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Rho-modifying bacterial protein toxins from *Photorhabdus* species

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ABSTRACT

Photorhabdus bacteria live in symbiosis with entomopathogenic nematodes. The nematodes invade insect larvae, where they release the bacteria, which then produce toxins to kill the insects. Recently, the molecular mechanisms of some toxins from *Photorhabdus luminescens* and *asymbiotica* have been elucidated, showing that GTP-binding proteins of the Rho family are targets. The tripartite Tc toxin PTC5 from *P. luminescens* activates Rho proteins by ADP-ribosylation of a glutamine residue, which is involved in GTP hydrolysis, while PaTox from *Photorhabdus asymbiotica* inhibits the activity of GTPases by N-acetyl-glucosaminylation at tyrosine residues and activates Rho proteins indirectly by deamidation of heterotrimeric G proteins.

1. Introduction

Many bacterial protein toxins, which act on mammalian organisms, affect target cells by modification of proteins of the Rho family. Rho proteins, which include ~20 proteins (best known are Rho, Rac and Cdc42 isoforms), are master regulators of the actin cytoskeleton and involved in numerous processes important for host protection against pathogens (Ridley and Hall, 1992; Jaffe and Hall, 2005; Aktories, 2011; Lemichez and Aktories, 2013; Popoff, 2014). Thus, Rho proteins are essentially involved in epithelial barrier function, phagocytosis, migration of immune cells, and immune cell signaling. Therefore, Rho proteins are preferred targets for bacterial toxins and effectors. The toxins inhibit or activate Rho protein functions by covalent modification of the switch proteins, including ADP-ribosylation (Aktories et al., 1989), glucosylation (Just et al., 1995a), AMPylation (adenylylation) (Yarbrough et al., 2009; Worby et al., 2009), deamidation (Schmidt et al., 1997; Flatau et al., 1997) and proteolytic cleavage (Shao et al., 2003). Bacterial toxins and effectors also modulate Rho protein function by mimicking the role of regulatory proteins like GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) (Aktories, 2011). Mechanistic basis for the manipulation of Rho proteins by bacterial toxins/effectors is their switch functions in almost all eukaryotic cells (Ridley and Hall, 1992; Jaffe and Hall, 2005; Bustelo et al., 2007). Rho proteins belong to the Ras-superfamily of GTP-binding proteins (Madaule and Axel, 1985; Wennerberg et al., 2005). Accordingly, they are inactive in the GDP-bound form and active after nucleotide exchange induced by GEFs. In their active form, they interact with numerous effectors and, thereby, switch-on signaling and metabolic functions. The active state is terminated (switch-off) by hydrolysis of the bound GTP. This is caused by GAPs. Moreover, Rho proteins are regulated by GDIs (guanine nucleotide dissociation inhibitors), which keep the proteins in their inactive GTP-bound form in the cytosol (Jaffe and Hall, 2005; Wittinghofer and Vetter, 2011).

2. Photorhabdus bacteria

Photorhabdus luminescens are Gram-negative bacteria, which live in the gut of entomopathogenic nematodes of the family *Heterorhabditis*. The nematodes invade insect larvae, where they release the bacteria by regurgitation (Waterfield et al., 2004, 2009; Ciche, 2007; Forst and Nealson, 1996). The bacteria produce a large array of different toxins, which eventually kill the larvae, thereby a food source for bacterial and nematodes is produced and bacteria as well as nematodes are able to propagate (ffrench-Constant et al., 2007). When the food source is exhausted, the bacteria are taken

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up by the nematodes that leave the cadaver of the larvae to invade a new insect. Therefore, nematodes carrying *P. luminescens* bacteria are used as biological insecticides (ffrench-Constant et al., 2007). At least three different species are known from the genus *Photorhabdus*: *P. luminescens*, *P. temperata* and *P. asymbiotica* (Waterfield et al., 2009). While *P. temperata* and *P. luminescens* are selective pathogens for insects, *P. asymbiotica* was shown to be a human pathogen as well (Peel et al., 1999; Gerrard et al., 2004). Moreover, it turned out that also *P. asymbiotica* is mutualistically associated with nematodes like the related species *P. luminescens* and *temperata* (Gerrard et al., 2004, 2006).

The life cycle of nematodes and *Photorhabdus* species essentially depends on the production of protein toxins by the bacteria. Many types of *Photorhabdus* toxins have been reported (ffrench-Constant and Bowen, 2000). One of the first *Photorhabdus* toxins described in detail were the "**m**akes **c**aterpillar **f**loppy" (MCF) toxins. The name refers to the phenotype of intoxicated insect larvae (Daborn et al., 2002) showing loss of insect body turgor. Other very potent toxins belong to a group of "toxin complex" (Tc) toxins, exhibiting masses of over 1.7 MDa (Waterfield et al., 2001; Bowen et al., 1998; Sheets et al., 2011; Gatsogiannis et al., 2013; Meusch et al., 2014). Tc toxins are released from the bacteria into the hemolymph of insect larvae, where they attack insect cells in an exotoxin like manner. Tc toxins are not only produced by insect-associated bacteria like *Photorhabdus* species, *Serratia entomophila* and *Xenorhabdus nematophilus*, genes encoding Tc toxin components are also found in the genome of *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, which are important human pathogens (Heermann and Fuchs, 2008).

Recent studies on *Photorhabdus* toxins showed that not only Rho proteins from vertebrate organisms but also insect Rho proteins are targets of bacterial protein toxins. These data are reviewed in the following sections.

3. Tc toxins

Tc toxins consist of three components: TcA, TcB and TcC (complex is also termed PTC, Table 1), which each of them occurs in several isoforms (Bowen et al., 1998; ffrench-Constant and Waterfield, 2006; Gatsogiannis et al., 2013; Meusch et al., 2014). Recently, the structure of the complete 1.7 MDa Tc complex has been determined (Fig. 1) (Meusch et al., 2014). In this study, the Tc isoforms TcdA1 (TcA), TcdB2 (TcB) and TccC3/C5 (TcC) were studied. The TcA component forms a pentamer and is composed of eight domains: six domains forming the outer shell and two domains forming the translocation channel. The shell, which is connected by a ~48 amino acid linker to the domains forming the inner channel, is composed of a large extended α-helical domain, four putative receptor-binding domains and a neuraminidase-like domain that closes the bottom of the pentameric shell (Meusch et al., 2014). The pore-forming region of TcA consists of a funnel formed by a region, which interacts with TcB and a large α-helical central channel. The linker connecting the shell and the channel acts as an entropic spring that drives the syringe-like injection of the TcA translocation channel into the membrane. The syringe/shell opens due to endosomal acidification (Fig. 1) (Gatsogiannis et al., 2013; Meusch et al., 2014). Interestingly, this happens also at high pH values (e.g. in the midgut of insects).

TcB and TcC components are built by β-sheets that form together a large hollow cocoon which is closed by a distorted six-bladed β-propeller (Meusch et al., 2014), which is also the side where TcB interacts with TcA. The TcC component possesses an aspartyl autoprotease domain with two aspartates as a typical catalytic dyad. Previous studies were performed with the TcC isoforms TccC3 and TccC5, which both harbor ADP-ribosyltransferase domains at their C-terminus (Lang et al., 2010). It is suggested that the C-terminal ADP-ribosyltransferase domains are cleaved by the auto-protease and reside unfolded inside the cocoon of the TcC/TcB/TcA (PTC) toxin complexes. Cryo-EM studies suggest that the ADP-ribosyltransferase domains of TcC components pass through the β-propeller gate and enter the translocation pore of TcA (Meusch et al., 2014). The syringe-like injection of the TcA translocation channel into the membrane results in translocation of the ADP-ribosyltransferases into the cytosol of the target cell. Here, TccC3 ADP-ribosylates actin at threonine-148 (Lang et al., 2010). Threonine-148 is positioned at the actin-binding site of and prevents the binding of thymosin-β4 to actin. Because binding of thymosin-β4 to actin inhibits actin polymerization, ADP-ribosylation of threonine-148 induces actin polymerization (Fig. 1A) (Lang et al., 2010). The ADP-ribosyltransferase of TccC5 modifies Rho proteins.

4. ADP-ribosylation of Rho proteins by TccC5 of *Photorhabdus luminescens*

Like TccC3, TccC5 causes death of larvae after injection of the whole toxin complex (PTC5) (Lang et al., 2010). In insect hemocytes and in HeLa cells, TccC5 induces polymerization of actin and formation of stress fibers. The combination of the actin-ADP-ribosylating toxins TccC3 and TccC5 enhances the effects on the cytoskeleton and causes a strong clustering of the actin cytoskeleton (Fig. 1A). Functional consequences are for example inhibition of phagocytosis of *Escherichia coli* particles by insect hemocytes.

Sequence comparisons with other toxin ADP-ribosyltransferases and mutational analyses of TccC5 indicate that the *Photorhabdus* toxin belongs to the clostridial toxin-like ADP-ribosyltransferase (ARTC) subfamily of ADP-ribosyltransferases (Hottiger et al., 2010; Lang et al., 2010; Pfaumann et al., 2015). Typical for this

Table 1
Characterization of *Photorhabdus* toxins.

Toxin/effector	Source	Structure	Toxin activity	Target (amino acid modified)	Functional consequences
PTC3	*Photorhabdus luminescens*	Tripartite toxin 5 × TcdA1 (TcA) + 1 × TcdB2 (TcB) + 1 × TccC3 (TcC)	TccC3: ADP-ribosylation	Actin (Thr148)	Actin polymerization
PTC5	*Photorhabdus luminescens*	Tripartite toxin 5 × TcdA1 (TcA) + 1 × TcdB2 (TcB) + 1 × TccC5 (TcC)	TccC5: ADP-ribosylation	Rho GTPases (Gln61/63)	Rho activation
PaTox	*Photorhabdus asymbiotica*	Single chain toxin	GlcNAcylation Deamidation	Rho GTPases (Tyr32/34) Heterotrimeric G proteins $G\alpha_i$, $G\alpha_q$ (Gln204/Gln209)	Rho inhibition Gαi, Gαq activation

PTC3 and PTC5 from *Photorhabdus luminescens* have a tripartite structure and consist of TcA, TcB and TcC components. The enzyme components TccC3 and TccC5 possess ADP-ribosyltransferase activity and modify actin and Rho GTPases, respectively, resulting in actin polymerization and Rho activation. PaTox from *Photorhabdus asymbiotica* is a single chain toxin with at least two enzyme activities. The glycosyltransferase domain of PaTox causes attachment of N-acetylglucosamine (GlcNAc) onto Rho GTPases thereby inhibiting Rho proteins. The deamidation domain causes deamidation of heterotrimeric G proteins thereby activating the G proteins.