cycle arrest	*S. dysenteriae*	Guerra et al. 2011
HdCDT	DNA	DNAse-I-like (B subunit)	Cell cycle arrest	*Haemophilus ducreyi*	Guerra et al. 2011
AaCDT	DNA	DNAse-I-like (B subunit) and PIP_3 phosphatase	Cell cycle arrest (lymphocyte apoptosis)	*Aggregatibacter actinomycetemcomitans*	Shenker et al., 2007; Guerra et al. 2011
HhCDT	DNA	DNAse-I-like (B subunit)	Cell cycle arrest	*Helicobacter hepaticus*	Guerra et al. 2011
CjCDT	DNA	DNAse-I-like (B subunit)	Cell cycle arrest	*Campylobacter jejuni*	Guerra et al. 2011
SubAB	BIP/GRP78	Serine protease	ER stress responses	STEC	Byres et al. 2008

Continued

Table 1. *Continued*

Toxin	Target	Activity	Effect	Bacteria	Reference
Toxins targeting cAMP and MAP kinase signaling components					
Ctx	$G\alpha_s$ (R201, activation)	ADP-ribosylation	cAMP induction	*V. cholerae*	Aktories et al. 2011
LT	$G\alpha_s$ (R201, activation)	ADP-ribosylation	cAMP induction	*E. coli*	Aktories et al. 2011
Ptx	$G\alpha_i$ (C351, inactivation)	ADP-ribosylation	cAMP induction	*B. pertussis*	Aktories et al. 2011
PMT	$G\alpha_q$, $G\alpha_i$, $G\alpha_{12/13}$ (Q209, Q205, or Q226, activation)	Deamidation	cAMP inhibition, RhoA activation, PLC activation	*Pasteurella multocida*	Aktories et al. 2011
ET	cAMP production	Adenylate cyclase	cAMP production	*Bacillus anthracis*	Leppla 1982
CyaA	cAMP production	Adenylate cyclase	cAMP production	*B. pertussis*	Ahuja et al. 2004
LT	MAP kinase kinases (except MEK5)	Metalloprotease	MAP kinase signaling	*B. anthracis*	Collier and Young 2003

BoNT, botulinum neurotoxin; CDT, cytolethal distending toxin; CNF, cytotoxic necrotizing factor; CST, *C. spiroforme* toxin; Ctx, cholera toxin; DNT, dermonecrotic toxin; DT, diphtheria toxin; EF2, elongation factor-2; eIF, translation initiation factor; ET, edema toxin; ETA, exotoxin A; HT, hemorrhagic toxin; LLO, listeriolysin O; LT, lethal toxin; MAP, mitogen-activated protein; MARTX, multifunctional autoprocessing repeats-in-toxin; PLC, phospholipase C; PMT, *P. multocida* toxin; PTX, pertussis toxin; SNAP, synaptosomal-associated protein; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; Stx, Shiga toxin; Sub, subtilase toxin; TeNT, tetanus toxin; VAMP, vesicle-associated membrane protein; VIP, vegetative insecticidal protein.

the matrix substratum and intercellular monolayer barrier integrity, as well as for immune cells to undergo migration and perform phagocytosis (Jaffe and Hall 2005). This group of bacterial weapons is thus particularly efficient for disruption of host epithelium barriers, for efficient bacterial dissemination, and for freezing distantly located immune cells to prevent both their migration to the site of infection and bactericidal action (Boquet and Lemichez 2003). Actomyosin cytoskeleton disruption by direct cross-linking of ADP-ribose on actin's arginine-177 residue is an enzymatic property of several clostridial toxins (Table 1) (Aktories et al. 1986, 2011). The ADP-ribosylated actin monomers are extremely toxic, although actin is a very abundant protein in cells. Indeed, ADP-ribosylated actin monomers act dominantly once incorporated into actin filaments by blocking addition of new monomers. This produces a disassembly of actin filaments, leading to cell retraction and collapse.

The property of disassembling actomyosin cytoskeleton is shared by several large clostridial toxins that modify Rho proteins (Aktories 2011). This group of key upstream regulators of actomyosin cytoskeleton organization and dynamic belongs to the superfamilly of small Ras GTPases (Jaffe and Hall 2005). Large glucosylating toxins catalyze the addition of a sugar molecule, such as glucose from UDP-glucose, on a key threonine residue of several small GTPases (Just et al. 1995). Addition of bulky glucose groups to this key amino acid residue poisons their biochemical activity and association with downstream effectors. Glucosylating toxins share as a common target Rac, a small Rho GTPase that orchestrates cellular innate immune responses against pathogens (Bokoch 2005; Boyer et al. 2011). This effector-binding domain of Rho proteins is subjected to several other PTMs by bacterial effectors and toxins, such as ADP-ribosylation and AMPylation (Table 1) (Worby et al. 2009; Yarbrough et al. 2009; Aktories 2011).

Photorhabdus luminescens organisms establish a symbiotic relationship with some entomopathogenic nematode species, conferring on them the capacity to kill susceptible insects. Exciting new findings on Tc toxin (TccC3 and TccC5 subunits) of *P. luminescens* has shed light on two new toxin-mediated PTMs of actin and Rho GTPases (Lang et al. 2010). Hence, determination of the structure of the closely homologous Tc toxin of *Yersinia pseudotuberculosis* has revealed its organization into large multisubunit complexes (Landsberg et al. 2011). RhoA is ADP-ribosylated by TccC5 on glutamine-63 (Q61 for Rac and Cdc42) (Lang et al. 2010). This glutamine-63 residue is a hot spot of modification by bacterial toxins given its key function in catalyzing the hydrolysis of the guanosine triphosphate into guanosine diphosphate for switching GTPases to an inactive form (Flatau et al. 1997; Schmidt et al. 1997; Lemonnier et al. 2007). Thus, ADP-ribosylation of RhoA by TccC5 triggers its activation and the resulting formation of actin stress fibers (Lang et al. 2010). The TccC3 toxin subunit ADP-ribosylates actin on threonine-148 instead of arginine-177, as for other known actin-targeting toxins (Table 1). This modification blocks actin sequestration by thymosin-β4, thereby resulting in actin polymerization and aggregation of actin filament into clusters scattered through the cytosol. Intoxication of cells by both factors leads to an aggravation of actin reorganization into clusters because of the combined activation of Rho GTPases and actin polymerization.

TOXINS CORRUPTING UBIQUITIN AND UBIQUITIN-LIKE SIGNALING

An efficient way to interfere with the function of proteins is to catalyze their proteolysis. Some bacterial metalloprotease toxins such as the lethal factor of *Bacillus anthracis* and the neurotoxins of clostridia directly catalyze the endoproteolytic cleavage of key host proteins (Table 1). Recent advances also indicate that some bacterial toxins exacerbate cellular protein degradation by the ubiquitin/proteasomal system (UPS) (Munro et al. 2007). PTM of proteins by ubiquitin and ubiquitin-like molecules, such as SUMO or NEDD8, control the fate and activity of large numbers of proteins and

are targeted by numerous bacterial virulence factors (Munro et al. 2007). The ubiquitylation reaction consists of the covalent attachment of ubiquitin, an 8-kDa polypeptide, to lysine residues on the target protein (Weissman 2001). This involves a cascade of transfer reactions between ubiquitin carrier proteins. Among these factors, the E3 ubiquitin ligase enzymes confer the specificity to the reaction by binding distinctively to a panel of target proteins. Additional molecules of ubiquitin can be subsequently attached to one of the seven lysines of the previously cross-linked ubiquitin molecule, leading to the formation of various types of mono-, multi-, or polyubiquitin chain assemblies, notably lysine-48 (K48) polyubiquitin chains for substrate targeting to proteasomal destruction (Dikic et al. 2009). Several toxins, by catalyzing the permanent activation of Rho proteins, in fact exacerbate the cellular UPS-mediated regulation of these GTPases (Doye et al. 2002; Visvikis et al. 2010). This represents a remarkable example of how toxins can be useful to unravel new cellular regulations and determine in this case E3 ubiquitin ligases that are responsible for Rho protein ubiquitylation. For instance, the study of cytotoxic necrotizing factor 1 (CNF1) recently revealed the critical function of HACE1 and implicated XIAP and cIAP1, E3 ubiquitin ligases, in the targeting of Rac1 to UPS (Oberoi et al. 2011; Torrino et al. 2011). The importance of UPS regulation of Rho proteins during infection remains to be fully determined, although it likely fosters endothelium invasion by bacteria (Doye et al. 2002; Torrino et al. 2011). In addition, some toxins target ubiquitin-like molecules. For instance, *Listeria* listeriolysin O acts on distant host cells to stimulate the degradation of Ubc9, a key enzyme of protein modification by SUMOylation (Ribet et al. 2010). Although bacterial toxins interfere only indirectly with UPS regulation of host cell proteins, several bacterial effectors catalyze a direct PTM of ubiquitin and ubiquitin-like molecules. Indeed, the type III secreted effectors CHBP from *Burkholderia pseudomallei* and Cif from enteropathogenic *E. coli* deamidate the ubiquitin-like protein NEDD8 (Cui et al. 2010; Taieb et al. 2011). This abolishes the activity of multimeric Cullin-RING ubiquitin ligases and impairs numerous major signaling pathways.

TOXINS TARGETING CELL TRANSLATIONAL MACHINERY

An efficient way to kill host cells is through inhibition of protein synthesis. Several pathogenic bacteria share the capacity to inhibit protein translation by PTM of translational factors, as, for example, elongation factor-2 (EF2), which is ADP-ribosylated by diphtheria toxin (DT) of *Corynebacterium diphtheriae* and exotoxin A (ETA) of *Pseudomonas aeruginosa* (Table 1) (Murphy 2011). Both DT and ETA target a PTM-modified histidine residue of EF2 called diphthamide, which is only present in eukaryotes and archaea (Zhang et al. 2010). Most recently, a report shows that the lethal toxin of *B. pseudomallei* (BPSL1549) targets the translation initiation factor 4A (eIF4A) (Cruz-Migoni et al. 2011). This toxin belongs to the growing family of deamidase virulence factors first identified for CNF1 toxin, converting a specific target glutamine into a glutamic acid residue (Flatau et al. 1997; Schmidt et al. 1997; Cui et al. 2010). Despite an absence of primary sequence homology with the CNF1 deamidase domain, BPSL1549 possesses a conservation of the amino acid residues within the catalytic site. The deamidase activity of BPSL1549 toward eIF4A glutamine-339 has an inhibitory effect on the helicase activity, thereby blocking translation (Cruz-Migoni et al. 2011).

The group of Stx and Shiga-like *N*-glycosidase toxins of *Shigella dysenteriae* and enterohemorrhagic strains of *E. coli* remove a specific adenine from 28S ribosomal RNA (Table 1) (Johannes and Romer 2010). Stx toxins play key role in the induction of hemolytic uremic syndrome and at sublethal doses induced inflammatory reactions (Sandvig et al. 1992; Johannes and Romer 2010). A remarkable property of these toxins is their ability to enter cells by triggering membrane deformation at high concentration and undergo a retrograde transport to the ER, where the catalytic domain exits to the cytosol by hijacking the ER-associated

degradation machinery (Sandvig et al. 1992; Johannes and Romer 2010).

TOXINS TARGETING SNARE MACHINERY

The clostridial neurotoxins (CNTs) include TeNT and the BoNTs, which are the most toxic proteins for humans but are also commonly used therapy for many human neurological disorders (Table 1). The neurologic specificity of the CNTs lies in their ability to bind neuron-specific receptors and then cleave neuron-specific soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. These highly specific interactions between BoNTs and synaptic vesicle proteins have been recently reviewed (Brunger et al. 2008). There are seven BoNT serotypes (A–G), and each serotype cleaves one or more proteins within the SNARE protein complex (Hill et al. 2007). SNARE protein cleavage inhibits neurotransmitter vesicle fusion to the plasma membrane and subsequent release of neurotransmitters. The unique pathologies elicited by TeNT and BoNTs are due to their intracellular trafficking in neurons. BoNTs bind gangliosides on resting motor neurons at the neuromuscular junction and enter neurons within cycling synaptic vesicles. SV2 is the protein receptor for BoNT A (Dong et al. 2006). Upon acidification of the synaptic vesicle, the catalytic domain of the BoNTs translocates into the cytoplasm to cleave SNARE proteins and inhibit neurotransmitter release at the neuromuscular junction to elicit flaccid paralysis. In contrast, TeNT binds gangliosides on the cell surface of resting motor neurons and enters endosomes that transcytose TeNT into the central nervous system, where TeNT enters inhibitory neurons to block neurotransmitter release and elicit spastic paralysis. TeNT appears to be transported via a novel neuronal compartment through specialized pH regulation (Bohnert and Schiavo 2005; Chen et al. 2009).

TOXINS TARGETING DNA AND INDUCING ER STRESS RESPONSES

In addition to playing key roles during infection, in some cases bacterial toxins may represent risk factors for cancer (Collins et al. 2011). For example, the genotoxic activity of cytolethal distending toxins (CDTs), a group of toxins produced by a large number of Gram-negative pathogenic bacteria, likely represents a risk factor for carcinogenesis (Table 1) (Lara-Tejero and Galan 2000; Guerra et al. 2011). Structural analysis of *Haemophilus ducreyi* CDT (HdCDT) shows interactions between the three globular subunits (CdtA, -B, and -C) (Nesic et al. 2004). CdtA and CdtC adopt a lectin-type conformation, with structural homologies with the cell-binding subunit of the plant toxin ricin, and with affinity to sphingomyelin. The catalytic subunit CdtB shares conserved amino acid residues with the active site of mammalian deoxyribonuclease (DNase) I (Lara-Tejero and Galan 2000) at residues that contribute to cleavage of the phosphodiester bond of DNA and within a pentapeptide motif (Elwell and Dreyfus 2000). Hence, CdtB of *Aggregatibacter actinomycetemcomitans* bears a phosphatidylinositol 3,4,5-triphosphate 5-phosphatase enzymatic activity, similar to that of PTEN or SHIP1 (Shenker et al. 2007). The relative contribution of both biochemical activities in CdtB-induced DNA damage responses, G_2M cell cycle arrest, and cell death may vary depending on cell types (for review, see Guerra et al. 2011).

TOXINS TARGETING cAMP SIGNALING COMPONENTS

Several pathogenic bacteria have evolved toxins to manipulate the cellular flux of cAMP (Table 1). This comprises Ca^{2+}/calmodulin-dependent adenylate cyclase toxins producing cAMP from ATP, such as edema toxin (ET) of *B. anthracis*, as well as a group of toxins targeting heterotrimeric G proteins, such as cholera toxin of *Vibrio cholera* and pertussis toxin of *Bordetella pertussis* (Table 1) (Ahuja et al. 2004). cAMP is a crucial mediator of cell signaling and is thus implicated in numerous cell biological and physiological functions through the activation of effector proteins such as protein kinase A and the Rap1 exchange factor EPAC. Consistently, manipulation of cAMP signaling confers on bacteria a large panel of features. For example, a recent

study shed light on how ET of *B. anthracis* manipulates macrophage genetic responses (Kim et al. 2008). Production of cAMP leads to a reprogramming of macrophage transcription toward a genetic program with close similarities to anti-inflammatory G-protein-coupled receptors, for induction of inflammation resolution, a phenomenon requiring active cell signaling (Kim et al. 2008). This comprises a CREB- and syndecan-1-dependent induction of macrophage motility and vascular endothelial growth factor-triggered lymphangiogenesis. Toxins inducing cAMP flux (notably cholera toxin) are used to manipulate immune responses, a feature that is also under study for Rho-activating toxins, to develop potent immunoadjuvants for mucosal vaccination (Munro et al. 2005; Fabbri et al. 2008).

Recent advances have determined that ET and lethal toxin (LT) of *B. anthracis* directly target endothelial cell actin cytoskeleton and corrupt the proper localization of adherens junction molecules, effects that likely account for the major vascular dysfunctions resulting from anthrax infection or toxemia (Guichard et al. 2010; Lemichez et al. 2010; Maddugoda et al. 2011). At early time periods of endothelial cell infection or intoxication, ET has a dominant effect over LT, inducing the formation of transendothelial cell macroaperture tunnels ~10 μm in diameter (Maddugoda et al. 2011). Formation of these transcellular tunnels likely contributes to the rupture of endothelium barrier function and triggers edema that is particularly visible in the intestine of mice intoxicated by ET (Maddugoda et al. 2011). The tunnels are induced by a group of toxins and exoenzymes, now referred to as tunnel-forming toxins, also comprising the RhoA ADP-ribosyltranferase EDIN of *Staphylococcus aureus* (Boyer et al. 2006; Lemichez et al. 2010). LT, through its proteolytic activity toward mitogen-associated protein (MAP) kinase kinases, induces at late time periods of intoxication the formation of thick actin cables, compromising endothelial cell junction integrity and possibly the viability of some endothelial cell types (Rolando et al. 2010). Rupture of endothelial cell junctions by both toxins at late time periods of intoxication also involves the targeting of the Rab11/Sec15 exocyst components, thereby inhibiting the recycling pathways for proper localization of the vascular endothelial cadherin critical adherens junction molecule at cell–cell junctions (Guichard et al. 2010).

TOWARD DECIPHERING THE ROLE OF TOXINS DURING THE COURSE OF BACTERIAL INFECTION

Major challenges to date consist in defining how toxins affect differentiated host cell types in order to define their contribution to the various steps of the infection process. This exciting challenge is rendered possible by defining toxins' biochemical activities, as described above. New challenges include the identification of host cell toxin receptors, a major determinant of host species and cell type specificity. The importance of receptor specificity was recently presented, whereby humans do not synthesize sialic acid *N*-glycolylneuraminic acid but metabolize this monosaccharide, which is abundant in some food products, thereby becoming susceptible to the subtilase toxin (SubAB) (Byres et al. 2008). This toxin is produced by some strains of the shigatoxigenic group of *E. coli* (STEC) associated with severe forms of hemolytic uremic syndrome.

Determination of the toxin's mode of action requires characterizing host cell receptors. For example, a difference of a few amino acid residues between mouse and human precursors of a heparin-binding epidermal growth factor-like growth factor (HB-EGF) defines resistance in mice to diphtheria toxin (Naglich et al. 1992). The use of new screening methods to define host factors involved in cell intoxication will certainly accelerate the identification of host factor receptors. Notably, the use of gene-trap retrovirus-driven insertional mutagenesis to produce a collection of null-allele cell mutants for screening (Carette et al. 2009) will help identify toxin receptors and also help define host factors involved in facilitating toxin enzymatic domains' translocation and activation into the host cell cytosol, as well as toxin cofactors. Such an elegant strategy identified TMEM181 as the host

cell receptor of HdCDT, together with sphingomyelin synthase-1, an enzymatic activity likely required to maintain proper organization of lipid rafts (Carette et al. 2009). Similarly, the lipolysis-stimulated lipoprotein receptor has also been recently identified as the receptor of the binary actin ADP-ribosylating toxin of hypervirulent strains of *C. difficile* (Papatheodorou et al. 2011).

An approach of major interest, although probably highly counterintuitive, is to define conditions in which bacterial toxins can trigger host antimicrobial responses and thus exert local or cell-type-specific "avirulence" effects. This likely will help decipher how the host senses and reacts properly to pathogenic bacteria rather than to commensals (Sansonetti 2011). One such interesting example is the infection of macrophages by LT of *B. anthracis* (Ali et al. 2011). The lethal factor enzymatic component of this toxin penetrates cells and cleaves the amino-terminal part of MAP kinase kinases, interrupting these signaling pathways (Duesbery et al. 1998). This induces a leakage of ATP through connexin channels as a result of impaired p38 MAP kinase kinase and AKT signaling pathways (Ali et al. 2011). Next, the sensing of extracellular ATP via P_2X_7 purinergic receptors leads to activation of the inflammasome system and the production of interleukin-1β, a critical factor in mounting antibacterial responses (Ali et al. 2011). Sensing of ATP leakage and potassium efflux induced by several pore-forming toxins are likely critical elements allowing host cells to properly decipher between commensal and pathogenic bacteria-producing toxins (Ali et al. 2011; Hamon and Cossart 2011). Also in line with this, a recent study conducted in *Drosophila* revealed that direct activation of Rac activity by the CNF1 toxin of uropathogenic *E. coli* triggers an antimicrobial response via Rip kinase (IMD in flies) (Boyer et al. 2011). Importantly, recombinant insects expressing CNF1 become resistant to infection by *Pseudomonas* entomopathogenic bacteria. This indicates that some bacterial toxins may favor infection of the host at specific stages, while triggering antimicrobial responses in other contexts.

CONCLUDING REMARKS

Protein toxins provide the bacterium with an advantage in host–pathogen interactions. Each protein toxin appears unique and provides the producing bacterial pathogen with a selective advantage in these host–pathogen interactions. Continued studies on these protein toxins will extend our understanding of bacterial pathogenesis and may identify novel applications of these toxins as therapies to treat human disease.

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