Insecticidal Toxin Complexes from *Photorhabdus luminescens*

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Abstract Various bacterial toxins have potent insecticidal activity. Recently, the Toxin complexes (Tc's) of *Photorhabdus* and *Xenorhabdus* species have become an increased focus of current research. These large tripartite toxins with molecular masses >1.4 megadaltons consist of three components termed A, B, and C (or TcA, TcB, and TcC). While TcA is involved in receptor binding and toxin translocation, TcC possesses the specific toxin enzyme activity and TcB is a linker between components TcA and TcC. Here, a structure function analysis of the toxins is described and the application of Tc toxins as potential insecticides is discussed.

Contents

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1 Introduction

1.1 The Life Cycle of Insecticidal Nematodes

Bacteria from the genus *Photorhabdus* and *Xenorhabdus* are found in symbiotic association with entopathogenic nematodes of the family *Heterorhabditidae* and *Steinernematidae,* respectively. These nematodes are used with limited success as effective biological control agents against important agricultural insect pests of the order Coleoptera and Lepidoptera, including white grubs, weevils, and codling moths (Lacey and Chauvin 1999; Lacey and Georgis 2012). Entopathogenic nematodes employ these bacteria that live within the gut of the nematode to kill their hosts after invading susceptible larvae either through oral, anal, or tracheal pathways. Once the nematodes enter their host, they release their bacteria, which kill the insect usually within 48 h. The rapid kill, along with the production of other virulence factors, helps to defeat the insect's innate immunological defenses allowing the nematodes to survive and reproduce within the insect carcass (Daborn et al. 2001; Silva et al. 2002; Forst and Nealson 1996). The larval carcass provides sufficient nutrients for the nematodes to complete a number of life cycles before the infected carcass ruptures and releases thousands of new nematode progeny that then search for new insect hosts to start the cycle over again (Bowen 2000; Forst et al. 1997). Live entomopathogenic nematodes as insect biological control agents require a narrow effective range of soil moisture, and temperature to work, and demand careful handling and refrigerated storage requirements. These requirements have restricted their effectiveness as biological insect control products and has limited their commercial applications (Lacey and Georgis 2012).

1.2 Specificity of Insecticidal Toxins

Photorhabdus luminescens and *Xenorhabdus nematophilus* bacteria produce a variety of protein toxins, some forming very large (>1.4 MDa) oligomeric tripartite toxin complexes (Tc) that have high levels of toxicity toward insect pests detrimental to important agricultural crops (Waterfield et al. 2001b; ffrench-Constant et al. 2007). Other bacteria, including *Photorhabdus asymbiotica*, *Xenorhabdus bovienii*, *Serratia entomophila*, *Yersinia entomophaga*, *Y. pestis*, and *Pseudomonas syringae pv*. also produce similar toxin complexes (Parkhill et al. 2001; Buell et al. 2003; Wilkinson et al. 2009; Hurst et al. 2000). All of these bacteria represent sources for new genes encoding potent insect toxins that can potentially be used in biological control strategies or in genetically modified plants as alternatives or supplements to insecticidal crystal (Cry) toxins from *Bacillus thuringensis* currently employed in agriculture. Like many Cry proteins, the toxin complex proteins from *Photorhabdus luminescens*, *Xenorhabdus nematophilus*, and *Yersinia entomophaga* appear to be toxic only to certain orders of insects. Although their complete spectra

of insecticidal activity have not been fully characterized, the toxin complexes from *Photorhabdus luminescens* are generally very potent against Coleopteran insects such as the Southern corn rootworm (*Diabrotica undecimpunctata howardi*), and the Colorado potato beetle (*Leptinotarsa decemlineata*), which are insect pests of significant agricultural importance (Waterfield et al. 2001a; Bowen and Ensign 1998). The toxin complex proteins from *Xenorhabdus nematophilus* have limited potency against Coleopteran insects, but have potent activity against crop damaging Lepidopteran insects, such as tobacco budworm (*Heliothis virescens*), corn earworm, (*Helicoverpa zea*), and beet armyworm (*Spodoptera exigua*) larvae (Morgan et al. 2001; Sergeant et al. 2006). The toxin complexes of *Yersinia entomophaga* have been reported to have oral activity against both Coleoptera and Lepidopteran insects (Hurst J. Bact. 2011). The biochemical source of the specificity is most likely due to specific receptor interactions with the toxin which is known to bind putative receptors located in the membrane of the midgut of susceptible insects (Lee et al. 2007; Sheets et al. 2011). Identification and characterization of these receptors and binding interactions have not been fully reported. Other differences in the biological character of the insect midgut, such as pH, lipids, and presence of different proteases in insects of different orders may also have a role in the specificity of the toxin complexes and for their safety toward other organisms. In addition, all of the toxin complexes so far characterized are active by both oral ingestion and injection into the haemocel of the insect, which implies that the toxin complexes may have target sites that are not simply located in the lumen of the insect midgut (Bowen and Ensign 1998; Bowen 2000).

We will focus on the toxin complexes found in *Photorhabdus luminescens* for the purpose of this chapter, since significant structural and functional information has recently been determined for a subset of Tc's from this bacterium. Where similar data are available for Tc's from other bacteria (esp. *Xenorhabdus nematophilus* and *Yersinia entomophaga*) corresponding comparisons will be made. Understanding how these insect toxins function can assist with the development of new genes for biological or transgenic insect pest control. In addition, the biochemical mechanism of these toxins can provide insight into the mechanism of action of other pathogenic bacteria that cause animal diseases, and provide insight for designing new therapeutic protein reagents.

2 'Toxin Complex' (Tc) Toxins

2.1 Nomenclature of Tc Toxins

The nomenclature applied to many of the different Tc genes and subunits is confusing. Initial nomenclature was based upon the order that the genes were found on four (*a*, *b*, *c*, and *d*) different loci in *Photorhabdus luminescens* strain W-14 (Bowen et al. 1998). Hence they were named, for example, *tcaA* for the toxin complex (*tc*)

gene on the '*a*' locus encoding the first or '*A*' protein. Later it was determined that all toxin complexes so far examined in detail consist of three functionally different types of proteins or subunits (named A, B, and C), and it became apparent that a unified nomenclature was required (Waterfield 2006). Similar nomenclature was applied for the toxins found in *Xenorhabdus luminescens*, using Xpt standing for *Xenorhabdus* particulate toxin (Sergeant et al. 2003). The *Xenorhabdus* genes were then termed A, B and C based on the order in which they were found in the genome, giving *XptA*, *XptB* and *XptC*. Unfortunately, for both *Photorhabdus* and *Xenorhabdus* Tc proteins, it was then not clear via this nomenclature if we were discussing a type A, B or C protein as defined by its function. For example, tcaC1 is in fact a functional type B protein, tccB a functional type A protein, xptB1 a functional type C protein, and xptC1 is a functional type B protein (Fig. 55.1). The nomenclature for the toxin complex encoding genes isolated from *Yersinia entomophaga* and *Serratia entomophila* was more straight forward, with *yenA and sepA* encoding type A proteins, *yenB* and *sepB*, encoding type B proteins and *yenC* and *sepC* genes encoding type C proteins (Hurst et al. 2000, 2011). Whilst it is not the purpose of this chapter to define a more understandable nomenclature for the toxin complex proteins and genes, it is clear that understanding if the gene encodes a functional type A, B, or C protein is paramount. Knowing what organism the toxin is derived from, and defining a tripartite toxin complex in terms of its bacterial origin and protein composition is thus also important. In this chapter, therefore, we will indicate if a protein is a functional type A (tcA-like), B (tcB-like), or C (tcC-like) protein, along with the bacterial source of the protein and give a unique name to a toxin complex of specifically associated proteins.

2.2 TcA-like Proteins of the Toxin Complex

2.2.1 Interaction of TcA-like Proteins with the Host Cell Membrane

Early biochemical studies on purified toxin complexes isolated from *Photorhabdus luminescens* and *Xenorhabdus nematophilus*, showed toxin complexes to be very large and composed of multiple classes of proteins (Bowen et al. 1998; Bowen and Ensign 1998; Sergeant et al. 2003, 2006). The first measured binding interaction of a TcA-like toxin component with host cells was reported with XptA1. This study showed that the XptA1 protein binds to brush border membrane vesicles (BBMV) prepared from *Pieris brassicae* and to insect derived Sf21 cells, both of which are susceptible to the toxicity of this protein. In contrast, the related XptA2 protein, which is not toxic to these organisms, does not bind to BBMV's from *P. brassicae* or to Sf21 insect cells (Lee et al. 2007). Additional binding interactions were demonstrated for XptA2 using surface plasmon resonance showing that it binds with a K_d of 0.2 nM to solubilized insect midgut BBMV's prepared from *Heliothis zea*, an insect that XptA2 is toxic against (Sheets et al. 2011). These results support earlier studies that the determinate of insect specificity resides with the type of A

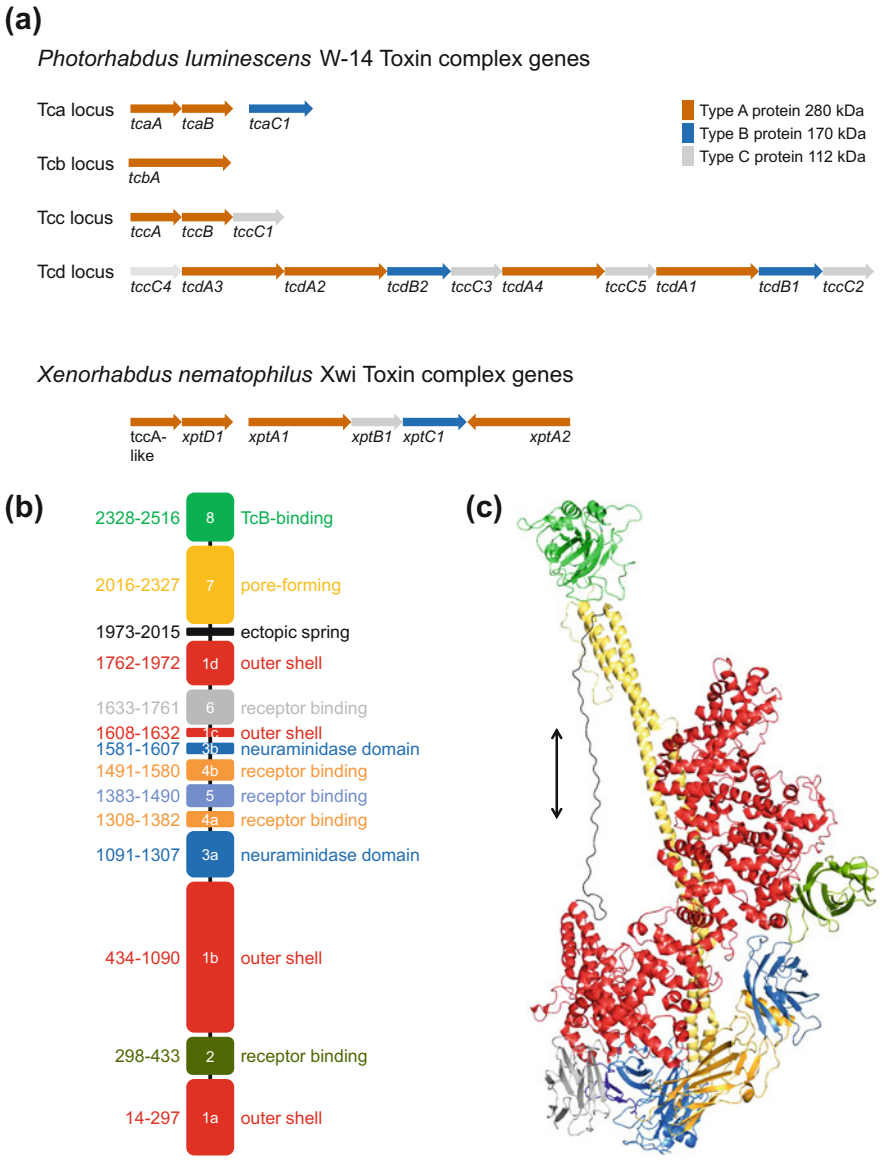


Fig. 55.1 Gene structures of Tc toxins and structure of the TcA-like toxin component TcdA1. (*A*). Gene structure for toxin complexes from *Photorhabdus luminescens* W-14, and *Xenorhabdus nematophilus* Xwi. Genes are colored according to the type of protein they encode (Type A, B, or C-like proteins). Note that tcaA + tcaB; tccA + tccB; and tccA-like + xptD1 are bifurcated type A proteins and presumably are expressed together to form a complete A protein. (*B*). Domain structure of the TcA-like toxin component TcdA1. (*C*). Crystal structure of TcdA1 [PDB 4o9y by PyMOL, modified from Meusch et al. 2014]. The double arrow shows the entropic spring module

protein contained by the toxin complex and that the additional B and C proteins function to potentiate the activity of the A protein (Sergeant et al. 2003; Waterfield et al. 2005).

Additional membrane interactions of the TcA-like protein from *Photorhabdus luminescens* demonstrated the ability of TcdA1 to form pores using $^{86}Rb^{+}$ release experiments on HT-29 cells (Lang et al. 2010). Similarly, TcdA1 forms ion-permeable channels in artificial lipid bilayer membranes with a single channel conductance of 125 pS in 150 mM KCl. The channels formed by TcdA1 were cation selective and the addition of B and C proteins (TcdB2-TccC3) to TcdA1 imbedded in the artificial membrane blocked the conductance of the channel by up to 75%. These results suggest that the binding site for the B-C proteins to the A protein is near the channel formed by the oligomeric A protein (Lang et al. 2013). Similar results were obtained with using the A protein XptA2 from *Xenorhabdus,* where adding XptA2 to one side of the black lipid membrane in the presence of 0.15 M KCl, increased the measured membrane current in a stepwise manner, indicative of formation of single channels with conductance of ~100 pS (Sheets et al. 2011). Meanwhile the interaction of the TcA-like protein TcdA1 with nanodiscs as a membrane model has been reported in near atomic detail (Gatsogiannis et al. 2016) (see below).

2.2.2 The Molecular and Atomic Structure of TcA-like Proteins

TcA-like proteins are large (~280 kDa) and associate into still larger sized oligomers (pentamers) (Figs. 55.1 and 55.2). In some cases, the gene encoding for a TcA protein appears to be bifurcated, presumably encoding the N-terminus and C-terminus portions of a full-sized class A protein by two separate open reading frames (Fig. 55.1a). The structure and organization of bifurcated TcA protein in *Photorhabdus* have not been fully characterized, but it is assumed that the expression of both the genes is required for them to associate into oligomers to produce a fully functional TcA protein. Examples of bifurcated TcA proteins are also known to exist in *Xenorhabdus nematophilus, Yersinia entomophaga,* and other bacteria-containing Tc genes (Morgan et al. 2001; Waterfield et al. 2001b; Hurst et al. 2011).

Recently, structures of a TcA protein from *Photorhabdus luminescens* (TcdA1) in both the pre-pore and pore state have been determined by cryoelectron microscopy (Gatsogiannis et al. 2016; Meusch et al. 2014; Gatsogiannis et al. 2013). TcdA1 forms a 1.41 MDa pentameric bell-shaped structure in the pre-pore state (Fig. 55.2). This structure is similar to the pentameric structure formed by the type A protein (YenA) from *Yersinia entomophaga* (Landsberg et al. 2011) The crystal structure of TcdA1 from *Photorhabdus luminescens* contains 8 different domains in each of the monomers forming the complete pentameric structure (Fig. 55.2). TcA toxin components consists of an inner pore-forming structure, which is built from domains 7 (amino acids 2016–2327) and 8 (amino acids 2328–2516) located at the C-terminus of TcA (Fig. 55.1b, c). Domain 7 consists of two ~200 Å long helices,

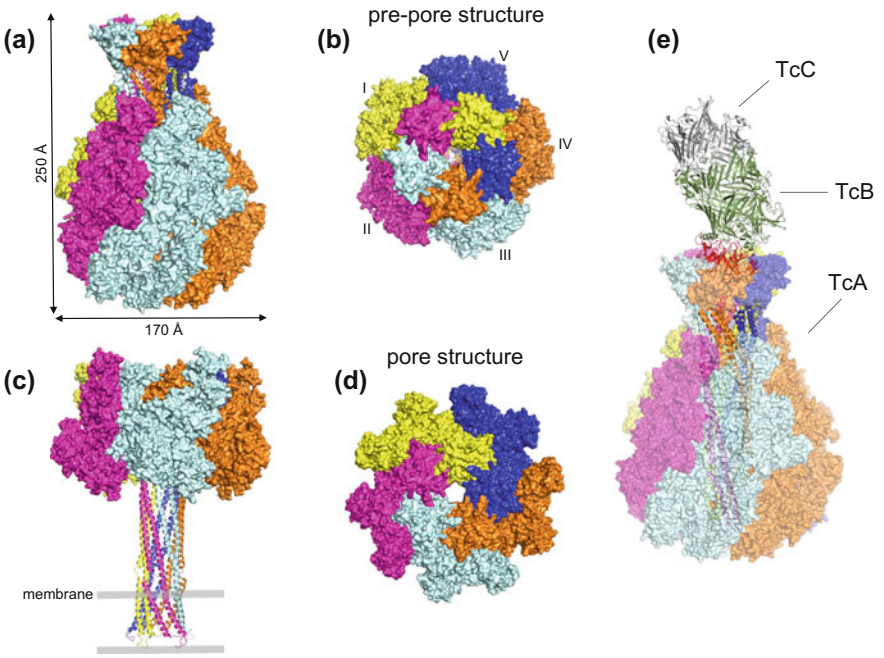


Fig. 55.2 Structures of Tc toxin oligomers as a pre-pore and pore complex. Molecular structure of TcdA1 [modified from Meusch et al. 2014 PDB 4o9y by PyMOL]. (*A*). Side view of pentameric TcdA1 in the pre-pore state. The different colors indicate each protomer. (*B*). Top view of TcdA1 pentamer in the pre-pore state, showing the interaction side with the TcB component. The various protomers are indicated by numbers and color. (*C*). Side view of the pentameric TcdA1 in the pore state, showing the interaction side with the TcB component. (*D*). Top view of the of the pentameric TcdA1 in the pore state. (*E*). Molecular structure of TcdB2-TccC3 on top of the pentameric TcdA1 complex [modified from Meusch et al. 2014]. TcdB2 and TccC3 form a large cage. The N-terminal region of TcdB2 has a β-propeller-like structure and interacts with the platform, which is formed by domain 8 of the TcdA1 pentamer. It is suggested that the ADP-ribosyltransferase domain, which is located in the C_{hvr} region of TccC3, is cleaved from the rest of the protein and lies in the cage in an unfolded conformation. Interaction with the TcA pentamer may open a gate for translocation of the unfolded ADP-ribosyltransferase into the TcA injection machine. TcdA1 is shown as above in a transparent view. (Pictures are modified from Meusch et al. (2014), PDB 409y and PDB4o9x by PyMOL)

one is descending and one is ascending) and both are connected by a loop region (residues 2140–2155). Together with the other eight helices from the other four protomers form the channel of the pore. The pore possesses a funnel-like region at the top, which is formed by domain 8 oligomers (Figs. 55.1 b, c and 55.2). This region builds the docking platform for the interaction with component TcB of the holotoxin (Fig. 55.2 b, d). The channel of TcA is encased by an outer shell, consisting of an all-helical domain (Fig. 55.1 b, c, regions 1a–1d), which has insertions for four putative receptor binding domains (domains 2, 4, 5 and 6 in Fig. 55.1 b, c) and one neuroaminidase-like domain (domain 3 in Fig. 55.1 b, c).

Like many other pore-forming toxins, the structure of TcdA1 can be described as a pre-pore and as a pore state. The pore state of TcdA1 (Fig. 55.2c, d) was obtained by reconstituting the complex into liposomes and nanodiscs, determining its structure by Cryo-EM and single-particle analysis. Transitioning from the pre-pore to pore state results in a large 12 nm movement of residues forming the central channel that is present in the interior of the toxin complex shielded by the outer surface of the toxin (Fig. 55.2). This corresponds to insertion of the α-helical pore into the membrane of the insect midgut, establishing a syringe-like mechanism that could be used to inject proteins into cells. The energy for the insertion event is entropic and is achieved through the action of a linker composed of 48 amino acid residues that connect the shell domains with the central channel-forming part of TcA (Fig. 55.1 b, c). This linker comprises an 'entropic spring' by changing from an extended to a compacted conformation thereby driving the central channel into the membrane. Based upon this structure, the TcA protein primarily functions as a syringe-like delivery system for the toxin complex (Meusch et al. 2014; Gatsogiannis et al. 2013, 2016). The interior lumen of the central channel is initially closed in the pre-pore state, but opens when in the pore state, allowing for injection of proteins through the channel into the insect gut (Gatsogiannis et al. 2016). Although the channel opens when in the pore state, it is still sufficiently narrow that it would require unfolding of the proteins to allow their passage through the pore and into a cell (Gatsogiannis et al. 2013). The structure shows how TcdA1 by itself can function in both a pore-forming role, and also as a protein toxin delivery system.

The identity of the receptors in the insect midgut that bind the toxin are not known, but the structure of the toxin complex in the pore state suggests that the receptor binding domains are about 125 Å from the surface of the membrane, requiring the receptors on the midgut cell surface to be both large and elongated to sufficiently interact with the receptor domains on the toxin complex (Meusch et al. 2014).

2.2.3 Is TcA per se Insecticidal?

Early insect bioassay studies suggested that the TcA protein (TcdA1) was the 'toxin' portion of the Tc, and that the addition of co-expressed TcB and TcC proteins function to 'potentiate' the activity of the TcA protein (Waterfield et al. 2005; Sergeant et al. 2003). Indeed, when the gene encoding TcdA1 from *Photorhabdus luminescens* was inserted into the *Arabidopsis thaliana* genome the resulting transgenic plants were toxic to tobacco hornworms (*Manduca sexta*), and inhibited the growth of southern corn rootworms (*Diabrotica undecimpunctata howardi*) (Liu et al. 2003). The ability of TcdA1 to bind and form pores in insect midgut membranes most likely accounted for the insecticidal activity of the TcdA1 expressed by the transgenic plants. Later studies showed that both the TcB and TcC proteins are needed for the toxin complex to exhibit its full insecticidal activity (Waterfield et al. 2005).

2.3 TcB-like Proteins of the Toxin Complex

TcB-like proteins are about 170 kDa in size and bind to both the TcA and TcC proteins to facilitate the formation of a complete Tc toxin. TcB proteins have not shown any toxicity against insects and little is known about their function as part of the toxin complex other than to connect the TcC protein to the TcA complex. They can be singly expressed as soluble proteins, but in practice are often co-expressed with the TcC protein or expressed as a fusion protein with the TcC protein attached to its C-terminus. The co-expression of both TcB and TcC proteins together facilitates the successful expression of TcC proteins, which are often difficult to express individually and typically form an insoluble full-length protein that poorly associates with the TcB protein. For this reason, it has been thought that the TcB protein may act as a chaperon for the TcC protein. Interestingly, genes encoding naturally fused TcB–TcC proteins have been found in some bacteria with toxin complex genes (Yang and Waterfield 2013). The crystal structure of two TcB–TcC fusion proteins (TcdB2–TccC3) from *Photorhabdus luminescens* has been solved at 2.35 Å resolution and shows TcdB2 having a large hollow cocoon-like structure (Meusch et al. 2014). The structure is highly similar to the structure of the TcB–TcC components of *Yersinia entomophaga* (Busby et al. 2013). The N-terminus portion of the TcB protein binds to the TcA protein and forms a β-propeller type structure that is positioned on top of the channel formed by the TcA protein (Meusch et al. 2014; Busby et al. 2013). The β-propeller is in an open conformation when the TcB–TcC protein is bound to the TcA protein allowing for a clear channel through the toxin complex (Meusch et al. 2014).

2.4 TcC-like Proteins of the Toxin Complex

The TcC-like proteins are about 112 kDa in size and are important for potent toxicity of the complex. As mentioned above, TcC proteins need to be co-expressed with the TcB protein in *E. coli* or other protein expression systems to properly associate with the TcA protein for production of a complete and biologically active toxin complex (Sergeant et al. 2003). For the toxin complexes from *Photorhabdus* and *Xenorhabdus* studied in detail so far, when the B and C proteins are co-expressed, or expressed as a B-C fusion product, the two proteins are isolated as a soluble binary complex where the C protein is cleaved into two parts as part of an apparent activation mechanism (Lang et al. 2010; Sheets et al. 2011). The structure of the C protein can be considered to be composed of two domains. The core domain comprises the predominant N-terminal section of the protein and contains *rearrangement hotspot* (RHS) repeats (Busby et al. 2013). These repeats are believed to form an aspartyl autoprotease in the C2 protein of *Y. entomophaga* and

in TccC3 of *P. luminescens* (Meusch et al. 2014; Busby et al. 2013). Thus, the TcC protein autoproteolyses when co-expressed with the TcB protein to form two separate proteins. The site of cleavage occurs approximately two-thirds down the protein toward the C-terminus at a highly conserved junction among different TcC-like proteins. Immediately after the site of cleavage, the amino acid sequences of different TcC proteins from various organisms radically diverge, and this domain referred to as the hypervariable region (hvr). Cleavage of the hvr from the TcC protein releases a protein fragment (TcC_{hvr}) of about 32 kDa that non-covalently associates with the TcB–TcC protein. As describe below, TcC_{hvr} contains enzymatic activity that defines the toxic activity of the toxin complex.

2.5 Structure of the Complete TC Complex

A complete toxin complex that is fully active against insects is composed of all three Tc proteins (TcA, TcB, and TcC) where the TcA protein oligomerizes into a pentamer in most cases, and binds a TcB protein which in turn is bound to a TcC protein. Although the C protein is cleaved in the complete toxin complex, the cleaved TcC_{hvr} must remain associated with the toxin complex for it to be active against insects. Mutant TcB–TcC fusion proteins having a deleted TcC_{hvr} still bind tightly to the TcA protein and form a complex, but the toxin complex lacks potent insecticidal activity (Busby et al. 2013). The crystal structure of TcdB2–TccC3 presents a hollow cocoon structure (Fig. 55.2e), similar to that described for Tc YenB–YenC2 proteins described at 2.35 Å resolution (Busby et al. 2013). The TcC_{hvr} protein is assumed to be encapsulated within the cocoon structure of the TcdB2–TccC3 complex in a non-covalent manner possibly unfolded due to the probable hostile environment composed of positive charges and hydrophobic patches in the interior of the B-C structure (Meusch et al. 2014).

Recently, an elegant body of X-ray crystallography and electron cryomicroscopy structural studies at 4 Å resolution has been reported for crystals of TcdA1 and of a complete *Photorhabdus* toxin complex (PTC3), having TccC3 as the type of C-protein in the complex. The structure was obtained through co-crystallization of purified A protein TcdA1 and the binary B-C proteins TcdB2-TccC3 expressed as a fused single gene product (Meusch et al. 2014). The binary TcdB2–TccC3 proteins bind tightly to the top of the TcdA1 pentamer at an approximate 45 ° angle to its longitudinal axis forming a distorted six-bladed β-propeller structure having pseudo fivefold symmetry (Fig. 55.2e). The β-propeller is in an open conformation when bound to the TcdA1 pentamer, providing a chamber for the C_{hvr} to pass through into the channel of the pentameric TcdA1, as evidenced by extra density found inside the pore of the toxin complex (Meusch et al. 2014). It is believed that C_{hvr} is in an unfolded state when inside the channel. The structure of the entire PTC3 complex has an analogous overall structure to the complete pentameric toxin

complex from *Yersinia entomophaga*, except that the *Yersinia* toxin complex (Yen-Tc) is reported to be associated with two different chitinase proteins (Chi1 and Chi2) bound to the outer surface of each YenA protein for a total of ten chitinases, along with two type C proteins (C1 and C2) associated with a single B protein (Landsberg et al. 2011). In addition, the A proteins of YenTc are encoded by two separate genes that essentially form a single TcdA-like protein. Although early structural studies indicated two C proteins were present for each B protein, later crystal structures of the BC proteins of YenTc showed them to form binary complexes very similar as seen for TccB2–TccC3 of *Photorhabdus* (Busby et al. 2013). Thus we suspect that the purified toxin complex protein reported (Landsberg et al. 2011) was most likely composed of two separate TCs, each sharing the same A and B proteins, but having different C proteins. Such a structure would conform to the A_5BC structure observed for PTC3 (Meusch et al. 2014).

The role of the chitinases in YenTc was originally thought to function to degrade the chitin-rich peritrophic membrane of the insect gut to facilitate entry of the toxin into the cells. Such a mechanism is not required of Tc's from *Photorhabdus* or *Xenorhabdus* since these Tc's do not contain chitinases yet are highly toxic toward insects. Chitinase genes have been found in close proximity with the toxin complex genes of *Xenorhabdus nematophilus*, but are not required for their biological activity (Morgan et al. 2001). Later characterization of the chitinases of YenTc showed optimum endochitinase activity at pH values of neutral to acidic, whereas the pH of the lumen of the gut of some insects that the toxin was active against was as high as 9, where these chitinases were found not to have any biological activity (Busby et al. 2012). The chitinases found on YenTc may instead have a role in toxicity after the complexes enter the cell and kills the insect. Certainly, making and characterizing YenTc mutants lacking the chitinase proteins would bring clarity to the role of these proteins in the toxicity mechanism.

The genomes of *Photorhabdus luminescens* contains multiple different genes encoding TcA–, TcB–, and TcC–like proteins, which allows for the formation of different toxin complexes depending upon which A, B, and C protein combinations are contained within the complex (Waterfield et al. 2005). Different TcB and TcC proteins can be combined with different TcA proteins to form a wide variety of different Tc's which can express different toxicity mechanisms and attack different hosts. Hybrid toxins can potentially be made that have unique insect specificity depending on the type of TcA, TcB, and TcC proteins contained in the toxin complex. Although a complete comparison has not been made, it has been shown that different pairs of TcB and TcC proteins can be co-expressed and they bind to the various TcA-like proteins to form mixtures of different toxin complexes. Indeed, TcB and TcC proteins from *Photorhabdus luminescens* (TccB2 and TccC3) have been co-expressed and added to a TcA protein from *Xenorhabdus nematophilus* (XptA2) to form a hybrid toxin complex from two different organisms. This toxin complex expresses the insect selectivity determined by XptA2, but the molecular toxicity is a result of the actions of the hypervariable region of TccC3 (Sheets et al. 2011).

3 Biological Activities of Tc Toxins

Early studies indicated that the maximal insect-killing activity of Tc toxins depends on the combination of all three Tc components, including TcA, TcB, and TcC (Waterfield et al. 2001a). The tripartite *Photorhabdus luminescens* Tc complexes were studied in hemocytes obtained from the hemocoel of *Galleria mellonella* larvae (Lang et al. 2010). These studies revealed inhibition of phagocytosis by the toxin complexes consisting of TcdA1, TcdB2, and TccC3 (PTC3) or TcdA1, TcdB2 and TccC5 (PTC5), while the individual proteins were without effects. It turned out that inhibition of phagocytosis was caused by effects of the toxin on the actin cytoskeleton. However, studies with hemocytes and also with mammalian cells showed that PTC3 and PTC5 each affect the actin cytoskeleton in a different manner. While PTC3 induces aggregation of F actin, PTC5 causes formation of stress fibers. The combination of both toxins results in strong clustering of F-actin accumulations with loss of the normal cell morphology. The underlying molecular mechanisms are the ADP-ribosyltransferase activities of the TccC3 and TccC5 components (Lang et al. 2010). However, the toxin targets and the functional consequences of toxin catalyzed ADP-ribosylation are different for TccC3 and TccC5 toxin components.

3.1 TccC3 Is an ADP-Ribosyltransferase that Targets Actin

In spite of a low overall sequence homology between most bacterial ADP-ribosylating toxins, their molecular structures are very similar (Hottiger et al. 2010; Simon et al. 2014; Fieldhouse and Merrill 2008; Vogelsgesang et al. 2007; Pinto and Schuler 2015). Moreover, essential amino acid residues critical for catalysis of the ADP-ribosyltransferase reaction are frequently conserved. Typical for a large group of bacterial ADP-ribosyltransferases is the so-called "RSE" motif, which represents three amino acids involved in NAD-binding (residues "R" and "S") and in catalysis (catalytic glutamate "E") (Hottiger et al. 2010). TccC3, which harbors the ADP-ribosyltransferase activity in its C_{hvr}, belongs to this family of RSE enzymes (Pfaumann et al. 2015). Mutational analyses revealed that Arg791 and Glu943 are the pivotal residues of the typical RSE motif. The crucial serine residue has not been determined with absolute certainty, however serine866 or serine871 are good candidates (Pfaumann et al. 2015).

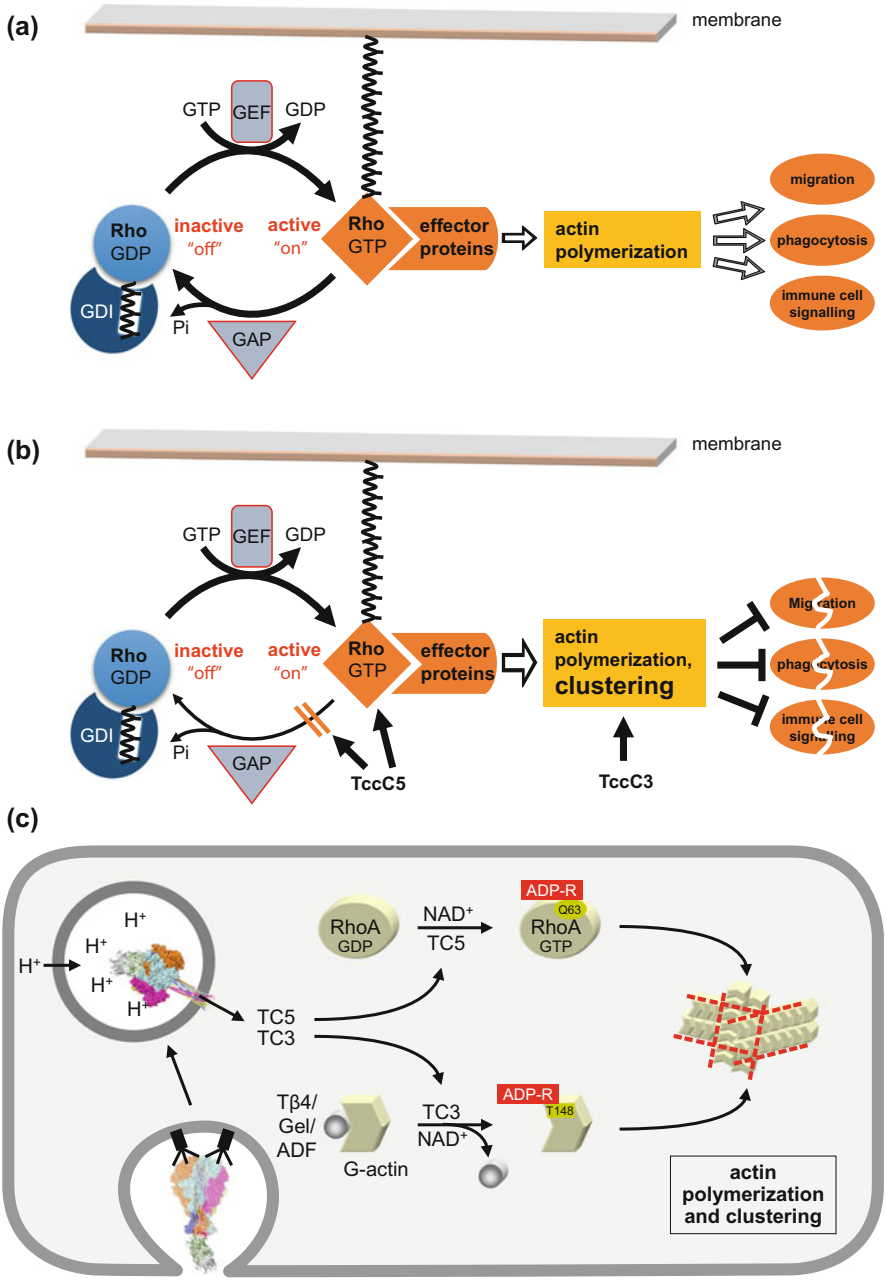
3.1.1 Modification of Actin by TccC3 at Threonine148

TccC3 mono-ADP-ribosylates actin at threonine148 (Lang et al. 2010). Modification of actin at this site has no effect on the native state of actin, thus ATP-binding is preserved and modified actin still inhibits DNAse I (Lang et al. 2016).

Moreover, the critical concentration for actin polymerization is not changed. However, threonine148 is located at the site where actin interacts with thymosin-β4 (Lang et al. 2010). The actin-binding protein thymosin-β4 (~5 kDa) belongs to a group of rather small peptides, consisting of 42–45 residues, which sequester G-actin and inhibits salt-induced actin polymerization (Mannherz and Hannappel 2009). ADP-ribosylation of actin at T148 blocks the interaction of thymosin-β4 with actin and prevents its inhibition of actin polymerization (Lang et al. 2010). Other actin-binding proteins that interact with actin involve threonine148 (Lang et al. 2016). For example, the interaction of gelsolin and of ADF/cofilin with actin ADP-ribosylated at threonine148 is impaired. Under physiological conditions, gelsolin and ADF/cofilin induce fragmentation of F-actin and thereby control the dynamics of actin treadmilling (Pollard and Cooper 2009; Dominguez and Holmes 2011). By contrast, the interaction of actin ADP-ribosylated at threonine148 with profilin is not affected. Profilin supports plus-end polymerization of actin and enhances formin-induced actin elongation. Thus, ADP-ribosylation of actin at threonine148 favors actin polymerization while actin fragmentation and severing is inhibited. Moreover, ADP-ribosylation at threonine148 appears to affect actin–actin interaction in favor of F-actin bundling and aggregation, eventually, resulting in destruction of the normal cell morphology and in cell death (Lang et al. 2010; Lang et al. 2016) (Fig. 55.3b).

3.1.2 *Photorhabdus* Toxin Photox Modifies Actin at Arginine177

Various bacterial toxins modify actin by ADP-ribosylation (Aktories et al. 2011) including the binary toxins *Clostridium botulinum* C2 toxin (Aktories et al. 1986), *C. perfringens* iota toxin (Schering et al. 1988), *C. difficile* transferase CDT (Gülke et al. 2001; Schwan et al. 2009) and *Bacillus cereus* vegetative insecticidal proteins (VIP) (Han et al. 1999). Moreover, various bacterial effectors like *Salmonella enterica* SpvB (Tezcan-Merdol et al. 2001) and *Aeromonas salmonicida* AexT (Vilches et al. 2008), which probably enter target cells by type III secretion, ADP-ribosylate actin. However, these toxins do not modify threonine148 but instead ADP-ribosylate actin at arginine177 (Vandekerckhove et al. 1987, 1988; Hochmann et al. 2006). This modification of actin at arginine177 inhibits the formation of actin filaments by steric hindrance and therefore blocks actin polymerization (Aktories et al. 1986; Aktories and Wegner 1989, 1992). Moreover, it causes F-actin depolymerization by an actin-capping effect at the plus-ends of actin filaments (Wegner and Aktories 1988). *Photorhabdus luminescens* produces a potential toxin called Photox, which possesses ADP-ribosyltransferase activity and modifies actin at arginine-177 (Visschedyk et al. 2010). Although the translocation mechanism of Photox into target cells is not known, it is remarkable that *Photorhabdus luminescens* produces toxins that cause polymerization of actin (e.g., TccC5-containing Tc complexes) and also release toxins (e.g., Photox) that induce depolymerization of actin filaments and of the actin cytoskeleton. Eventually, both toxin effects are detrimental for targeted cells. However, a precise spatial—and


(a)
membrane
GTP
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inactive
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Rho
GTP
effector
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actin
polymerization
migration
phagocytosis
immune cell
signalling
GDI
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(b)
membrane
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Rho
GTP
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GDI
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GAP
TccC5
TccC3
(c)
H+
RhoA
GDP
NAD+
TC5
ADP-R
Q63
RhoA
GTP
TC5
TC3
Tβ4/
Gel/
ADF
G-actin
TC3
NAD+
ADP-R
T148
actin
polymerization
and clustering

◂**Fig. 55.3** Mode of action of Tc toxins. (*A*) TccC5 activates Rho proteins by ADP-ribosylation at glutamine63. **a** Rho proteins are regulated by a GTPase cycle, are activated by GEFs and inactivated by GTP hydrolysis, which is facilitated by GAPs (GTPase activating proteins). GDIs (guanine nucleotide dissociation inhibitors) keep Rho proteins in the cytosol. GTP-bound Rho activates multiple effectors. A major effect of active Rho is the organization of the actin cytoskeleton involved in phagocytosis, migration and immune cell signaling. **b** TccC5 ADP-ribosylates RhoA in glutamine63, thereby GTP hydrolysis is blocked and Rho is persistently active, resulting in enhanced actin polymerization and deregulation of actin functions. In addition, TccC3 ADP-ribosylates actin causing actin clustering (see below). (*B*). Model for the action of *Photorhabdus* Toxin complex. The toxin complex proteins come together to form a complete toxin complex which binds to membrane bound receptors on midgut cells and is endocytosed into the cell. At low pH in the endosome, the Tc forms a pore that releases, through a syringe-like mechanism, the C_{hvr} of the TcC protein into the cytosol. The C_{hvr} of TccC3 ADP-ribosylates actin at threonine-148, which inhibits actin interaction with actin-binding proteins like thymosin β-4 (Tβ-4), gelsolin (Gel) or ADF and induces actin polymerization and clustering. The C_{hvr} of TccC5 ADP-ribosylates Rho GTPases resulting in its persistent activation leading to actin polymerization. Both toxins largely redistribute the actin cytoskeleton and cause actin clustering leading to toxicity

time-dependent control of the toxins' actions may provide an advantage for *Photorhabdus luminescens* and its specific life cycle.

3.2 *TccC5 Is an ADP-Ribosyltransferase that Targets Rho Proteins*

TccC5 also harbors ADP-ribosyltransferase activity in its C_{hvr}. The RSE motif for TccC5 ADP-ribosyltransferase activity comprises arginine774, serine809 and glutamate886. Mutations of these residues result in inhibition of the ADP-ribosyltransferase activity (Pfaumann et al. 2015). Biochemical studies and mass spectrometric analysis showed that Rho proteins are the substrates of TccC5 (Lang et al. 2010).

3.2.1 Rho Proteins Are Substrates of TccC5

Rho proteins belong to the superfamily of Ras proteins and are regulated by a GTPase cycle (Cherfils and Zeghouf 2013; Jaffe and Hall 2005; Heasman and Ridley 2008) (Fig. 55.3a). They are active in the GTP-bound form and inactive after GTP hydrolysis. GTP-binding is achieved by guanine nucleotide exchange factors (GEFs) (Garcia-Mata and Burridge 2007), which induce the release of GDP bound to Rho proteins. Because the concentration of GTP in the cytosol is higher than the concentration of GDP, release of GDP results in subsequent binding of GTP. The activate state of Rho proteins is turned off by hydrolysis of bound GTP, a process which is facilitated by GTPase activating proteins (GAPs) (Scheffzek et al. 1998). In the GTP-bound form, Rho proteins interact with numerous effectors including various protein kinases, adaptor, and regulatory proteins (Thumkeo et al.

2013). A prominent role of Rho proteins is the regulation of the actin cytoskeleton and of actin-dependent processes. Thus, Rho proteins regulate multiple cellular motile functions like cell migration, phagocytosis, vesicle traffic and cytokinesis. However, Rho proteins are also involved in transcriptional control and cell cycle regulation (Hanna and El-Sibai 2013). Moreover, Rho proteins are preferred substrates of bacterial protein toxins and effectors (Aktories 2011; Lemichez and Aktories 2013).

3.2.2 Functional Consequences of Rho ADP-Ribosylation by TccC5

Preferred in vitro substrates of TccC5 are RhoA, RhoB and RhoC, Rac1, 2 and 3, Cdc42 and Tc10 (Pfaumann et al. 2015; Lang et al. 2010). Modification occurs at glutamine63 of RhoA and glutamine61 of Rac and Cdc42. This residue is located in the switch II region of the small GTPases and is essential for GTP hydrolysis (Cherfils and Zeghouf 2013). Thus, toxin-induced ADP-ribosylation at this position inhibits GTP hydrolysis by the small GTPase and turns the GTP-binding protein into a persistently active protein (Lang et al. 2010) (Fig. 55.3a). The consequence of toxin-caused activation of RhoA is the strong formation of stressfibres, which is a typical cell culture response following RhoA activation. Thus, in cell culture, the RhoA effect appears to be dominant. However, when both active TcC components (e.g., TccC3 and TccC5) were added to cell cultures as part of the complete Tc complexes, stressfibers are no longer visible but gross actin clustering is observed, indicating that modification of threonine148 by TccC3 is dominant (Lang et al. 2010). Activation of Rho and Rac proteins by TccC5 was studied in comparison with the cytotoxic necrotizing factor CNF1 of *Escherichia coli*, which activates Rho proteins by deamidation of glutamine63 (Schmidt et al. 1997). Surprisingly, activation of RhoA by deamidation is transient and returns to normal Rho activation levels after 18 h, while TccC5 causes RhoA activation for more than 18 h (Pfaumann et al. 2015). Similar results are observed with Rac proteins. Activation of Rac by CNF1 is transient with subsequent degradation of Rac, while TccC5 causes persistent activation over 18 h without degradation of Rac.

In congruence with TccC3, which directly ADP-ribosylates actin at threonine148, ADP-ribosylation of RhoA by TccC5 favors actin polymerization (Lang et al. 2010; Pfaumann et al. 2015). Accordingly, both toxins share a similar effect on gene transcription regulation by myocardin-related transcription factor A (termed MAL or MRTF). MAL is an essential coactivator of the transcription factor *serum responsive factor* (SRF) (Medjkane et al. 2009; Posern and Treisman 2006). The transcriptional regulator Mal interacts with G-actin thereby inhibiting its transcriptional activity. Polymerization of actin releases MAL from G actin and supports MAL-mediated transcriptional activation resulting in increased expression of multiple proteins involved in organization of the cytoskeleton, adhesion and in motile functions including actin itself (Medjkane et al. 2009; Posern and Treisman 2006). Studies showed that TccC3 as well as TccC5 strongly induce MAL

activation (Pfaumann et al. 2015). Thus, manipulation of MAL-dependent function including signaling, migration and adhesion of *Photorhabdus* toxins target cells may play an important role in host–pathogen interaction in larvae.

4 Conclusions

Recent studies have shed light on the structure and function of the huge and highly complex toxin complex toxins from *Photorhabdus luminescens*. These tripartite toxins are a paradigm of a novel toxin delivery system using a syringe-like mechanism to transport an enzymatic toxin into a cell. Because Tc toxins are found in various species from different bacterial genera, these recent findings will help us better understand toxin-dependent host–pathogen interactions caused by these proteins. There are still many outstanding questions concerning the binding of the TcA component of the toxin complex to target cells, the delivery into, and the action of the biological active components (TcC) in host cells. For example, identification of the toxin receptor (or receptors) would be of major importance. Moreover, numerous TcC components have been identified in the genome of *Photorhabdus*, and related bacteria, which share high sequence similarity in their N-terminus but largely differ at the C-terminus, where the biological activity is located. Sequence analyses clearly show that these hypervariable region most likely harbor different biological activities. Therefore, it would be exciting to analyze these activities in detail. Insecticidal bacterial protein toxin play a still increasing role in pest control and parasite management. The various toxins of *Photorhabdus* species including the Tc toxins may increase our armamentarium against various types of insect pests. One prerequisite for the efforts to obtain novel, even more effective insecticidal agents against insect pests is the detailed understanding of the mode of action of these toxins.

References

Aktories K (2011) Bacterial protein toxins that modify host regulatory GTPases. Nat Rev Microbiol 9(7):487-498. doi: 10.1038/nrmicro2592, [pii] nrmicro2592

Aktories K, Bärmann M, Ohishi I, Tsuyama S, Jakobs KH, Habermann E (1986) Botulinum C2 toxin ADP-ribosylates actin. Nature 322:390–392

Aktories K, Lang AE, Schwan C, Mannherz HG (2011) Actin as target for modification by bacterial protein toxins. FEBS J 278(23):4526–4543. doi:10.1111/j.1742-4658.2011.08113.x

Aktories K, Wegner A (1989) ADP-ribosylation of actin by clostridial toxins. J Cell Biol 109:1385–1387

Aktories K, Wegner A (1992) Mechanisms of the cytopathic action of actin-ADP-ribosylating toxins. Mol Microbiol 6:2905–2908

Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R (1998) Insecticidal toxins from the bacterium *Photorhabdus luminescens*. Science 280(5372):2129–2132

Bowen DJ, Ensign JC (1998) Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium photorhabdus luminescens. Appl Environ Microbiol 64(8):3029–3035

Buell CR, Joardar V, Lindeberg M, Selengut J, Paulsen IT, Gwinn ML, Dodson RJ, Deboy RT, Durkin AS, Kolonay JF, Madupu R, Daugherty S, Brinkac L, Beanan MJ, Haft DH, Nelson WC, Davidsen T, Zafar N, Zhou L, Liu J, Yuan Q, Khouri H, Fedorova N, Tran B, Russell D, Berry K, Utterback T, Van Aken SE, Feldblyum TV, D'Ascenzo M, Deng WL, Ramos AR, Alfano JR, Cartinhour S, Chatterjee AK, Delaney TP, Lazarowitz SG, Martin GB, Schneider DJ, Tang X, Bender CL, White O, Fraser CM, Collmer A (2003) The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas* syringae pv. tomato DC3000. Proc Natl Acad Sci USA 100(18):10181–10186. doi:10.1073/pnas.1731982100

Busby JN, Landsberg MJ, Simpson RM, Jones SA, Hankamer B, Hurst MR, Lott JS (2012) Structural analysis of Chi1 Chitinase from Yen-Tc: the multisubunit insecticidal ABC toxin complex of *Yersinia entomophaga*. J Mol Biol 415(2):359–371. doi:10.1016/j.jmb.2011.11.018

Busby JN, Panjikar S, Landsberg MJ, Hurst MR, Lott JS (2013) The BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device. Nature 501(7468):547–550. doi: 10.1038/nature12465, [pii] nature12465

Cherfils J, Zeghouf M (2013) Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiol Rev 93(1):269–309. doi: 10.1152/physrev.00003.2012, [pii] 93/1/269

Daborn PJ, Waterfield N, Blight MA, Ffrench-Constant RH (2001) Measuring virulence factor expression by the pathogenic bacterium *Photorhabdus luminescens* in culture and during insect infection. J Bacteriol 183(20):5834–5839. doi:10.1128/JB.183.20.5834-5839.2001

Dominguez R, Holmes KC (2011) Actin structure and function. Annu Rev Biophys 40:169–186. doi:10.1146/annurev-biophys-042910-155359 [doi]

ffrench-Constant R, Waterfield N (2006) An ABC guide to the bacterial toxin complexes. Adv Appl Microbiol 58:169–183

ffrench-Constant RH, Bowen DJ (2000) Novel insecticidal toxins from nematode-symbiotic bacteria. Cell Mol Life Sci 57(5):828–833

ffrench-Constant RH, Dowling A, Waterfield NR (2007) Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon 49 (4):436–451

Fieldhouse RJ, Merrill AR (2008) Needle in the haystack: structure-based toxin discovery. Trends Biochem Sci 33(11):546–556. doi: 10.1016/j.tibs.2008.08.003, [pii] S0968-0004(08)00188-6

Forst S, Dowds B, Boemare N, Stackebrandt E (1997) *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annu Rev Microbiol 51:47–72

Forst S, Nealson K (1996) Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Microbiol Rev 60(1):21–43

Garcia-Mata R, Burridge K (2007) Catching a GEF by its tail. Trends Cell Biol 17(1):36–43. doi: 10.1016/j.tcb.2006.11.004, [pii] S0962-8924(06)00326-6

Gatsogiannis C, Lang AE, Meusch D, Pfaumann V, Hofnagel O, Benz R, Aktories K, Raunser S (2013) A syringe-like injection mechanism in *Photorhabdus luminescens* toxins. Nature 495 (7442):520–523. doi: 10.1038/nature11987, [pii] nature11987

Gatsogiannis C, Merino F, Prumbaum D, Roderer D, Leidreiter F, Meusch D, Raunser S (2016) Membrane insertion of a Tc toxin in near-atomic detail. Nat Struct Mol Biol. doi:10.1038/nsmb.3281

Gülke I, Pfeifer G, Liese J, Fritz M, Hofmann F, Aktories K, Barth H (2001) Characterization of the enzymatic component of the ADP-ribosyltransferase toxin CDTa from *Clostridium difficile*. Infect Immun 69(10):6004–6011

Han S, Craig JA, Putnam CD, Carozzi NB, Tainer JA (1999) Evolution and mechanism from structures of an ADP-ribosylating toxin and NAD complex. Nature Struct Biol 6:932–936

Hanna S, El-Sibai M (2013) Signaling networks of Rho GTPases in cell motility. Cell Signal 25 (10):1955–1961. doi: 10.1016/j.cellsig.2013.04.009, [pii] S0898-6568(13)00124-1

Heasman SJ, Ridley AJ (2008) Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol 9(9):690–701. doi: 10.1038/nrm2476, [pii] nrm2476

Hochmann H, Pust S, von FG, Aktories K, Barth H (2006) *Salmonella enterica* SpvB ADP-ribosylates actin at position arginine-177-characterization of the catalytic domain within the SpvB protein and a comparison to binary clostridial actin-ADP-ribosylating toxins. Biochemistry 45(4):1271–1277

Hottiger MO, Hassa PO, Luscher B, Schuler H, Koch-Nolte F (2010) Toward a unified nomenclature for mammalian ADP-ribosyltransferases. Trends Biochem Sci 35(4):208–219. doi: 10.1016/j.tibs.2009.12.003, [pii] S0968-0004(09)00242-4

Hurst MR, Glare TR, Jackson TA, Ronson CW (2000) Plasmid-located pathogenicity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photorhabdus luminescens*. J Bacteriol 182(18):5127–5138

Hurst MR, Jones SA, Binglin T, Harper LA, Jackson TA, Glare TR (2011) The main virulence determinant of *Yersinia entomophaga* MH96 is a broad-host-range toxin complex active against insects. J Bacteriol 193(8):1966–1980. doi:10.1128/JB.01044-10

Jaffe AB, Hall A (2005) Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol 21:247–269

Lacey LA, Chauvin RL (1999) Entomopathogenic nematodes for control of diapausing codling moth (Lepidoptera: Tortricidae) in fruit bins. J Econ Entomol 92(1):104–109

Lacey LA, Georgis R (2012) Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J Nematol 44(2):218–225

Landsberg MJ, Jones SA, Rothnagel R, Busby JN, Marshall SD, Simpson RM, Lott JS, Hankamer B, Hurst MR (2011) 3D structure of the *Yersinia entomophaga* toxin complex and implications for insecticidal activity. Proc Natl Acad Sci USA 108(51):20544–20549. doi: 10.1073/pnas.1111155108, [pii] 1111155108

Lang AE, Konukiewitz J, Aktories K, Benz R (2013) TcdA1 of *Photorhabdus luminescens*: Electrophysiological Analysis of Pore Formation and Effector Binding. Biophys J 105(2):376–384. doi: 10.1016/j.bpj.2013.06.003, [pii] S0006-3495(13)00675-9

Lang AE, Qu Z, Schwan C, Silvan U, Unger A, Schoenenberger CA, Aktories K, Mannherz HG (2016) Actin ADP-ribosylation at Threonine148 by *Photorhabdus luminescens* toxin TccC3 induces aggregation of intracellular F-actin. Cell Microbiol. doi:10.1111/cmi.12636

Lang AE, Schmidt G, Schlosser A, Hey TD, Larrinua IM, Sheets JJ, Mannherz HG, Aktories K (2010) *Photorhabdus luminescens* toxins ADP-ribosylate actin and RhoA to force actin clustering. Science 327(5969):1139–1142. doi: 10.1126/science.1184557, [pii] 327/5969/1139

Lee SC, Stoilova-McPhie S, Baxter L, Fulop V, Henderson J, Rodger A, Roper DI, Scott DJ, Smith CJ, Morgan JA (2007) Structural characterisation of the insecticidal toxin XptA1, reveals a 1.15 MDa tetramer with a cage-like structure. J Mol Biol 366(5):1558–1568. doi: 10.1016/j.jmb.2006.12.057, [pii] S0022-2836(06)01747-5

Lemichez E, Aktories K (2013) Hijacking of Rho GTPases during bacterial infection. Exp Cell Res 319(15):2329–2336

Liu D, Burton S, Glancy T, Li ZS, Hampton R, Meade T, Merlo DJ (2003) Insect resistance conferred by 283-kDa *Photorhabdus luminescens* protein TcdA in *Arabidopsis thaliana*. Nat Biotechnol 21(10):1222–1228

Mannherz HG, Hannappel E (2009) The beta-thymosins: intracellular and extracellular activities of a versatile actin binding protein family. Cell Motil Cytoskeleton 66(10):839–851. doi:10.1002/cm.20371[doi]

Medjkane S, Perez-Sanchez C, Gaggioli C, Sahai E, Treisman R (2009) Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis. Nat Cell Biol 11(3):257–268. doi: 10.1038/ncb1833, [pii] ncb1833

Meusch D, Gatsogiannis C, Efremov RG, Lang AE, Hofnagel O, Vetter IR, Aktories K, Raunser S (2014) Mechanism of Tc toxin action revealed in molecular detail. Nature 508(7494):61–65. doi: 10.1038/nature13015, [pii] nature13015

Morgan JA, Sergeant M, Ellis D, Ousley M, Jarrett P (2001) Sequence analysis of insecticidal genes from *Xenorhabdus nematophilus* PMFI296. Appl Environ Microbiol 67(5):2062–2069. doi:10.1128/AEM.67.5.2062-2069.2001

Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebaihia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG (2001) Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature 413(6858):848–852

Pfaumann V, Lang AE, Schwan C, Schmidt G, Aktories K (2015) The actin and Rho-modifying toxins PTC3 and PTC5 of *Photorhabdus luminescens*: enzyme characterization and induction of MAL/SRF-dependent transcription. Cell Microbiol 17(4):579–594. doi:10.1111/cmi.12386 [doi]

Pinto AF, Schuler H (2015) Comparative structural analysis of the putative mono-ADP-ribosyltransferases of the ARTD/PARP family. Curr Top Microbiol Immunol 384:153–166. doi:10.1007/82_2014_417

Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. Science 326 (5957):1208–1212. doi: 10.1126/science.1175862, [pii] 326/5957/1208

Posern G, Treisman R (2006) Actin' together: serum response factor, its cofactors and the link to signal transduction. Trends Cell Biol 16 (11):588–596. doi: 10.1016/j.tcb.2006.09.008, [pii] S0962-8924(06)00269-8

Scheffzek K, Ahmadian MR, Wittinghofer A (1998) GTPase-activating proteins: helping hands to complement an active site. Trends Biochem Sci 23(7):257–262. [pii] S0968-0004(98)01224-9

Schering B, Bärmann M, Chhatwal GS, Geipel U, Aktories K (1988) ADP-ribosylation of skeletal muscle and non- muscle actin by *Clostridium perfringens* iota toxin. Eur J Biochem 171:225–229

Schmidt G, Sehr P, Wilm M, Selzer J, Mann M, Aktories K (1997) Gln63 of Rho is deamidated by *Escherichia coli* cytotoxic necrotizing factor 1. Nature 387:725–729

Schwan C, Stecher B, Tzivelekidis T, van HM, Rohde M, Hardt WD, Wehland J, Aktories K (2009) *Clostridium difficile* toxin CDT induces formation of microtubule-based protrusions and increases adherence of bacteria. PLoS Pathog 5(10):e1000626. doi:10.1371/journal.ppat.1000626 [doi]

Sergeant M, Baxter L, Jarrett P, Shaw E, Ousley M, Winstanley C, Morgan JA (2006) Identification, typing, and insecticidal activity of *Xenorhabdus* isolates from entomopathogenic nematodes in United Kingdom soil and characterization of the xpt toxin loci. Appl Environ Microbiol 72(9):5895–5907. doi:10.1128/AEM.00217-06

Sergeant M, Jarrett P, Ousley M, Morgan JA (2003) Interactions of insecticidal toxin gene products from *Xenorhabdus nematophilus* PMFI296. Appl Environ Microbiol 69(6):3344–3349

Sheets JJ, Hey TD, Fencil KJ, Burton SL, Ni W, Lang AE, Benz R, Aktories K (2011) Insecticidal toxin complex proteins from *Xenorhabdus nematophilus*: structure and pore formation. J Biol Chem 286(26):22742–22749. doi: 10.1074/jbc.M111.227009, [pii] M111.227009

Silva CP, Waterfield NR, Daborn PJ, Dean P, Chilver T, Au CP, Sharma S, Potter U, Reynolds SE (2002) Bacterial infection of a model insect: *Photorhabdus luminescens* and *Manduca sexta*. Cell Microbiol 4(6):329–339. doi:194 [pii]

Simon NC, Aktories K, Barbieri JT (2014) Novel bacterial ADP-ribosylating toxins: structure and function. Nat Rev Microbiol 12(9):599–611. doi: 10.1038/nrmicro3310, [pii] nrmicro3310

Tezcan-Merdol D, Nyman T, Lindberg U, Haag F, Koch-Nolte F, Rhen M (2001) Actin is ADP-ribosylated by the *Salmonella enterica* virulence-associated protein SpvB. Mol Microbio 39:606–619

Thumkeo D, Watanabe S, Narumiya S (2013) Physiological roles of Rho and Rho effectors in mammals. Eur J Cell Biol 92(10–11):303–315. doi: 10.1016/j.ejcb.2013.09.002, [pii] S0171-9335(13)00058-7

Vandekerckhove J, Schering B, Bärmann M, Aktories K (1987) *Clostridium perfringens* iota toxin ADP-ribosylates skeletal muscle actin in Arg-177. FEBS Lett 225:48–52

Vandekerckhove J, Schering B, Bärmann M, Aktories K (1988) Botulinum C2 toxin ADP-ribosylates cytoplasmic β/g-actin in arginine 177. J Biol Chem 263:696–700

Vilches S, Wilhelms M, Yu HB, Leung KY, Tomas JM, Merino S (2008) *Aeromonas hydrophila* AH-3 AexT is an ADP-ribosylating toxin secreted through the type III secretion system. Microb Pathog 44(1):1–12. doi: 10.1016/j.micpath.2007.06.004, [pii] S0882-4010(07)00084-8

Visschedyk DD, Perieteanu AA, Turgeon ZJ, Fieldhouse RJ, Dawson JF, Merrill AR (2010) Photox, a novel actin-targeting mono-ADP-ribosyltransferase from *Photorhabdus luminescens*. J Biol Chem 285(18):13525–13534. doi: 10.1074/jbc.M109.077339, [pii] M109.077339

Vogelsgesang M, Pautsch A, Aktories K (2007) C3 exoenzymes, novel insights into structure and action of Rho-ADP-ribosylating toxins. Naunyn Schmiedebergs Arch Pharmacol 374(5–6):347–360

Waterfield N, Dowling A, Sharma S, Daborn PJ, Potter U, ffrench-Constant RH (2001a) Oral toxicity of *Photorhabdus luminescens* W14 toxin complexes in Escherichia coli. Appl Environ Microbiol 67(11):5017–5024

Waterfield N, Hares M, Yang G, Dowling A, ffrench-Constant R (2005) Potentiation and cellular phenotypes of the insecticidal Toxin complexes of *Photorhabdus* bacteria. Cell Microbiol 7 (3):373–382

Waterfield NR, Bowen DJ, Fetherston JD, Perry RD, ffrench-Constant RH (2001b) The tc genes of *Photorhabdus*: a growing family. Trends Microbiol 9(4):185–191

Wegner A, Aktories K (1988) ADP-ribosylated actin caps the barbed ends of actin filaments. J Biol Chem 263:13739–13742

Wilkinson P, Waterfield NR, Crossman L, Corton C, Sanchez-Contreras M, Vlisidou I, Barron A, Bignell A, Clark L, Ormond D, Mayho M, Bason N, Smith F, Simmonds M, Churcher C, Harris D, Thompson NR, Quail M, Parkhill J, ffrench-Constant RH (2009) Comparative genomics of the emerging human pathogen *Photorhabdus asymbiotica* with the insect pathogen *Photorhabdus luminescens*. BMC Genomics 10:302. doi: 10.1186/1471-2164-10-302, [pii] 1471-2164-10-302

Yang G, Waterfield NR (2013) The role of TcdB and TccC subunits in secretion of the *Photorhabdus* Tcd toxin complex. PLoS Pathog 9(10):e1003644. doi:10.1371/journal.ppat.1003644