Bacterial protein toxins that modify host regulatory GTPases

Klaus Aktories

Abstract | Many bacterial pathogens produce protein toxins to outmanoeuvre the immune system of the host. Some of these proteins target regulatory GTPases such as those belonging to the RHO family, which control the actin cytoskeleton of the host cell. In this Review, I discuss a diversity of mechanisms that are used by bacterial effectors and toxins to modulate the activity of host GTPases, with a focus on covalent modifications such as ADP-ribosylation, glucosylation, adenylylation, proteolysis, deamidation and transglutamination.

GTPases
GTP-binding proteins that have GTP hydrolysis activity and act as molecular switches, dependent on the bound nucleotide (GDP-bound proteins are in the off state, and GTP-bound proteins are in the on state).

Isoprenylated tail
The carboxy-terminal tail of the protein to which an isoprenyl moiety has been attached at the carboxy-terminal cysteine residue as a post-translational modification. Isoprenylation of many small GTPases, including RHO-family proteins, is essential for membrane binding. A C_{20} isoprenyl moiety (geranylgeranyl) is usually attached to RHO-family proteins.

RAB
A family of small GTPases that are involved in membrane and vesicle traffic.

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universität Freiburg, Albertstr. 25, 79104 Freiburg, Germany.
e-mail: klaus.aktories@pharmakol.uni-freiburg.de
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The cytoskeleton of eukaryotic cells has a pivotal role in host–pathogen interactions. It is essential for epithelial and endothelial barrier functions, which prevent and limit the invasion and dissemination of pathogens in tissues[1,2]. It also has key roles in different processes carried out by immune cells, including migration[3], phagocytosis[4,5], formation of the immune synapse and immune cell signalling[6,7]. For these reasons, the cytoskeleton is normally kept under a tight control, in which GTPases of the RHO family play a major part[8]. RHO-family proteins are molecular switches that are involved in many of the signalling pathways which are needed for effective immune responses, such as the development, activation and function of B and T cells[6,7], polarized migration and phagocytosis in leukocytes[4], the sensing of chemotactic gradients, and the regulation of degranulation and NADPH oxidase activity in phagocytes.

To fulfil all these functions, the ~20 RHO-family proteins, including the much studied RHOA, cell division cycle 42 (CDC42) and the RAC proteins, are tightly regulated[9,10]. Most RHO-family GTPases switch between an active GTP-bound form and an inactive GDP-bound state (FIG. 1). The inactive form is activated by GDP–GTP exchange, which is induced by members of a large array (~80 proteins) of guanine nucleotide exchange factors (GEFs)[11]. This activation takes place at the cell membrane, to which the GTPase is attached through an isoprenyl moiety that is post-translationally added to the carboxyl terminus of the protein[12]. The active GTP-bound state is terminated by GTP hydrolysis facilitated by certain GTPase-activating proteins (GAPs), a group that includes at least 60 members[13]. A third family of regulatory proteins (comprising only three members) consists of guanine nucleotide dissociation inhibitors (GDIs), which keep RHO-family GTPases in the cytosol by a close interaction with the isoprenylated tail of the GTPases, and thereby prevent their activation[14]. In the GTP-bound state, RHO-family proteins modulate the activity of many cellular effectors (such as protein kinases, phospholipases and adaptor proteins), which are at least partly specific for certain RHO-family proteins[9].

Over 20 years ago, it was found that some bacterial protein toxins covalently modify RHO-family GTPases[15–17]. This modification affects the regulatory functions of the GTPases and interferes with the corresponding host signalling pathways[18]. As a result, many immune functions are blocked and some physiological processes of the host cells (for example, endocytosis) are hijacked by the action of the toxins, helping the pathogen to establish a niche for proliferation in a hostile environment. Moreover, the modification and subsequent modulation of the activities of RHO-family proteins by bacterial toxins were key for the elucidation of the physiological functions of these GTPases[19,20]. However, bacterial pathogens can also affect the activity of host GTPases by mechanisms that do not involve covalent modifications; this is the case for various bacterial effectors that are secreted by type III secretion (T3S) or type IV secretion (T4S) systems and mimic endogenous regulators of the GTPase cycle. Here, I review the mechanisms that are used by bacterial pathogens to alter the activity of host GTPases. After a brief summary of non-covalent modulation of GTPases by effectors that are injected into the host cell by cell-bound bacteria, this Review focuses on toxin-catalysed covalent modifications of RHO-family GTPases and members of related GTPase families such as RAB and ARF.

Modulation of GTPases by bacterial effectors

Various bacterial T3S and T4S effectors modulate the GTPase cycle of proteins belonging to the RHO, RAB

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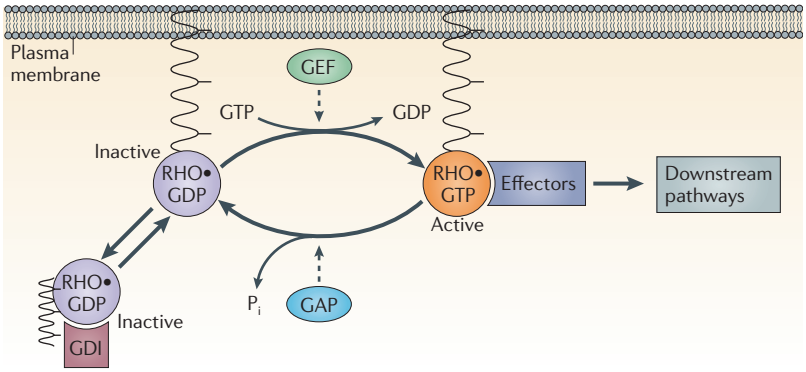

Figure 1 | **Regulation of the RHO-family GTPase cycle.** RHO-family proteins are inactive when bound to GDP and active when bound to GTP. Guanine nucleotide dissociation inhibitors (GDIs) prevent nucleotide exchange and keep the GTPases in the cytosol. Guanine nucleotide exchange factors (GEFs) activate RHO-family GTPases by inducing GDP–GTP exchange. In the active, GTP-bound form, RHO-family proteins interact with and regulate the activity of endogenous effectors. GTPase-activating proteins (GAPs) inactivate RHO-family proteins by facilitating GTP hydrolysis. RHO-family proteins are isoprenylated, which allows membrane binding. Figure is modified from REF. 17 © (2005) Macmillan Publishers Ltd. All rights reserved.

and ARF families by mimicking the functions of host regulators (see TABLE 1).

Activation of GTPases. The *Salmonella* pathogenicity island 1 (SPI-1) locus of *Salmonella enterica* subsp. *enterica* serovar Typhimurium encodes two T3S effectors, SopE and SopE2, that activate RAC proteins and CDC42 in a GEF-like manner[21]. Both effectors contain a Gly-Ala-Gly-Ala motif that binds to the GDP-loaded RHO-family proteins between the switch I and II regions and destabilizes the binding of a key Mg^{2+} and of GDP, leading to GDP release[22]. The subsequent binding of GTP, which has a higher cellular concentration than GDP, induces a conformational change in the GTPase to yield the active form. The effector BopE from *Burkholderia pseudomallei* is similar to SopE and SopE2 and also acts as a GEF analogue[23].

Various proteins belonging to the family of WXXXE effectors are produced by *Shigella flexneri* (IpgB1 and IpgB2)[24–26], *Escherichia coli* (Map and EspM2)[25,27], *Citrobacter rhodentium* (EspT)[27,28] and *S.* Typhimurium[29] (SifA and SifB). At first, these proteins were suggested to be active mimics of RHO-family proteins[25,30], but they have since been identified as GEFs and shown to share a common V-shaped three-dimensional structure with the SopE effectors. The WXXXE motif (which is absent in the SopE effectors) is not directly involved in the catalysis of nucleotide exchange but is important for the stability of the catalytic loop. Remarkably, the structural changes that are induced in RHO-family proteins by bacterial effectors of the WXXXE and SopE families are very similar and resemble those that are induced by host cell GEFs of the DBL family, indicating that they use the same molecular mechanism to induce nucleotide exchange[26].

The T4S effector DrrA (also known as SidM) from *Legionella pneumophila* is a very efficient GEF for RAB1A[31,32], increasing the nucleotide exchange rate by more than five orders of magnitude and leading to the release of RAB1A from GDIs[33,34]. DrrA also possesses adenylylation activity for RAB1A (see below).

EspG is a T3S effector from enterohaemorrhagic *E. coli* (EHEC) that uses a different mechanism to keep GTPases of the ARF family in the active state. It binds in a chaperone-like manner to ARF•GTP, thereby inhibiting the binding of endogenous ARF-specific GAPS (ARFGAPS) (for example, as seen for the ARFGAP ASAP3). Because GTP hydrolysis and nucleotide exchange on ARFs are necessary for proper vesicle transport function, EspG blocks Golgi traffic[35].

GAP-like inhibitors. Most eukaryotic GAP proteins facilitate GTP hydrolysis by GTPases of the RHO and RAS families by stabilizing the switch I and II regions and by providing an arginine residue (also called an arginine finger) that, together with a highly conserved glutamine residue of the GTPase, is involved both in the proper positioning of GTP and in the nucleophilic attack by a water molecule to allow the GTP hydrolysis reaction[36]. Similarly to eukaryotic GAPs, the *S.* Typhimurium T3S effector SptP, which consists of an amino-terminal RHOGAP and a C-terminal tyrosine phosphatase domain, provides an arginine finger for rapid GTP hydrolysis by RHO-family proteins[37]. The effectors YopE from *Yersinia pseudotuberculosis*[38], exoenzymes ExoS and ExoT from *Pseudomonas aeruginosa*[39,40] and AexT from *Aeromonas salmonicida* facilitate GTP hydrolysis of RHO-family proteins by the same mechanism. Remarkably, whereas YopE has a single-domain structure, the other bacterial GAPs possess additional ADP-ribosyltransferase domains that target RAS-related proteins, among other proteins. For example, HRAS, RALA, and the RAB5, RAB9 and RAB11 groups of proteins are ADP-ribosylated by ExoS[41], CRK proteins are modified by ExoT[41] and actin is ADP-ribosylated by AexT[42].

The T4S effector LepB from *L. pneumophila* possesses RABGAP activity and disturbs vesicle traffic in the host cell. Owing to the large size of the protein (149 kDa), several other functions are expected. Moreover, the localization of the GAP function in the protein is not clear, as no sequence similarity with eukaryotic RABGAPs is evident[43].

Alteration of host GTPases by covalent modification

In addition to the non-covalent modifications of host GTPases discussed in the previous section, a growing number of bacterial protein toxins are being shown to affect host GTPase activity by catalysing covalent modifications of the target proteins (TABLE 2). As discussed in the following sections, the mechanisms involved in these modifications are diverse and include ADP-ribosylation, glucosylation, adenylylation, proteolysis, deamidation and transglutamination. Many bacterial toxins of this group are released from the pathogen into the environment and enter the eukaryotic target cell through their autonomous membrane translocation systems. Strictly speaking,

ARF
(ADP-ribosylating factor). A family of small GTPases that are involved in membrane and vesicle traffic. These factors were initially recognized as cofactors for the ADP-ribosylation that is catalysed by the cholera toxin.

Switch I and II regions
Regions of GTP-binding proteins that change the conformation of these proteins between the GDP-bound (inactive) and GTP-bound (active) state. Accordingly, effectors recognize these regions in particular. In RHOA, the switch I region covers residues 27–42, and the switch II region, residues 59–73.

DBL
A family of typical guanine nucleotide exchange factors acting on the RHO-family GTPases.

RAS
A family of small GTPases that are involved in proliferation and differentiation. This term is also sometimes (but not here) used to refer to a protein superfamily of small GTPases that includes the RAS, RAB, ARF (ADP-ribosylating factor), RHO and RAN protein families, among others.

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Table 1 | **Bacterial secreted effectors that modulate the activities of small GTPases by mimicking host regulators**

Effectors	Source organisms	Mode of action	Target GTPases	Refs
Activators				
SopE and SopE2	*Salmonella enterica* subsp. *enterica* serovar Typhimurium	GEF	RAC proteins and CDC42	21
BopE	*Burkholderia pseudomallei*	GEF	RAC proteins and CDC42	146
IpgB1	*Shigella flexneri*	GEF	RAC proteins and CDC42	24,25
IpgB2	*S. flexneri*	GEF	RHOA, RAC proteins and CDC42	26
Map	*Escherichia coli* (EHEC and EPEC)	GEF	CDC42	147
EspM1, EspM2 and EspM3	*E. coli* (EHEC and EPEC) and *Citrobacter rodentium*	GEF	RHOA	27,148
EspT	*E. coli* (EPEC) and *C. rodentium*	GEF	RAC proteins and CDC42	28
SifA and SifB	S. Typhimurium	GEF	RHO proteins?	29
DrrA (also known as SidM)	*Legionella pneumophila*	GEF	RAB1A	33,34
EspG	*E. coli* (EHEC)	Blocks ARFGAP binding	ARF1	35
Inhibitors				
SptP	S. Typhimurium	GAP	RAC proteins and CDC42	37
YopE	*Yersinia pseudotuberculosis*, *Yersinia enterocolitica* and *Yersinia pestis*	GAP	RHO proteins, RAC proteins and CDC42	38,87
ExoS and ExoT	*Pseudomonas aeruginosa*	GAP	RHO proteins, RAC proteins and CDC42	39,40
AexT	*Aeromonas salmonicida*	GAP	RHO proteins, RAC proteins and CDC42	42
LepB	*L. pneumophila*	GAP	RAB1	43

ARF1, ADP-ribosylating factor 1; ARFGAP, ARF-specific GAP; CDC42, cell division cycle 42; EHEC, enterohaemorrhagic *E. coli*; EPEC, enteropathogeni *E. coli*; Exo, exoenzyme; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor.

only these factors are bacterial toxins (exotoxins), whereas other virulence factors that are introduced into target cells by T3S or T4S systems are often called bacterial effectors.

ADP-ribosylation of RHO-family GTPases

C3-like inhibitory toxins. The C3 exoenzyme of *Clostridium botulinum* was the first toxin shown to modify RHO-family GTPases[44,45] (FIG. 2a). The toxin catalyses the ADP-ribosylation of RHOA, RHOB and RHOC at Asn41 (FIG. 2b), whereas RAC proteins and CDC42 are very poor substrates of the toxin[46]. At least seven C3-related toxins are known (TABLE 2), and they have ~35–90% sequence identity. All these toxins are small (~25 kDa) proteins and consist of only an ADP-ribosyltransferase domain, without any specific modules for membrane translocation. Therefore, the cell accessibility of C3-like toxins is often very low when they are applied to cell cultures. However, the C3-like toxin C3stau (also known as Edin) of *Staphylococcus aureus* does not need to be translocated to reach its target, as the bacterium invades the host cell and, once within the host cytoplasm, secretes the toxin[47]. Moreover, monocytes and macrophages take up *C. botulinum* C3 toxin very efficiently, probably from a low pH-dependent compartment, suggesting that these host cells are preferred targets for the toxin[48]. The same is true for other toxins (see below). However, the exact mechanism of uptake is not clear.

What are the functional consequences of RHO-family ADP-ribosylation mediated by the C3-like toxins? Although the residue modified by C3 (Asn41) is located at the border of the switch I region of RHO-family proteins (a region that is essential for the interaction of the GTPases with their effector proteins) (FIG. 2b), the modified RHO-family proteins are still able to bind to some of their effectors[49]. However, it has been shown that ADP-ribosylated RHOA exhibits a tight binding to GDI[50] (FIG. 2a), which inhibits membrane–cytosol cycling and prevents activation by GEFs[51]. Thus, RHOA signalling is blocked, resulting in the destruction of stress fibres and the redistribution of the actin cytoskeleton, for example[52,53]. In addition, endothelial cells treated with C3-like toxins form macroapertures, or large transcellular tunnels[54]. The formation of these transient transcellular holes depends on the inactivation of RHOA. It has been suggested that these structures are involved in dissemination of the toxin-producing pathogens (for example, *S. aureus* producing C3stau) during bacteriemia[55]. Similar effects could be induced by the inhibition of RHOA expression using a small interfering RNA. Unrelated to its ADP-ribosylating activity, C3 binds tightly to the GTPase RALA and inhibits its function[56]. However, the biological role of this effect is not clear.

***The activating toxins of* P. luminescens.** In contrast to the inhibitory activity of C3-like toxins, the toxin complex (Tc) from *Photorhabdus luminescens* (a symbiont of

RALA
One of the RAL proteins, a group of GTP-binding proteins in the RAS family. RAL proteins are involved in proliferation, motility and protein sorting.

CRK
(CT10 regulator of kinase). A family of adaptor proteins that mediate the signalling involved in focal adhesion and the regulation of phagocytosis.

Stress fibres
Contractile bundles of polymerized actin that also contain myosin motor proteins. The formation of stress fibres is induced by activation of RHOA.

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Table 2 | **Bacterial proteins that affect the activities of host GTPases by covalent modification**

Toxins	Source organisms	Modification	Target GTPase	Acceptor amino acid	Refs
Activators					
TccC5	*Photorhabdus luminescens*	ADP-ribosylation	RHO proteins, RAC proteins and CDC42	Gln61 or Gln63	59
Ctx	*Vibrio cholerae*	ADP-ribosylation	$G\alpha_s$ proteins	Arg201	121
LT	*Escherichia coli*	ADP-ribosylation	$G\alpha_s$ proteins	Arg201	149
DrrA (also known as SidM)	*Legionella pneumophila*	Adenylylation	RAB1B	Tyr77	31,32,81
Cnf1, Cnf2 and Cnf3	*E. coli*	Deamidation	RHO proteins (preferred by Cnf3), RAC proteins and CDC42	Gln61 or Gln63	102,110, 150
Cnf	*Yersinia pseudotuberculosis*	Deamidation	RHOA	Gln63	102,110, 150
Pmt	*Pasteurella multocida*	Deamidation	$G\alpha_q$ and $G\alpha_i$ proteins, and $G\alpha_{13}$*	Gln209, Gln205 or Gln226 (respectively)	123
Dnt	*Bordetella pertussis* and *Bordetella parapertussis*	Deamidation and transglutamination	RHO-family proteins	Gln61 or Gln63	151
Inhibitors					
C3 (two strain variants)	*Clostridium botulinum*	ADP-ribosylation	RHOA, RHOB and RHOC	Asn41	44,45
C3	*Clostridium limosum*	ADP-ribosylation	RHOA, RHOB and RHOC	Asn41	44,45
C3cer	*Bacillus cereus*	ADP-ribosylation	RHOA, RHOB and RHOC	Asn41	44,45
C3stau (also known as Edin; three strain variants)	*Staphylococcus aureus*	ADP-ribosylation	RHOA, RHOB and RHOC	Asn41	44,45
Ptx	*B. pertussis*	ADP-ribosylation	$G\alpha_i$ proteins	Cys351	152
Toxins A and B (also known as TcdA and TcdB, respectively)	*Clostridium difficile*	Glucosylation	RHO-family proteins	Thr35 or Thr37	63
Lethal toxin	*Clostridium sordellii*	Glucosylation	RAS-family proteins, and RAC proteins	Thr35 or Thr37	64,65
Haemorrhagic toxin	*C. sordellii*	Glucosylation	RHO-family proteins	Thr35 or Thr37	153
α-toxin	*Clostridium novyi*	*N*-acetylglucosamination	RHO-family proteins	Thr35 or Thr37	66
TpeL	*Clostridium perfringens*	*N*-acetylglucosamination (also glucosylation)	RAS, RAL, RAC and RAP proteins	Thr35 in RAC proteins	67
VopS	*Vibrio parahaemolyticus*	Adenylylation	RHO-family proteins	Thr35 or Thr37	72
IbpA	*Histophilus somni*	Adenylylation	RHO-family proteins	Tyr32 or Tyr34	75
YopT	*Yersinia* spp.	Proteolytic cleavage	RHO-family proteins	Cys200 in RHOA	83
LopT	*P. luminescens*	Proteolytic cleavage	RHO-family proteins	Cys200 in RHOA?	92

Cnf, cytotoxic necrotizing factor; Ctx, cholera toxin; Dnt, dermonecrotizing toxin; $G\alpha_i$, adenylyl cyclase-inhibiting guanine-nucleotide-binding protein subunit-α (Gα); $G\alpha_q$, phospholipase Cβ-activating Gα; $G\alpha_s$, adenylyl cyclase-stimulating Gα; IbpA, immunoglobulin-binding protein A; LT, heat-labile enterotoxin; Pmt, *Pasteurella multocida* toxin; Ptx, pertussis toxin; Tc, toxin complex. *A G protein subunit of the $G_{12/13}$ family that leads to activation of RHOA.

nematodes and a pathogen of insects) catalyses an ADP-ribosylation of RHO-family proteins that results in their persistent activation (FIG. 2a,b). These bacteria produce several protein toxins, including the Tc proteins. These complexes, with masses of >1 MDa, consist of at least three different protein types called TcA, TcB and TcC. The genome of *P. luminescens* encodes several homologues for each of the three protein types. TcA proteins appear to be involved in membrane binding and translocation, and TcC proteins carry the biological activity (FIG. 3), whereas the role of TcB proteins is not clear. One of the TcC proteins, TccC5, is an ADP-ribosyltransferase that modifies RHOA at Gln63, and RAC proteins and CDC42 at Gln61. This glutamine residue is essential for the intrinsic and GAP-catalysed GTP hydrolysis (that is, the turn-off reaction) of the RHO-family GTPases[57,58].

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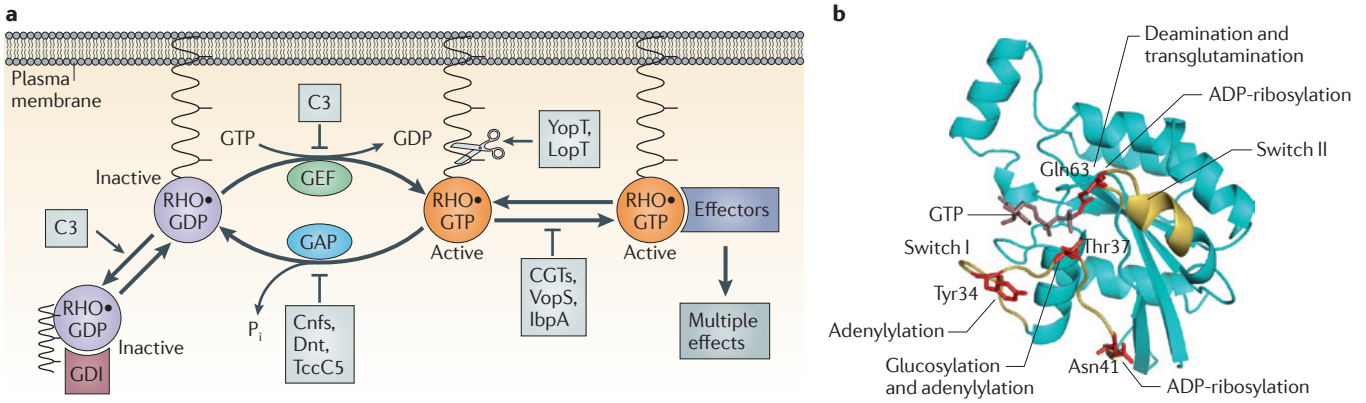

Figure 2 | **Bacterial effectors that modify RHO-family GTPases. a** | ADP-ribosylation of RHO-family proteins by C3-like toxins (C3) of various bacteria induces tight binding of guanine nucleotide dissociation inhibitors (GDIs) to the GTPase and inhibition of RHO-family activation by guanine nucleotide exchange factors (GEFs). The toxins YopT from *Yersinia* spp. and LopT from *Photorhabdus luminescens* are cysteine proteases that cleave the carboxy-terminal isoprenylated cysteine from RHO-family GTPases, leading to release of these proteins from membranes and their inactivation. Clostridial glucosylating toxins (CGTs) attach glucose or *N*-acetylglucosamine to RHO-family proteins, which then cannot switch into their active conformation. The toxins VopS from *Vibrio parahaemolyticus* and IbpA (immunoglobulin-binding protein A) from *Histophilus somni* catalyse the adenylylation (that is, attachment of AMP) of RHO-family proteins, thereby preventing their interaction with their cellular effectors. The cytotoxic necrotizing factors (Cnfs) from *Escherichia coli* and dermonecrotizing toxin (Dnt) from *Bordetella* spp. cause deamidation and transglutamination, respectively, of RHO-family GTPases; as a result, GTP hydrolysis (that is, the turn-off reaction) is blocked, and the RHO-family proteins are constitutively active. The TccC5 toxin component from the toxic complex (Tc) of *P. luminescens* activates RHO-family proteins by attaching an ADP-ribosyl moiety. **b** | The structure of GTP-bound RHOA, showing the target sites for toxin-catalysed modification. RHO-family proteins interact with their endogenous effectors via the switch I and switch II regions (yellow). ADP-ribosylation by C3-like toxins occurs at Asn41, which is located at the end of the switch I region. Tyr34, which is located in the switch I region, is adenylylated by IbpA. Thr37 is glucosylated by CGTs and adenylylated by VopS. Deamidation and transglutamination by Cnfs and Dnt, respectively, occur at Gln63. Gln63 is also modified by TccC5-induced ADP-ribosylation. The Protein Data Bank accession for RHOA is 1A2B.

ADP-ribosylation blocks this function and causes persistent activation of the RHO-family proteins. In line with this, the toxin causes extensive formation of stress fibres in target cells (for example, in haemocytes of insect larvae and in mammalian HeLa cells). Although RHOA, RAC1 and CDC42 were shown to be activated in target cells after treatment with TccC5, the cellular phenotype can be explained by activation of RHOA alone[59]. In addition, another TcC protein, TccC3, affects the cytoskeleton directly by ADP-ribosylation of actin at Thr148 (REF. 59). Thereby, the interaction of actin with thymosin β4 (which sequesters monomeric globular actin and therefore prevents actin polymerization) is blocked, leading to enhanced actin polymerization[59]. The combined actions of TccC3 and TccC5 induce clustering of the actin cytoskeleton, which inhibits phagocytosis of bacteria by insect haemocytes, for example.

Glucosylating toxins from *Clostridium* spp.

RHO-family GTPases can be inhibited by several toxins that catalyse mono-*O*-glucosylation or *N*-acetylglucosamination — that is, the covalent addition of a glucose or an *N*-acetylglucosamine to the GTPase, respectively (BOX 1; FIG. 2b). The prototypes of this toxin family are *Clostridium difficile* toxins A and B (also known as TcdA and TcdB, respectively), which are the major virulence factors used by this bacterium to cause antibiotic-induced diarrhoea and pseudomembranous colitis[60–62]. The toxins from *C. difficile* and the lethal toxin from *Clostridium sordellii* use uridine diphosphate (UDP)-glucose as the sugar donor for the reaction[63–65], whereas the alpha-toxin from *Clostridium novyi* uses UDP-*N*-acetylglucosamine as the donor[66]. The recently discovered toxin TpeL from *Clostridium perfringens* can use both sugar donors[67]. The clostridial glucosylating toxins display different preferences towards their target GTPases. Whereas toxins A and B from *C. difficile* can glucosylate many RHO-family GTPases, *C. sordellii* lethal toxin modifies RAC proteins but not other RHO-family proteins. However, it can modify additional small GTPases such as RAL, RAP and RAS proteins of the RAS family[64,65]. RAS-family proteins are also the main target of *C. perfringens* TpeL[67].

All clostridial glucosylating toxins modify RHO- and RAS-family proteins at a highly conserved threonine residue (for example, Thr35 in RAC proteins and CDC42, and Thr37 in RHOA, RHOB and RHOC) that is involved in the binding of GTP and Mg^{2+} (REF. 63). Glucosylated GTPases are no longer activated by GEFs and are also GAP insensitive[51]. Most important, glucosylation prevents the change into the active conformation in RHO- and RAS-family proteins, even after GTP binding[68,69]. Glucosylated RHO-family proteins do not cycle; they are bound to membranes and are not released into the cytosol[70]. Thus, glucosylation results in the functional inactivation of RHO- and

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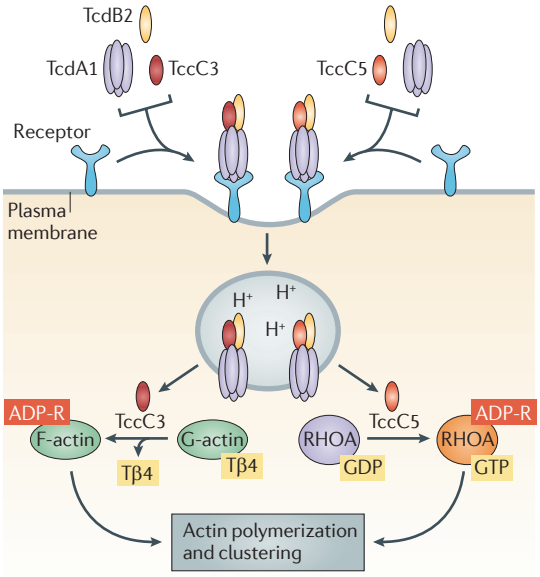

Figure 3 | ***Photorhabdus luminescens* toxins act on the cytoskeleton.** Toxin complex (Tc) toxins of *Photorhabdus luminescens* consist of three types of components, TcA, TcB and TcC, which interact with each other in the ratio 4:1:1. TcA is involved in membrane binding, TcB has unknown functions (perhaps as a linker of the other two components) and TcC possesses the ADP-ribosyltransferase activity. Several homologues of these Tc proteins are encoded in the genome of *P. luminescens*. The molecular mechanisms of the TcC proteins TccC3 and TccC5 have been elucidated[59]. After cell binding by the TcA protein TcdA1 and endocytosis of the tripartite complex consisting of TcdA1, TcdB2 and TccC3 or TccC5, the TccC3 or TccC5 component enters the cytosol from the acidic endosome. In the cytosol, TccC5 adds an ADP-ribosyl (ADP-R) group to RHO-family proteins at Gln63 (in some proteins, this residue is Gln61). This causes persistent activation of the GTPases and rearrangement of the cytoskeleton. In addition, TccC3 affects the cytoskeleton by ADP-ribosylation of actin at Thr148. Thymosin β4 (Tβ4), which sequesters monomeric globular actin, is therefore unable to bind actin, and so actin polymerization is enhanced. Together, TccC3 and TccC5 induce clustering of the cytoskeleton, which results in an inhibition of the phagocytosis of bacteria by insect haemocytes.

RAS-family proteins and the blockade of their signalling functions (for more details on the structure and uptake of clostridial glucosylating toxins, see BOX 1).

It is not only small GTPases such as RHO- and RAS-family proteins that are targeted by bacterial glucosyltransferases, but also larger GTPase-like host elongation factors (EFs). For example, the *L. pneumophila* effector glucosyltransferases Lgt1, Lgt2 and Lgt3, which are related to clostridial glucosylating toxins, modify Ser53 of EF1α1 and EF1α2, thereby inhibiting protein synthesis in host cells[71].

Adenylylation of host GTPases

Inhibition of RHO-family proteins. GTPases can be modified by toxin-catalysed adenylylation (also known as 'AMP-ylation'), whereby an AMP moiety derived from ATP is attached to the GTPase[72]. Recently, this type of modification was identified as the molecular mechanism of various bacterial T3S and T4S effectors that contain either a Fic (filamentation induced by cyclic AMP) domain or a doc (death of curing) domain[72]. Fic and doc domains, collectively known as Fido domains, are found in >2,000 bacterial proteins[73], but their biological function was enigmatic until recently. *Vibrio parahaemolyticus*, a halophilic bacterium that is responsible for food-borne gastroenteritis, produces the T3S effector VopS, which harbours a Fic domain at the C terminus. This effector causes rounding of target cells and inhibits RHOA both in intact cells and *in vitro*[74]. VopS increases the molecular mass of RHO and RAC proteins and CDC42 by 329 Da, which is consistent with the attachment of AMP[72]. Labelling of RHO-family proteins with VopS in the presence of [α-^{32}P]ATP further proved the adenylylation reaction. VopS catalyses the attachment of AMP to the highly conserved Thr37 (or Thr35) of RHO-family GTPases (the same site that is glucosylated by other toxins; see above), thereby inhibiting downstream signalling of these RHO-family proteins[72] (FIG. 2). The preferred substrates for modification by VopS are probably the active forms of RHO-family proteins, despite these active forms having the hydroxyl group of Thr35 (or Thr37) directed into the protein (whereas this hydroxyl group is solvent exposed in the GDP-bound form).

Fic domains consist of at least eight α-helices and a core motif of HPFX(D/E)GN(G/K)R with an invariant histidine residue[72,73]. Changing this histidine blocks adenylylation and the cytopathic effects induced by VopS. Excitingly, Fic domains are conserved from bacteria to humans, and the human Huntingtin interacting protein E (HYPE; also known as FICD) is capable of adenylylating RHO-family proteins *in vitro*[75].

IbpA (immunoglobulin-binding protein A) from *Histophilus somni*, a pathogen of livestock, is a bacterial virulence factor that contains two consecutive Fic domains[75] at its C terminus. Deletion studies showed that both Fic domains, when independently expressed in target cells, are able to induce disruption of the actin cytoskeleton[75]. Pull-down assays revealed that IbpA directly modifies RHO and RAC proteins and CDC42. Surprisingly, IbpA adenylylates RHO-family proteins not at Thr35 or Thr37, like VopS, but at Tyr32 or Tyr34 (REF. 75). This tyrosine residue is highly conserved and located in the switch I region of the RHO-family GTPases (FIG. 2b). Thus, the functional consequence is similar to the modification of Thr35 or Thr37 by VopS, resulting in blockade of downstream RHO-family-mediated signalling. As with the modification by VopS, adenylylation by IbpA appears to depend on the active form of the GTP-binding protein; the AMP linkage can be cleaved by a phosphodiesterase, indicating a phosphodiester bond[75].

Recently, the crystal structure was solved for the second Fic domain of IbpA (Fic2) complexed with CDC42. Fic2 consists of 14 α-helices and is divided into a catalytic core that harbours the Fic motif, and an N-terminal subdomain that might be involved in

Pull-down assays
Precipitation experiments in which one protein or domain of interest is coupled to beads as a bait to pull down those proteins that interact with it. In the case of RHO-family proteins, their interactions can be studied by using the RHO-binding domains of their effectors as bait.

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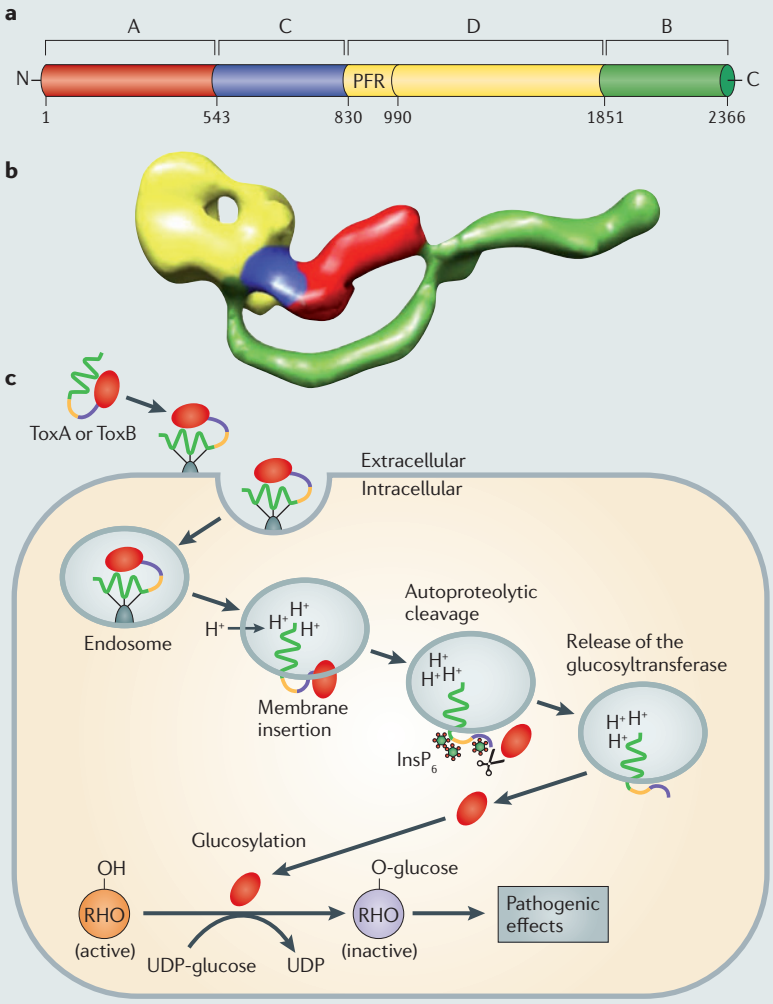
Box 1 | ***Clostridium difficile* toxins A and B**

Clostridium difficile toxins A and B (ToxA and ToxB; also known as TcdA and TcdB, respectively) consist of at least four domains (the ABCD model) (see the figure, parts **a**,**b**). The amino-terminal region harbours the biologically active glucosyltransferase domain A (red)[137], whereas the carboxy-terminal 'binding' domain B (green) is involved in binding to a cell surface receptor[138]. A cysteine protease ('cutting') domain C (blue) is located downstream of domain A[139]. Between domains C and B, the 'delivery' domain D (yellow) is involved in translocation of the toxin into the cytosol[140]. The toxin binds with its C terminus to the cell surface receptor, resulting in endocytosis of the toxin–receptor complex (see the figure, part **c**). The low pH of endosomes results in a structural change in the toxin, allowing membrane insertion. A small part of the D domain (the pore-forming region (PFR)) is involved in pore formation[140]. After translocation of the glucosyltransferase and protease domains into the cytosol, the cysteine protease domain is activated by binding to inositol hexakisphosphate ($InsP_6$)[139,141]. The protease domain autocatalytically cleaves the toxin and releases the glucosyltransferase domain into the cytosol[142], where RHO-family proteins are then glucosylated[63]. Part **b** of the figure shows a three-dimensional model of the structure of *C. difficile* toxin A from images obtained by negative-stain electron microscopy. Part **b** image is reproduced, with permission, from REF. 143 © (2010) American Association for the Advancement of Science.



UDP, uridine diphosphate.

recognition of the RHO-family proteins. Interestingly, the Fic2–CDC42 complex formed dimers consisting of two Fic2 and two CDC42 molecules. The structure of CDC42 in this complex adopts the conformation found in the GDI–CDC42 complex. In RAC1, the switch II region with the motif YLL (Tyr64, Leu67 and Leu79) is required for recognition by IbpA. The same YLL motif seems to be essential for the recognition of RHO-family proteins by VopS, which modifies the GTPases at threonine residues. The crystal structure data suggest that the invariant histidine residue of Fic domains interacts with the hydroxyl group of the tyrosine acceptor of the GTPase to facilitate nucleophilic attack of ATP by the tyrosine residue of the RHO-family protein[76].

***Adenylylation by* L. pneumophila *effectors*.** The T4S effector DrrA from *L. pneumophila*, the cause of Legionnaires' disease, adenylylates the host GTPase RAB1B, which is involved in vesicle transport from the endoplasmic reticulum to the Golgi and in intra-Golgi transport. After phagocytosis by macrophages, *L. pneumophila* cells redesign the host phagosome into a replication-permissive vacuole, and control host cell metabolism and intracellular organelle biogenesis and organization[77,78]. To this end, the pathogen translocates >150 different effectors with a variety of activities into the host cytosol via the Dot/Icm (defect in organelle trafficking/intracellular multiplication) T4S system. DrrA, one of the best characterized among the *L. pneumophila* effectors, possesses RABGEF activity[32,79] (as discussed in a previous section) and a C-terminal phosphatidylinositol-4-phosphate-binding domain for membrane localization[80]. Remarkably, the N-terminal part of DrrA possesses a similar structure to that of glutamine synthetase adenylyltransferase, which uses adenylylation to regulate the activity of glutamine synthetase in bacteria[81]. DrrA adenylylates RAB1B at Tyr77, which is located in the switch II region of the GTPase. The modification of RAB1B by AMP attachment prevents the stimulation of GTP hydrolysis by GAPs, thereby inhibiting the turn-off reaction. Adenylylated RAB1B cannot interact with its endogenous effector, MICAL3 (which is suggested to be involved in vesicle traffic), but it does interact with another *L. pneumophila* effector, LidA[81]. Thus, AMP attachment to Tyr77 of RAB1B selectively activates the GTPase for functioning with *L. pneumophila* proteins.

The AnkX effector of *L. pneumophila* possesses a Fic domain; it prevents proliferative vacuole–lysosome traffic and induces Golgi fragmentation. Importantly, the invariant histidine residue of the AnkX Fic domain is essential for its activity[82]. However, so far neither adenylylation activity nor a direct target has been identified.

Toxin-induced proteolysis of RHO-family proteins

The outer protein YopT from *Yersinia* spp. is a T3S effector that destructs the host cytoskeleton through its cysteine protease activity[83]. YopT cleaves RHO-family proteins directly upstream of the C-terminal cysteine residue, to which the isoprenyl moiety is attached (FIG. 2a). As a result, the GTPase is released from the membrane and therefore inactivated[83–85]. For substrate recognition, YopT needs the isoprenyl group and the C-terminal polybasic region of RHO-family proteins, but the nucleotide-binding state of the GTPase does not seem to be important. YopT acts *in vitro* on

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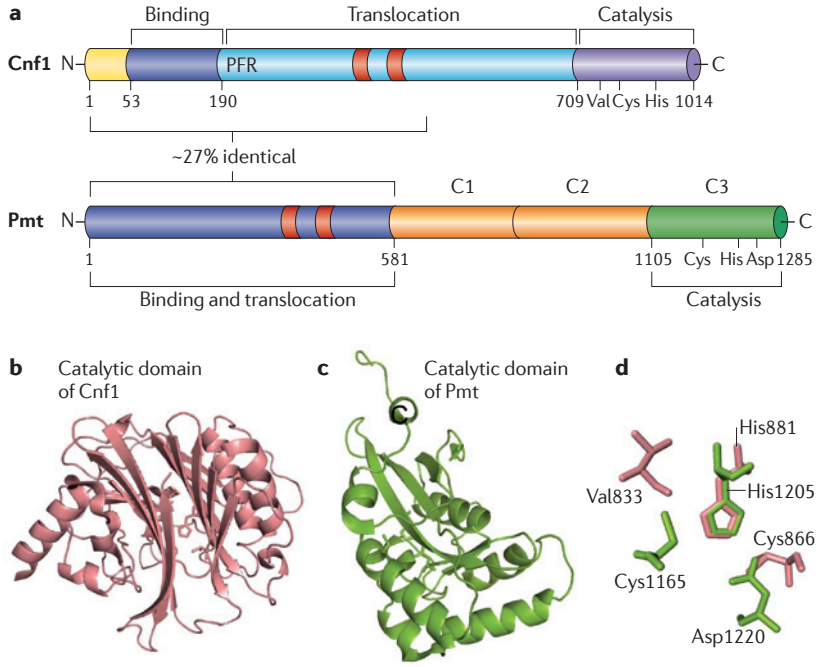

Figure 4 | **Structure of *Escherichia coli* cytotoxic necrotizing factor 1 and *Pasturella multocida* toxin. a** | The amino termini of *Escherichia coli* cytotoxic necrotizing factor 1 (Cnf1) and *Pasturella multocida* toxin (Pmt) are similar (~27% sequence identity) and contain two hydrophobic helices (red) that participate in membrane insertion and translocation. After endocytosis of Cnf1, only the part of the toxin harbouring the carboxy-terminal catalytic deamidase domain reaches the cytosol. The catalytic amino acids are indicated (Cys, His, Val and Asp). The C terminus of Pmt consists of three domains, C1, C2 and C3 (REF. 145). C1 is involved in membrane localization, the function of C2 is unclear and C3 possesses deamidase activity. Although the molecular mechanisms (deamidation) of Cnf1 and Pmt are the same, the catalytic domains have different structures. **b**,**c** | The structures of the catalytic domains of Cnf1 (part **b**) and Pmt (part **c**). **d** | The catalytic amino acids of Pmt (Cys1165, His1205 and Asp1220; green) have a different position to those of Cnf1 (Val833, Cys866 and His881; pink). Protein Data Bank accessions for Cnf11 and Pmt are 1HQ0 and 2EBF.

various RHO-family GTPases, although RHOA appears to be its preferred substrate in HeLa cells and human umbilical vein endothelial cells (HUVEC)[86], whereas it can also cleave RHOG and RAC proteins in COS1 cells. Interestingly, after being released from membranes by YopT, RAC proteins are translocated into the nucleus[87]. The functional consequences of this are unclear, although nuclear RAC proteins may play a part in cell division[88] and in β-catenin signalling[89], which regulates the actin cytoskeleton and is a key factor in WNT signalling. Moreover, nuclear localization of RAC proteins is essential for their degradation[90].

YopT is a member of a specific group of papain-like cysteine proteases that possess a catalytic site composed of cysteine, histidine and aspartate and are inhibited by the protease inhibitor E64 (REF. 91). Other bacterial virulence factors such as LopT from *P. luminescens* also belong to this protein family. LopT targets RHO-family proteins in mammalian and insect cells and releases RAC proteins and RHOA from membranes[92]. The same type of protease is found in many avirulence factors, such as AvrPphB and NopT from the plant pathogens *Pseudomonas syringae* and *Rhizobium* sp., respectively[83,93].

WNT signalling
A pathway that transfers a signal from membrane receptors to the nucleus and is involved in embryogenesis and tumour development.

Avirulence factors
Bacterial factors that induce a strong antimicrobial response in host plants, with the consequence that the plant is protected. The factors seem to be virulence factors under certain conditions.

Laminin receptor
A cell membrane protein that is the receptor for the extracellular matrix glycoprotein laminin and is involved in cell adhesion to the basement membrane.

Activation of host GTPases by deamidation

Cnfs. RHO-family GTPases are persistently activated by various cytotoxic necrotizing factors (Cnfs) such as Cnf1, Cnf2 and Cnf3 from *E. coli* and Cnf from *Y. pseudotuberculosis*[94–97]. Cnfs are ~115 kDa proteins consisting of an N-terminal domain that is involved in target cell binding and membrane translocation, and a C-terminal catalytic domain[98]. It has been proposed that *E. coli* Cnf1 binds to the laminin receptor and/or the laminin receptor precursor at the host cell surface[99], although *Y. pseudotuberculosis* Cnf seems to bind to a different, as-yet-unidentified receptor[100]. Owing to inherent proteolytic activity of the toxin, only the C-terminal part of the toxin seems to reach the cytosol[101]. Cnfs activate RHO proteins, RAC proteins and CDC42 by deamidation of a glutamine residue (Gln63 or Gln61), which as a result becomes glutamic acid[102,103] (FIG. 2). This amino acid change blocks GTP hydrolysis and locks the RHO-family protein in the active state. Note that this modification occurs at the same glutamine residue that is ADP-ribosylated by TccC5 from *P. luminescens* (FIGS 2,3).

The catalytic site of Cnf1 is composed of Val833, Cys866 and His881 (REF. 104) (FIG. 4). Cnf1 seems to recognize only a small part of the RHO-family protein, as a 20-amino acid peptide covering the switch II region of RHOA is a substrate for deamidation[105]. The activation of RHO-family proteins by Cnf1 in cells causes the formation of stress fibres, lamellipodia and filopodia; this is the result of the activation of RHOA, RAC proteins and CDC42, respectively[94,106]. In addition, Cnf1 induces the formation of multinucleate cells[107] (probably by inhibiting cytokinesis) and the uncoupling of S phase from mitosis[108], and inhibits mitotic catastrophe[109]. Although all Cnfs can act on a variety of RHO-family GTPases *in vitro*, their effects on live cells can differ: whereas *E. coli* Cnf1 and Cnf2 activate several RHO-family GTPases, *E. coli* Cnf3 and *Y. pseudotuberculosis* Cnf are largely specific for RHOA[110]. The reasons for this specificity are unclear.

The persistent activation of RHO-family proteins as a result of the deamidation catalysed by the various Cnfs induces the subsequent degradation of the GTPases by polyubiquitylation and proteasomal cleavage[111–113]. The degradation of RHO-family proteins may result in a decreased inflammatory response, which favours bacterial infection[114]. Moreover, depending on the different efficiencies of cells in triggering RHOA, RAC proteins or CDC42, ubiquitylation and degradation result in variations in the actin phenotype that is induced by the various Cnfs[112].

Deamidation and transglutamination by* Bordetella *Dnt. *Bordetella parapertussis* and *Bordetella bronchiseptica* produce a 146 kDa dermonecrotizing toxin (Dnt)[115] that is related to Cnfs and harbours the catalytic domain (~30% identical to that of Cnf1) at the C terminus. The 54 N-terminal amino acids are thought to be involved in receptor binding[116], whereas the middle part of the protein is involved in membrane translocation. The toxin is most probably cleaved by furin-like proteases during

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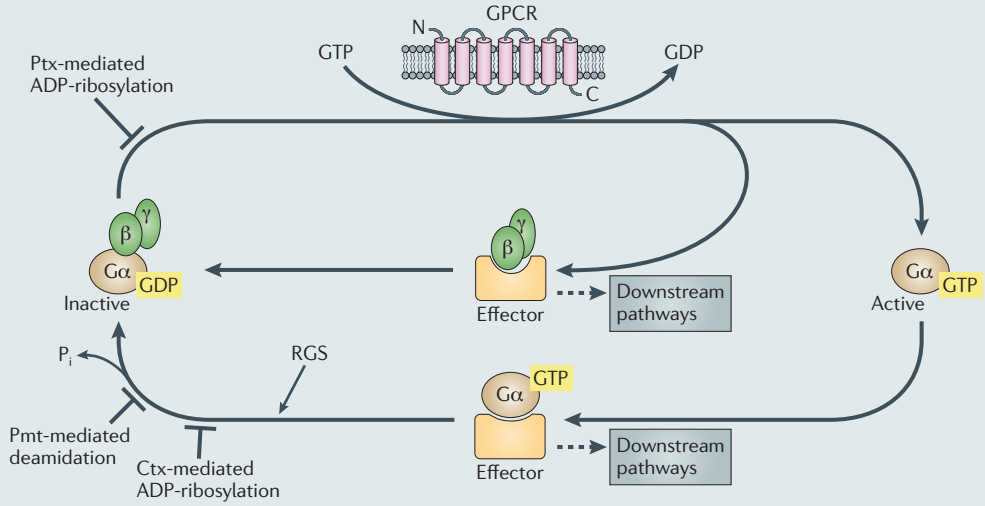
Box 2 | **Heterotrimeric guanine-nucleotide-binding proteins as targets of bacterial toxins**

Heterotrimeric guanine-nucleotide-binding (G) proteins are signal-coupling factors that are usually activated by numerous heptahelical membrane receptors known as G protein-coupled receptors (GPCRs), such as adrenoceptors or serotonin receptors (see the figure). G proteins consist of α-, β- and γ-subunits with masses of ~45, ~35 and ~7 kDa, respectively. The α-subunit binds GTP and possesses GTPase activity. The β-subunit is usually in a tight complex with the γ-subunit. In the inactive, GDP-bound form, Gα associates with the βγ complex. GPCRs, similarly to the GEFs that act on small GTPases, activate G proteins by inducing a GDP–GTP exchange in the α-subunit, accompanied by release of the βγ complex. Subsequently, GTP-bound Gα binds to and regulates the activity of endogenous effector proteins such as adenylyl cyclases or phospholipase Cβ (PLCβ). The βγ complex, which is released upon Gα activation, interacts with cellular effectors such as phosphoinositide-3-kinases (PI3Ks). Hydrolysis of GTP terminates the active state and causes re-association of the subunits to form the inactive Gαβγ complex. GTP hydrolysis is stimulated by regulator of G protein signalling (RGS) proteins. About 20 different G proteins are known, and these are grouped into at least four major families according to their α-subunits. Typical effects of G proteins of the different classes are stimulation of adenylyl cyclase by G_s proteins, inhibition of adenylyl cyclase by G_i proteins, activation of PLCβ by G_q proteins and activation of RHOA by $G_{12/13}$ proteins[128,144].

Examples of bacterial toxins acting on heterotrimeric G proteins are *Pasteurella multocida* toxin (Pmt), which deamidates a specific glutamine residue of Gα subunits[123], and cholera toxin (Ctx), which ADP-ribosylates $Gα_s$ (REF. 121). Both toxins lock G proteins in their active state. In addition, pertussis toxin (Ptx) ADP-ribosylates $Gα_i$ at the carboxyl terminus, thereby inhibiting the interaction with the GPCR and, therefore, inhibiting the activity of the G protein[41].



Lamellipodia
Broad, membrane ruffling-like processes that contain actin and are induced by the activation of RAC proteins.

Filopodia
Thin, finger-like cell protrusions that contain actin and are induced by activation of the GTPase cell division cycle 42 (CDC42).

Mitotic catastrophe
Mitotic-linked cell death as a consequence of the inappropriate entry of cells into mitosis.

Polyubiquitylation
The attachment of several ~8 kDa ubiquitin peptide molecules onto lysine residues of target proteins by a three-enzyme cascade. It is often the prerequisite for the proteasomal degradation of the target proteins.

Furin
A serine endoprotease that cleaves endogenous precursor proteins. Furin is also involved in the processing of anthrax toxin, *Pseudomonas* exotoxin A and envelope proteins of various viruses.

Dynamin
A large GTPase that is regulated by oligomerization and forms a collar-like structure around invaginations of membranes during the pinch-off process of vesicle formation.

Polyamines
Metabolites such as putrescine, spermine and spermidine that are derived from arginine and methionine. They seem to be involved in many cellular processes, such as proliferation and migration.

RHO kinases
Endogenous effectors of RHO-family proteins; RHO kinases are activated by the GTP-bound form of RHO-family GTPases and regulate smooth muscle contraction and many signalling processes that are related to the cytoskeleton.

dynamin-dependent endocytosis; as a result of this, only a part of the toxin is released into the cytosol[117]. In the cytosol, Dnt acts as a Cnf-like deamidase but also as a transglutaminase, leading to deamidation or polyamination of RHO-family GTPases[118,119]. The toxin attaches polyamines such as spermidine, spermine or putrescine to RHOA, RAC proteins and CDC42 at the Gln61 or Gln63 position[119]. This is the same site that is deamidated by Cnfs (FIG. 2). In fact, Dnt catalyses a deamidation reaction in the absence of polyamines, and Cnf is capable of transglutamination *in vitro* at high concentration of poylamines[120]. Dnt-modified RHO-family proteins are active and induce similar cytoskeletal effects as Cnf-modified proteins. After Dnt treatment, RHOA can interact with RHO kinases without GTP loading, suggesting that transglutamination forces the active conformation of RHOA[119].

Pmt activates heterotrimeric G proteins. It is not only small GTPases such as the RHO-family proteins that are targets of bacterial toxins, but also larger heterotrimeric guanine-nucleotide-binding (G) proteins (see BOX 2 for details about these proteins). G proteins are well-known targets of cholera toxin (Ctx) from *Vibrio cholerae*, heat-labile enterotoxin (LT) from *E. coli* and pertussis toxin (Ptx) from *Bordetella pertussis*. Whereas Ctx causes persistent activation of G_s proteins by ADP-ribosylation (see below)[121], Ptx-induced ADP-ribosylation of G_i proteins inhibits their receptor-mediated activation[41]. Recently, it has been shown that the ~145 kDa *Pasteurella multocida* toxin (Pmt)[122], which is produced by *P. multocida*, a pathogen of livestock and sporadically of humans, activates heterotrimeric G proteins by deamidation[123] (BOX 2). Substrates of Pmt are G_q and G_i proteins, and G_{13}. The toxin deamidates Gln205 of $Gα_{i2}$ and Gln209 of $Gα_q$ to form glutamic acid[123]. These highly conserved glutamine residues are functionally equivalent to Gln63 of RHOA, which is deamidated by Cnfs. Accordingly, Gln205 of $Gα_i$ and Gln209 of $Gα_q$ play pivotal parts in the hydrolysis of GTP, which is the turn-off reaction of the G proteins[124,125]. Deamidation of these residues blocks GTP hydrolysis and locks $Gα_i$ and $Gα_q$ in the active state. As mentioned above, heterotrimeric G proteins (for example, G_s proteins) are also persistently activated by Ctx-catalysed ADP-ribosylation[121]. In these G proteins, Arg201 is ADP-ribosylated[126,127]. This arginine residue stabilizes the GTP hydrolysis transition state in a complementary manner with the pivotal glutamine residue of Gα subunits[128]. In small GTPases such as the RHO-family proteins, the equivalent arginine residue is

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not present but is provided for GTP hydrolysis by the 'arginine finger' of their GAPs.

Although Pmt and Cnfs catalyse the same biochemical reaction and possess considerable sequence similarity in their N termini (which are probably responsible for toxin up-take), they completely differ in the folding of the catalytic domain (FIG. 4).

The activation of various G proteins by Pmt results in a variety of functional effects. Activation of G_q proteins by Pmt stimulates phospholipase Cβ (PLCβ), resulting in cleavage of phosphatidylinositol bisphosphate, and the production of diacylglycerol and inositol trisphosphate[129]. Diacylglycerol activates protein kinase C, and inositol trisphosphate mobilizes intracellular calcium, resulting in multiple cellular responses. Probably because of G_q activation, the toxin activates the JAK–STAT (Janus kinase–signal transduction and activator of transcription) pathway, which is involved in cell proliferation[130]. Pmt-induced activation of G_i proteins inhibits adenylyl cyclase and activates phosphatidylinositol 3-kinase via Gβγ[131]. Activation of phosphatidylinositol 3-kinase appears to be involved in the anti-apoptotic effects of Pmt[132]. The effects of Pmt on G_{13} cause indirect activation of RHOA by stimulation of RHOGEFs (such as $p115^{RHOGEF}$) and formation of stress fibres[133]. Activation of G_q proteins is also capable of activating RHOA. Pmt is one of the most potent mitogenic factors and stimulates extracellular regulated kinases (ERKs), which mediate proliferation signals from growth factor receptors to the nucleus[134,135]. This effect of Pmt is apparently caused by persistent activation of various G proteins such as G_q and G_i proteins, and G_{13} (REF. 136).

Concluding remarks

Our knowledge of the mechanisms underlying the modification of host GTPases by bacterial toxins has expanded in recent years, but many questions remain. An intriguing aspect of GTPase modification by toxins is target selectivity. For example, RHO-family GTPases are inactivated by adenylylation or by glucosylation at the Thr35 (or Thr37) residue, which is conserved in almost all GTPases and essential for nucleotide binding. So, why are only certain GTPases modified?

Moreover, the inhibition of GTP hydrolysis is a common mechanism used by several toxins to activate GTPases. Cnfs, Pmt and TccC3 modify the same or functionally equivalent amino acids to block the turn-off mechanism of GTPases, although different methods (for example, deamidation and ADP-ribosylation) are involved. All these toxins interfere in the same way with the transition state of the GTP hydrolysis reaction. Moreover, this molecular mechanism is shared by Ctx, which ADP-ribosylates an arginine residue of G proteins that is essential for GTP hydrolysis.

Thus, these data strengthen the proposal that bacterial protein toxins preferentially target GTP-binding proteins. This is especially true for small GTPases, but also applies for heterotrimeric G proteins and for large GTPases such as the EF1α proteins. Why are GTP-binding proteins preferred targets? A probable explanation is that these proteins function as molecular switches for the orchestration of diverse cell responses in host–pathogen interactions. Therefore, we can expect the list of bacterial toxins that interfere with eukaryotic GTPases to further increase.

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Competing interests statement
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