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# Bidirectional attack on the actin cytoskeleton. Bacterial protein toxins causing polymerization or depolymerization of actin

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ABSTRACT

The actin cytoskeleton is one of the major targets of bacterial protein toxins. The family of binary actin-ADP-ribosylating toxins, including *Clostridium difficile* transferase CDT, *Clostridium perfringens* iota toxin and *Clostridium botulinum* C2 toxin, modifies arginine-177 of actin. Thereby actin polymerization is blocked. By contrast, actin polymerization is facilitated by the tripartite *Photorhabdus luminescens* toxin complex including TccC3, which modifies actin at threonine-148. The review discusses both toxin families in respect to recent findings.



## 1. Introduction

Studies from the last 20 years have provided exciting insights into the role of the cytoskeleton of target cells in host–pathogen interactions. An increasing number of bacterial toxins and effectors were shown to alter the host actin cytoskeleton by diverse mechanisms, resulting in time and space-controlled depolymerization or polymerization of actin. Why is the cytoskeleton a frequent target of bacterial toxins and effectors? The answer to this question is plausible and straight forward. Many cellular and molecular functions of cytoskeletal components are essentially involved in host–pathogen interactions. For example, the cytoskeleton is involved in adhesion, adherence and colonization of bacteria in the host organism. Many bacteria (e.g. *Salmonella*, *Listeria* or *Shigella*) invade host cells by inducing endocytosis or stimulating phagocytosis with the aim to reach an intracellular niche (Dramsi and Cossart, 1998; Galan, 2001; Gruenheid and Finlay, 2003; Ireton and Cossart, 1997; Patel and Galan, 2005), where they can escape the immune system of the host. Pathogen-induced phagocytosis and endocytosis as well as intracellular motility and pathogen traffic depend on the cytoskeleton. On the other hand, extracellular pathogens have to turn down immune responses. They inhibit migration and signaling of immune cells, block phagocytosis by macrophages or try to invade host tissues by alteration of epithelial barriers. All these depend on manipulation of the host cytoskeleton.

Many pathogens target the cytoskeleton indirectly by interfering with regulators of the actin cytoskeleton. Rho family proteins (e.g., Rho, Rac and Cdc42), which are master regulators of the cytoskeleton, and their signal pathways are prototypically manipulated by bacterial toxins and effectors (see recent reviews Aktories, 2011; Aktories and Barbieri, 2005; Boquet, 1999; Boquet, 2000; Boquet and Lemichez, 2003; Davies et al., 2011; Fiorentini et al., 2003; Genth et al., 2008; Gruenheid and Finlay, 2003; Lemichez et al., 2010; Lemonnier et al., 2007). Other

*Abbreviations:* CDT, *C. difficile* transferase; CHO, Chinese hamster ovary cells; LSR, lipolysis-stimulated lipoprotein receptor; PA, protective antigen; +TIPs, plus-end-tracking proteins; Tc, toxin complex; Tβ4, thymosin-β4.

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pathogens directly modify actin, one of the major components of the cytoskeleton. Here we will discuss toxins that modify functions of the cytoskeleton by direct ADP-ribosylation of actin thereby either inducing depolymerization or polymerization of the actin cytoskeleton.

## 2. Actin as target of bacterial protein toxins

Actin is involved in multitude cellular processes, which are key to establish structure, morphology and motility of cells and cell components. As an essential part of the cytoskeleton, it is involved in epithelium barrier function, migration and cell locomotion, phagocytosis, signaling of immune cells and intracellular trafficking. Thereby, the actin cytoskeleton plays an essential role in host–pathogen interactions.

Actin is highly conserved in all eukaryotic cells and one of the most abundant proteins. It consists of 4 subdomains and has firmly bound 1 molecule of ATP (Fig. 1). Its physiological role largely depends on the ability to polymerize and to form filamentous F-actin. F-actin consists of two strands characterized by a two-start left handed double helix (Holmes et al., 1990; Kabsch and Vandekerckhove, 1992; Pollard, 1990). Upon actin polymerization, ATP is randomly hydrolyzed to ADP and Pi, which is slowly released. Thereby, ATP- and ADP-actin is formed, which differs in polymerization properties. F-actin is polar in structure with a fast growing plus or barbed end and a minus or pointed end. The plus end preferably harbors ATP-actin and tends to polymerize and the pointed end harbors preferably ADP-actin and tend to depolymerize. The steady state (plus end polymerization and minus end depolymerization) is called treadmilling of actin (Wegner, 1976). Actin binds a large array of proteins so-called actin binding proteins, which modulate the functional properties of actin among these are proteins, which sequester monomeric actin (e.g. β-thymosins, profilin and cofilin), sever and cap F-actin (e.g., gelsolin and severin), crosslink F-actin (α-actinin and fimbrin) or nucleate actin polymerization (formins and Arp2/3 complex) (Pollard and Cooper, 1986, 2009; Van et al., 1999).

## 3. Actin-depolymerizing toxins

The first bacterial protein toxin, which was identified to modify actin was *Clostridium botulinum* C2 toxin (Aktories et al., 1986; Ohishi and Tsuyama, 1986). This toxin is produced by type C and D strains of *C. botulinum*, which also produce botulinum neurotoxins (e.g. BoNT/C or D) and the Rho-modifying *C. botulinum* ADP-ribosyltransferase C3 (Aktories et al., 1987; Ohishi, 2000; Ohishi et al., 1980; Ohishi and Odagiri, 1984). C2 toxin is the prototype of the family of binary actin-ADP-ribosylating toxins (Barth et al., 2004) also including, *Clostridium difficile* transferase CDT (Perelle et al., 1997), *Clostridium perfringens* iota toxin (Stiles and Wilkens, 1986) and *Clostridium spiroforme* toxin (CST) (Simpson et al., 1989) and the vegetative insecticidal toxin VIP from *B. cereus* (Han et al., 1999) All these toxins consist of two separated components and are called binary toxins.

## 4. Structure of binary actin ADP-ribosylating toxins

One component, the biologically active A-component, is involved in modification of actin by ADP-ribosylation (Aktories et al., 1986) and the B-component is involved in binding and translocation of the ADP-ribosyltransferase into the cytosol (Ohishi et al., 1981) (Fig. 2). The binding components have molecular masses of ~100 kDa and are activated by proteolytic cleavage (Ohishi, 1987). Thereby an about 20 kDa fragment is released to allow the oligomerization of the major part of the binding components to form heptamers (Barth et al., 2000). The binding components of the actin-ADP-ribosylating toxins are highly related to the protective antigen PA, which is the binding component of anthrax toxin (Young and Collier, 2007). Therefore, the rapid progress in our knowledge about PA was essential for the understanding of the structure and functions of the binding component of the binary actin-ADP-ribosylating toxins.

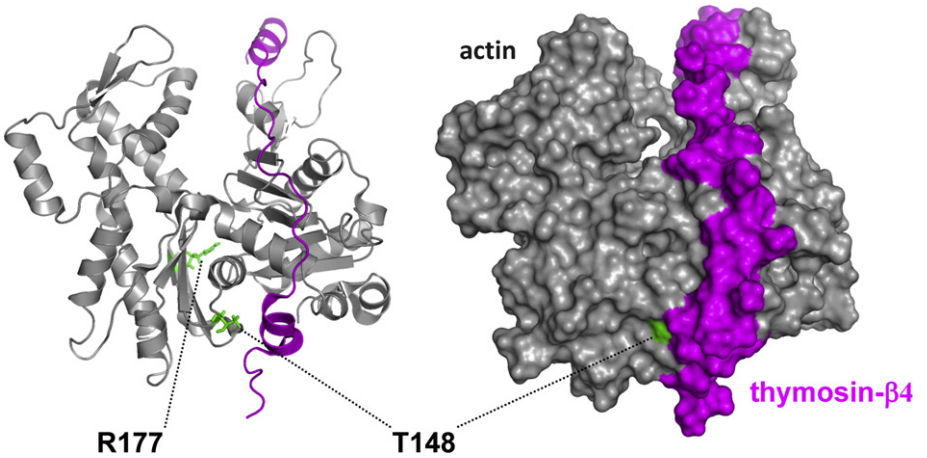


**Fig. 1.** Structure thymosin-β4 bound to actin. Left, ribbon blot of the Tβ4-actin complex by using PyMOL software. Whereas ADP-ribosylation of arginine-177 (R177) by binary toxins (e.g. *C. difficile* transferase) causes depolymerization of actin, ADP-ribosylation of threonine-148 (T148) by *P. luminescens* toxin TccC3 leads to polymerization of actin. Right, surface model of the Tβ4-actin complex. Note, T148 is located in the interaction site of thymosin-β4 with actin. The data are from Protein Data Bank 1UY5. For details see text.