Photorhabdus luminescens Toxins TccC3 and TccC5 Affect the Interaction of Actin with Actin-Binding Proteins Essential for Treadmilling

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Abstract Actin is one of the most abundant cellular proteins and an essential constituent of the actin cytoskeleton, which by its dynamic behavior participates in many cellular activities. The organization of the actin cytoskeleton is regulated by a large number of proteins and represents one of the major targets of bacterial toxins. A number of bacterial effector proteins directly modify actin: Clostridial bacteria produce toxins, which ADP-ribosylate actin at Arg177 leading to inhibition of actin polymerization. The bacterium *Photorhabdus luminescens* produces several types of protein toxins, including the high molecular weight Tc toxin complex, whose component TccC3 ADP-ribosylates actin at Thr148 promoting polymerization and aggregation of intracellular F-actin leading to inhibition of several cellular functions, such as phagocytosis. Here, we review recent findings about the functional consequences of these actin modifications and for the Thr148-ADP-ribosylated actin the subsequent alterations in the interaction with actin-binding proteins. In addition, we describe the effects of ADP-ribosylation of Rho GTPases by the TccC5 component.

Contents

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1 Introduction

A large number of bacterial toxins target actin probably because of its intracellular abundance, highly conserved structure, and participation in many cellular functions. Intracellularly actin exists in monomeric (G-actin) or polymerized, filamentous form (F-actin). Actin filaments are essential for cell migration, phagocytosis, secretion, adhesion, intracellular traffic, cytokinesis, gene regulation, and the maintenance of cell morphology and cell contacts (Pollard and Cooper 2009; Dominguez and Holmes 2011; Pollard et al. 1994; Lanzetti 2007; Posern and Treisman 2006; Castellano et al. 2001). Due to its dynamic behavior, i.e., its constant polymerization and depolymerization cycles, the modification of actin may result in dramatic consequences for these many cellular functions. Bacterial toxins are able to hijack actin functions in order to support their cellular uptake, survival, and intracellular or intercellular transport (see contribution by Kühn and Mannherz).

Many eukaryotic and prokaryotic enzymes posttranslational modify actin by methylation, phosphorylation, glycosylation, ubiquitination, and ADP-ribosylation (Terman and Kashina 2013). In addition, a number of bacterial toxins modulate actin functions by ADP-ribosylation or cross-linking (Aktories et al. 2011; Satchell 2009; Sheahan et al. 2004). The bacterial toxin-induced ADP-ribosylation of actin at Arg177, leading to depolymerization of F-actin and inhibition of polymerization, was the first described toxin-induced modification of actin (Aktories et al. 1986a, b; Ohishi and Tsuyama 1986). Actin Arg177-specific ADP-ribosyltransferases are produced by *Clostridia* (*difficile*, *perfringens*, and *botulinum*), which cause diarrhea, food poisoning, and/or gas gangrene. Most members of this toxin group are binary actin-ADP-ribosylating exotoxins (e.g., *Clostridium botulinum* C2 toxin), although several bacterial ADP-ribosyltransferases (e.g., *Salmonella enterica* SpvB) are not binary in structure (Aktories et al. 2011; see also contribution by Kühn and Mannherz). Actin is also ADP-ribosylated at Thr148 by the *Photorhabdus luminescens* toxin TccC3. These toxins transfer ADP-ribose from NAD$^+$ to the toxin-specific residues of actin and modify its polymerization behavior (Aktories et al. 2011; Lang et al. 2011). Whereas ADP-ribosylation at Arg177 leads to inhibition of actin polymerization, ADP-ribosylation at Thr148 induces actin polymerization and aggregation. TccC3-catalyzed Thr148-ADP-ribosylation and subsequent actin polymerization appears to be aggravated through ADP-ribosylation of Rho GTPases by the *P. luminescens* TccC5 toxin (Lang et al. 2010). Both toxins are part of the tripartite Photorhabdus Tc (toxin complex) toxins, which consist of the three components TcA, TcB, and TcC. These Tc toxins are highly toxic for insects.

Here, we will briefly review the life cycle of *P. luminescens*, the modified interaction of Thr148-ADP-ribosylated actin with actin-binding proteins (ABPs) and the functional consequences of the toxin-induced polymerization of F-actin on the cytoskeleton and actin treadmilling. In addition, we will summarize the effects of the TccC5-catalyzed ADP-ribosylation of Rho GTPases on the actin cytoskeleton.

2 Life Cycle and Tc Toxins of *Photorhabdus luminescens*

Photorhabdus luminescens are motile Gram-negative entomopathogenic enterobacteria, which live in symbiosis with nematodes of the family *Heterorhabditidae* (Forst et al. 1997; Joyce et al. 2006; Waterfield et al. 2009). The nematodes, carrying the Photorhabdus bacteria in their gut, invade insect larvae, where the bacteria are released from the nematode gut by regurgitation into the open circulatory system (hemocele) of the insect (Fig. 1). In the larvae, the bacteria replicate and start to produce a large number of different virulence factors, including various toxins that kill the insect host within few days (Ciche et al. 2008; Waterfield et al. 2009). Then, the cadaver is used as a food source for the bacteria and the nematodes (Ffrench-Constant et al. 2003; Waterfield et al. 2009). After the death of the insect and advanced tissue degradation, the characteristic bioluminescence of
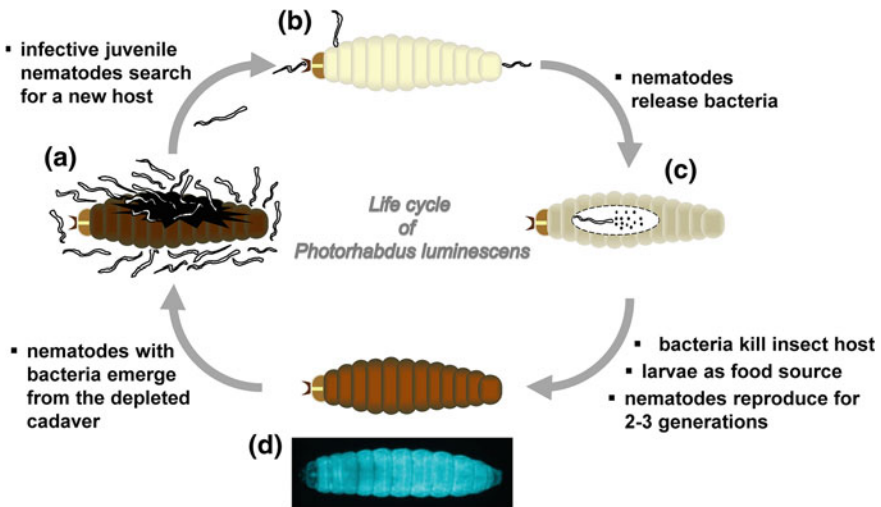

Fig. 1 Life cycle of *Photorhabdus luminescens*. Nematodes with *Photorhabdus luminescens* in their gut leave a dead insect larva (a) and search for new prey. They are taken up by a new larva or intrude it (b). Within the insect hemocoel, they release the bacteria by regurgitation. The bacteria release their toxins which kill the insect larva (c) and a luciferase leading to the bioluminescence of the infected insect larva (d). For further details see the text

P. luminescens can be detected (Daborn et al. 2001) (Fig. 1d). When the insect cadaver is depleted, the replicated nematodes take up bacteria again and leave the insect cadaver to find and invade new insect prey. Therefore, nematodes harboring *P. luminescens* are also used as biological insecticides.

Photorhabdus luminescens produces a large array of toxins, which are only partially characterized. One of the most potent agents produced by the bacteria is the tripartite Tc (toxin complex) toxin, which occurs in several homologues and isoforms. These high molecular mass toxin complexes (~ 1.7 mDa) consist of three components TcA, TcB, and TcC (Ffrench-Constant and Waterfield 2006; Meusch et al. 2014). TcA is the pentameric binding and membrane translocation component, which inserts into the endosomal membrane after acidification of endosomes. Membrane insertion is triggered not only at low pH, but also at high pH, explaining why TcA is able to act directly through the midgut of insects (Bowen et al. 1998; Gatsogiannis et al. 2013). TcB and TcC form together a large cage-like structure (cocoon), which harbors the TcC components (Meusch et al. 2014). The TcC components TccC3 and TccC5 posses at their C-terminal domains an ADP-ribosyltransferase, which are auto proteolytically cleaved and reside inside the cocoon before their intracellular translocation through the TcA complex (Meusch et al. 2014). TccC3 ADP-ribosylates actin at Thr148 while the component TccC5 modifies Rho proteins at Gln61/63 (Lang et al. 2010). Thus, ADP-ribosylated Rho proteins are persistently activated and strongly induce the formation of stress fibers and lamellipodia. Both modifications alter the dynamic behavior of the actin cytoskeleton leading to an increased polymerization and clustering of F-actin, which finally inhibit crucial cellular functions such as phagocytosis (Lang et al. 2010).

Tc toxins were first described in the insect pathogens *Photorhabdus* and *Xenorhabdus*. Due to the increasing number of genome sequencing projects, it became obvious that Tc gene homologues are widely distributed among the other pathogens such as the Gram-negative human pathogens *Yersinia* and *Burkholderia* or Gram-positive insect pathogens such as *Paenibacillus* and *B. thuringiensis* (Yang and Waterfield 2013). Homologues of TcB and TcC proteins are also found in other bacteria sucha as *Wolbachia* or *Mycobacteria*, and even in fungi, indicating a much wider role of this toxin family beyond insect toxicity (Yang and Waterfield 2013). Even infections of humans by Photorhabdus have been reported (Peel et al. 1999).

3 ADP-Ribosylation of Actin by *P. luminescens* TccC3

3.1 *Thr148-ADP-Ribosylation Promotes Actin Polymerization*

In contrast to Arg177-ADP-ribosylation, Thr148-ADP-ribosylated actin is still able to polymerize. Arg177-ADP-ribosylation is performed by a number of toxins from different bacterial origins. Arg177 is located in subdomain 3 of actin facing the
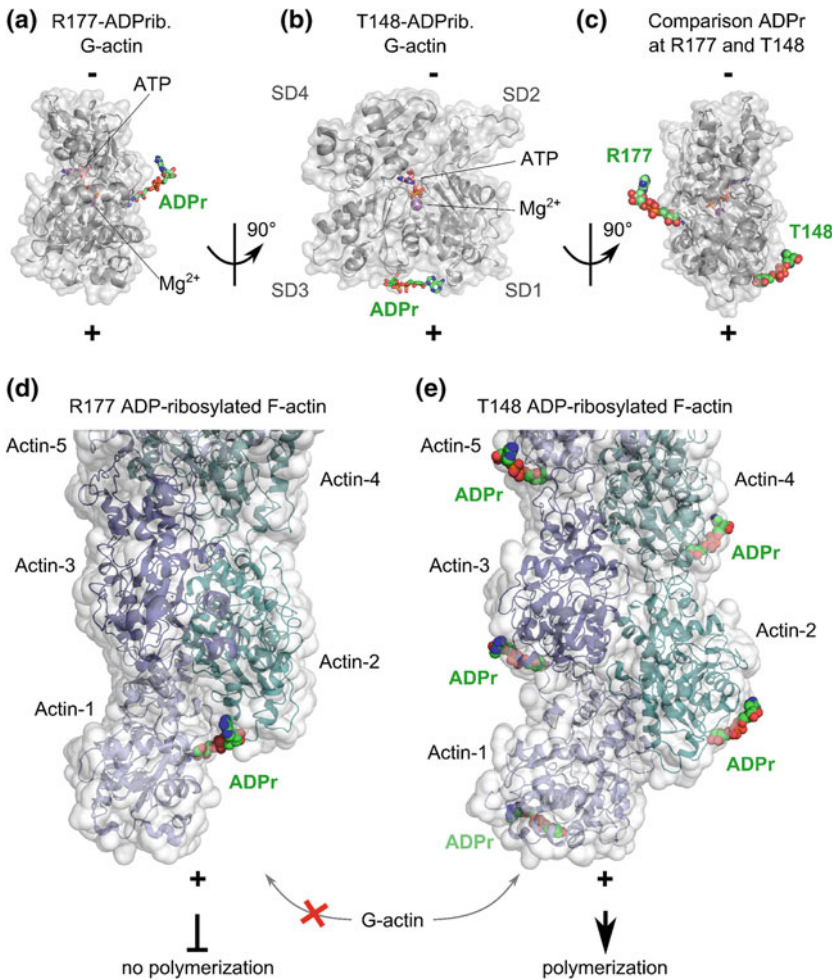
Fig. 2 Model of ADP-ribosylated G-actin and its effect on F-actin polymerization. **a** 'Side-view' of Arg177-ADP-ribosylated (R177-ADPrib.) G-actin (*light gray*) in cartoon-surface representation. The ADP-ribosyl moiety (ADPr) has been modeled on R177/T148 (PDB 1ATN) to illustrate the position of this modification on actin. The pointed (−) and barbed face (+) of G-actin are indicated. **b** 'Front-view' of Thr148-ADP-ribosylated (T148-ADPrib.) G-actin. The ADP-ribosyl modification is located at the hydrophobic cleft between actin subdomains SD1 and SD3. **c** Comparison of the locations of the ADP-ribosyl moieties attached to either Arg177 or Thr148. **d, e** Effect of actin-ADP-ribosylation on its polymerization behavior. Depicted are F-actin filaments (PDB 4A7N) in cartoon-surface representation. **d** Arg177-ADP-ribosylated G-actin binds the barbed end (+) of F-actin but prevents the addition of G-actin monomers like a capping protein (Aktories and Wegner 1988). It potentially impairs the formation of interstrand contacts between actin protomer 1 of F-actin (actin-1) and the incoming G-actin. **e** In contrast, Thr148-ADP-ribosylated actin is able to polymerize into F-actin filaments (Lang et al. 2010) with the ADP-ribosyl moiety decorating the outer surface of the filament. Thr148-ADP-ribosylation appears to not interfere with the interstand and intrastrand actin-actin contacts in F-actin

opposite parallel strand (Fig. 2a–c). After its ADP-ribosylation, actin polymerization is impaired because the adenine–ribose moiety appears to interrupt the interstrand actin-actin contacts (Fig. 2d).

In contrast, Thr148-ADP-ribosylated actin shows a high tendency to polymerize. It retains its native state as judged by its ability to inhibit the enzymatic activity of deoxyribonuclease I (DNase I) in monomeric state (Lang et al. 2016). No difference was observed in the critical concentration of polymerization between native and Thr148-ADP-ribosylated actin (Lang et al. 2010). The ADP-ribose moieties covalently attached to Thr148 are located on the outer surface of F-actin and therefore do not interfere with the actin-actin contacts within the filament (Fig. 2c, e). Upon polymerization, Thr148-ADP-ribosylated F-actin is even able to stimulate the myosin subfragment 1 ATPase activity (unpublished data).
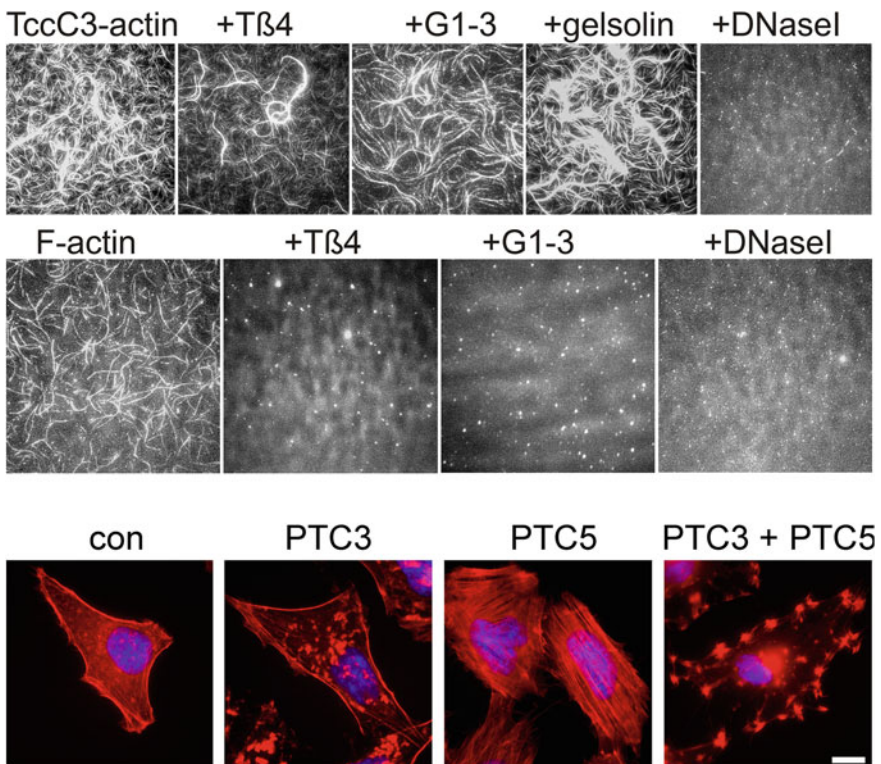

Fig. 3 Polymerization behavior of Thr148-ADP-ribosylated actin as determined by TIRF microscopy. *Upper row* Thr148-ADP-ribosylated actin at 5 μM was polymerized by addition of 1 mM MgCl$_2$ and 50 mM KCl (Lang et al. 2016). The extent of F-actin polymerization is shown 15 min after initiating without an ABP or with addition of 10 μM of Tß4, 5 μM gelsolin G1–3, 3.66 μM full length gelsolin, or 5 μM DNase I as indicated. *Middle row* Identical experiment with unmodified actin. *Lower row* Effect of intoxicating HeLa cells with *P. luminescens* toxins (PTC3, PTC5 and both simultaneously). Images show TRITC-phalloidin staining of HeLa cells 4 h after intoxication. Note F-actin aggregate formation after TccC3 (PTC3) intoxications and the increase in cytoplasmic stress fibers after TccC5 (PTC5) intoxication

When the polymerization behavior of Thr148-ADP-ribosylated actin was visualized by total internal reflection fluorescence (TIRF) microscopy (Fig. 3; Lang et al. 2016), it was noted that the rapid polymerization of Thr148-ADP-ribosylated actin at concentrations above 5 μM was accompanied by the formation of curled F-actin bundles. The strong tendency for bundle formation was not observed for
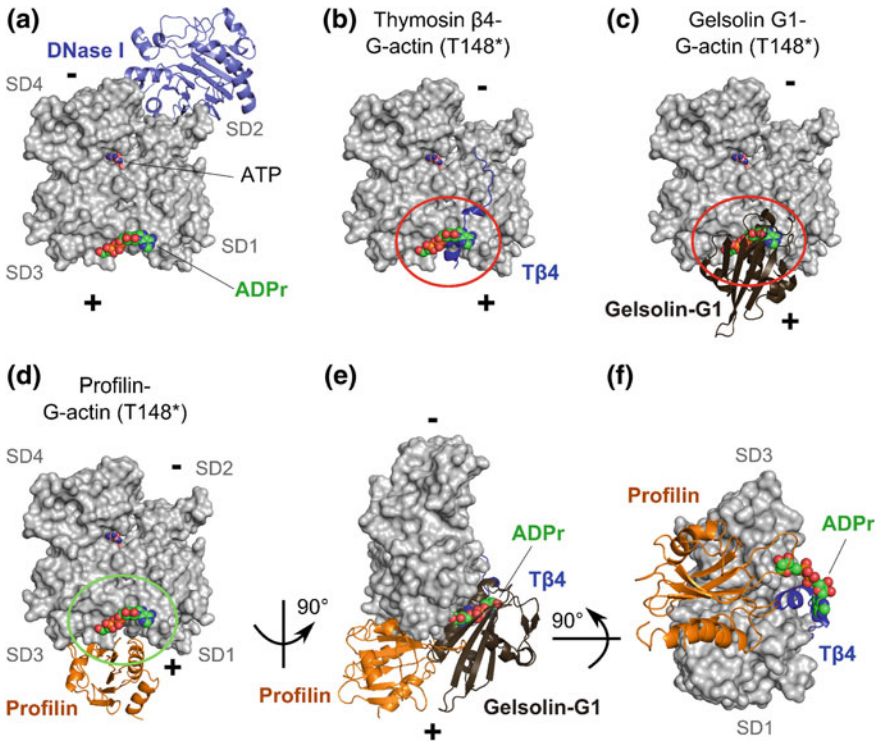

Fig. 4 Thr148-ADP-ribosylation of actin interferes with its binding to certain actin-binding proteins (ABPs). **a** The binding site of DNase I (*slate blue*, PDB 1ATN) on the pointed face of G-actin is separated from the ADP-ribosylated Thr148 amino acid side chain of actin (T148*). Therefore, the ADP-ribosyl moiety (ADPr) does not prevent the formation of the actin: DNase I complex. The ADPr was modeled on T148 as in Fig. 2 to illustrate the location of the modification in the actin: ABP complex. **b** Tß4 (*blue*, PDB 4PL8) binds with its amphipathic helix into the hydrophobic cleft between actin subdomains SD1 and SD3. The impaired binding of Tß4 to Thr148-ADP-ribosylated actin seems to occur due to its steric hindrance with ADPr (see also **f**). **c** The N-terminal G1 segment of gelsolin (*dark brown*, PDB 1EQY) interacts like Tß4 with the same surface at the barbed face of actin. Its binding to actin appears likewise to be impaired by the ADP-ribosylated Thr148. **d** In contrast, biochemical data indicate that Thr148-ADP-ribosylation does not impair binding of profilin (*orange*, PDB 2PBD) to actin. This may be explained by a further rearward binding of profilin into the cleft between SD1 and SD3. The different binding regions of Gelsolin-G1 and profilin are further demonstrated by a side-view of actin (**e**) and between profilin and Tß4 on a direct view onto the actin's barbed face (**f**). The translational rotation axes are indicated. *Red circle* clash between ABP and ADP-ribosylation, *green circle* no steric hindrance between the ABP and the ADP-ribosyl moiety

native F-actin under otherwise identical conditions (Fig. 3). The polymerization of Thr148-ADP-ribosylated actin, however, is not inhibited by thymosin-ß4 (Tß4), a small actin-binding protein that normally inhibits actin polymerization [as shown by the fluorescence assay using pyrene-actin (Lang et al. 2010)] or TIRF microscopy (Fig. 3; Lang et al. 2016). Indeed TIRF microscopy revealed no change in the polymerization behavior or bundle formation of Thr148-ADP-ribosylated actin in the presence of Tß4. Only DNase I was able to inhibit the polymerization of Thr148-ADP-ribosylated actin like native actin (Fig. 3) that was most probably due to its binding to the so-called D-loop, which is spatially separated from the Thr148-ADP-ribose moiety (Fig. 4a).

Bundle formation was predominant in the test tube when using purified actin; however, it was only transiently detected in HeLa cells intoxicated with the TccC3 toxin. Instead, the cellular actin condensed into aggregates of varying sizes, which were positively stained by TRITC-phalloidin suggesting the aggregation of short actin filaments (Fig. 3; Lang et al. 2010, 2016). The different behavior of F-actin condensation (predominantly bundles with purified actin versus aggregates in cells) might be due to additional regulatory mechanisms present in intact cells like, for instance, other ABPs or further modified not yet identified target proteins aiming at the disposal of the Thr148-ADP-ribosylated actin.

3.2   Impaired Interactions of Thr148-ADP-Ribosylated Actin with a Number of Actin-Binding Proteins

Thr148 is located at the base of subdomain 3 (SD3) of actin close to the hydrophobic cleft between SD1 and SD3 (Fig. 4; see also contribution of Kühn and Mannherz), which constitutes a main binding area for a number of actin-binding proteins (ABPs) (Dominguez and Holmes 2011), in particular for ABPs, which sever F-actin or maintain actin in monomeric, non-polymerized state. The bulgy ADP-ribose attached to this residue might hinder the access of these ABPs to their binding region (Fig. 4). Therefore, the interaction of a number of ABPs targeting this area of native and Thr148-ADP-ribosylated actin was further analyzed (see below).

This appeared to be indeed the case for ABPs with G-actin sequestering or F-actin fragmenting activity. The first ABP, for which reduced affinity to Thr148-ADP-ribosylated actin was shown, was thymosin-ß4 (Tß4). Tß4 is a small peptide of 5 kDa and the main intracellular monomeric actin sequestering protein (Fechheimer and Zigmond 1993). It forms a 1:1 complex with G-actin, whereby actin polymerization is inhibited. Since, however, it binds G-actin with relatively low affinity (Kd about 1–3 µM), it is easily displaced by other ABPs with higher affinity to G-actin (Mannherz and Hannappel 2009; Mannherz et al. 2010) or by free barbed ends of F-actin or F-actin fragments. Only due to its high intracellular concentrations (up to 500 µM; Cassimeris et al. 1992), Tß4 can appreciably sequester G-actin (Fechheimer and Zigmond 1993). Thr148-ADP-ribosylated actin

was shown to have an eightfold reduced affinity to Tß4 resulting in a marked reduction in its ability to inhibit the polymerization of Thr148-ADP-ribosylated actin (Lang et al. 2010). This effect is most probably due to the structural proximity of the ADP-ribose moiety attached to Thr148 to the N-terminal helix of Tß4 responsible for binding to the SD1–SD3 cleft of actin (Fig. 4b). Therefore, Thr148-ADP-ribosylation will shift the intracellular equilibrium between G- and F-actin (normally 1 to 1 in quiescent cells) toward F-actin. Indeed, TIRF microscopy demonstrated that Tß4 had no polymerization inhibitory effect on Thr148-ADP-ribosylated actin in contrast to native actin (Fig. 3; Lang et al. 2016).

Intracellularly, the polymerization promoting effect by the TccC3 toxin might be further enhanced by the reduction in affinity of ABPs to Thr148-ADP-ribosylated actin that have F-actin severing activity. Gelsolin and ADF (actin depolymerizing factor)/cofilin and profilin were tested as examples for this class of ABPs (Lang et al. 2016). These experiments were in most cases performed using purified skeletal muscle actin, since no difference in the rate and extent of Thr148-ADP-ribosylation of skeletal muscle and cytoplasmic ß-actin had been detected (Lang et al. 2016). The F-actin fragmenting activity of gelsolin is mainly dependent on the high affinity binding of its N-terminal segment G1 to the target area between SD1 and SD3 (McLaughlin et al. 1993; Nag et al. 2013). Comparing the extent of TccC3-catalyzed Thr148-ADP-ribosylation of free actin or after complexing with gelsolin and its fragments, it was shown that intact gelsolin and the gelsolin fragments comprising G1 inhibited Thr148-ADP-ribosylation (Lang et al. 2016). A similar inhibition was shown for ADF and cofilin. Again these effects will be most probably due to steric clashes between the ADP-ribose attached to Thr148 and the binding helices of gelsolin G1 and ADF/cofilin (McLaughlin et al. 1993; Paavilainen et al. 2008).

In contrast, complexing of actin with profilin did not inhibit Thr148-ADP-ribosylation, although it also binds to the cleft between subdomain 1 and 3 (Schutt et al. 1993). A detailed structure analysis may explain these diverse effects. The ADP-ribose attached to Thr148 is located at the entrance of this cleft. Since G1 and ADF/cofilin were shown to bind to the front part of this cleft, a structural clash between the ADP-ribose and G1 (Fig. 4c) or ADF/cofilin is easily conceivable (not shown). In contrast, profilin binds to the rear part of this cleft (Schutt et al. 1993). Therefore, profilin binding to actin will not be affected by Thr148-ADP-ribosylation (see Fig. 4d–f). Of note, the profilin:actin complex is preferentially added to the plus end of growing actin filaments or used by, for instance, the nucleating proteins of the formin family for F-actin elongation (Goode and Eck 2007). Indeed, profilin is still able to bind to Thr148-ADP-ribosylated actin as shown by chemical cross-linking (Lang et al. 2016). Therefore, profilin binding to Thr148-ADP-ribosylated actin might further support its general tendency to polymerize.

Conversely, polymerized Thr148-ADP-ribosylated actin is resistant against the severing activity of gelsolin or its fragment G1-3 and cofilin as shown by TIRF (total internal reflection fluorescence) microscopy (Fig. 3; Lang et al. 2016). Furthermore, Thr148-ADP-ribosylated F-actin appears to possess a lower rate of

cycling or treadmilling as indicated by a lower ATPase activity and rate to exchange its bound nucleotide (ADP) (Lang et al. 2016). In contrast to the effects of these severing proteins on Thr148-ADP-ribosylated F-actin is the observed effect of deoxyribonuclease I (DNase I). DNase I binds G-actin with high affinity by interacting with the so-called D-loop of subdomain 2 (Kabsch et al. 1990) and is able to depolymerize F-actin (Mannherz et al. 1980). Therefore, DNase I binding is not impaired by Thr148-ADP-ribosylation, and consequently, DNase I is able to depolymerize Thr148-ADP-ribosylated F-actin (Fig. 3; Lang et al. 2016). However, DNase I is an extracellular protein and therefore not able to reverse the intracellular apparently irreversible actin polymerization and aggregation after Thr148-ADP-ribosylation.

4 ADP-Ribosylation of Rho GTPases by *Photorhabdus luminescens* TccC5

Rho proteins are a family of small GTP-binding proteins with about 20 family members (Hall 1993; Nobes and Hall 1994). Best studied are Rho, Rac, and Cdc42 isoforms. They are the master regulators of the cytoskeleton and involved in motility functions, cellular traffic, but also transcription or proliferation (Jaffe and Hall 2005). Rho proteins are regulated by a GTPase cycle (Hall 1994) (Fig. 5). They are active in the GTP-bound form and inactive with GDP-bound form. Activation occurs by GDP to GTP exchange, which is facilitated by guanine nucleotide exchange factors (GEFs) and allows interaction with effectors like the formins or Rho-dependent kinases, which then cause polymerization of actin and formation of stress fibers (Schmidt and Hall 2002; Pruyne et al. 2002; Kimura et al. 1996). The inactive state is induced by GTP hydrolysis, caused by their endogenous GTPase activity, and accelerated by GTPase-activating proteins (GAPs) (Fig. 5). Rho-regulating proteins (GDIs) stabilize the inactive GDP-bound form of Rho proteins and keep Rho proteins in inactive form in the cytosol (DerMardirossian and Bokoch 2005). Because of their multifunctional regulatory roles are Rho proteins preferred targets for bacterial toxins and effectors.

   TccC5 as part of the Photorhabdus toxin complex PTC5 ADP-ribosylates Rho GTPases leading to their irreversible activation (Lang et al. 2010) that indirectly should promote polymerization of actin and formation of stress fibers. This assumption is indeed supported by data obtained after selective intoxication of HeLa cells with TccC5, which led to a prominent increase in long stress fibers within their cytoplasm (see Fig. 3). Though the exact mechanism of this effect is still unclear, it appears highly likely that the activated Rho proteins stimulate actin nucleation and elongation activating proteins such as formins and the Arp2/3 complex (see contribution by Lemichez). In combination with *P. luminescens* TccC3, TccC5 will thus increase the effects on the cytoskeleton. It was, however,
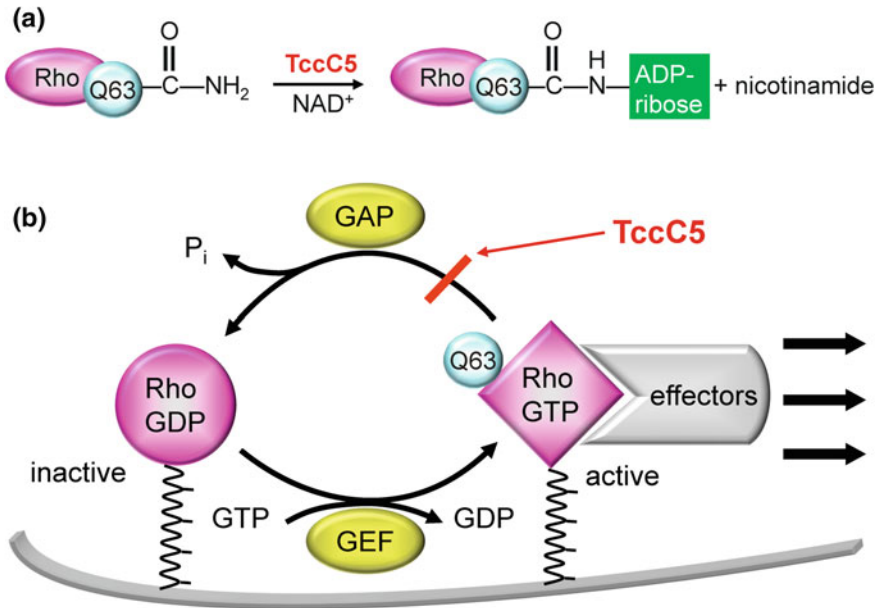
Fig. 5 Mechanism of Rho-ADP-ribosylation by TccC5 toxin. **a** TccC5 ADP-ribosylated Rho at Gln63 (Gln61, not shown), which (**b**) leads to permanent activation of the Rho-GTPase, because binding of Rho-GAP (GTPase-activating protein) is inhibited by the attached ADP-ribosyl moieties. The Rho-GTPase remains in the GTP-bound, i.e., active state

noted that only TccC3 intoxication alone or in combination with TccC5 led to strong clustering of the intracellular actin (Fig. 3; Lang et al. 2010).

Like TccC3, TccC5 functions as an ADP-ribosyltransferase, but instead of modifying actin, TccC5 modifies Rho GTPases (Lang et al. 2010; Pfaumann et al. 2015). The ADP-ribosyltransferase domain of TccC5 belongs to the subfamily of ADP-ribosyltransferase also designated as ARTC family of ADP-ribosylating toxins (Hottiger et al. 2010; Lang et al. 2010; Pfaumann et al. 2015). This subfamily contains a conserved RSE motif with an arginine and serine residue involved in NAD-binding and the so-called catalytic glutamate (Domenighini et al. 1994). By mutational analyses, we recently showed that in TccC5 these residues are Arg774, Ser809 and Glu886 (Pfaumann et al. 2015).

Rho proteins belong to the Ras superfamily of GTP-binding proteins and the Rho family comprises about 20 proteins, of which the Rho, Rac, and Cdc42 iso-forms are best known (Madaule and Axel 1985; Wennerberg et al. 2005). Of these, RhoA and B, Rac1–3, Cdc42, and the plant Rac-like protein Rop4 are major substrates of TccC5-induced ADP-ribosylation. Minor substrates are RhoC and TC10 (Pfaumann et al. 2015). Interestingly, TccC5-catalyzed ADP-ribosylation of Rho proteins occurs at Gln63 and Gln61, which is also modified by deaminating cytotoxic necrotizing factors (CNFs) from *E. coli* and *Yersinia* species (Schmidt et al. 1997; Flatau et al. 1997; Hoffmann and Schmidt 2004). This glutamine

residue is essential for GTP hydrolysis by Rho proteins (Wittinghofer and Vetter 2011). ADP-ribosylation of Gln63/61 prevents GTP hydrolysis by Rho proteins even in the presence of GAPs (Fig. 5). Therefore, Rho proteins are persistently in the active state and cause activation of effector proteins such as Rho kinase and formins, which cause polymerization of actin and formation of stress fibers or lamellipodia (the latter due to Rac activation; Lang et al. 2010; Pfaumann et al. 2015). Thereby, TccC5 will also contribute to the inhibition of phagocytosis by insect hemocytes and death of larvae (Lang et al. 2010).

5   Conclusions

Among the many bacterial toxins, which modulate the intracellular actin cytoskeleton, two groups of bacterial toxins directly and covalently modify actin by ADP-ribosylation. Actin Arg177-specific ADP-ribosyltransferases are produced by *Clostridium difficile*, *perfringens*, and *botulinum*, which cause diarrhea, food poisoning, and gas gangrene. Bacterial toxins, which transfer adenine–ribose to Arg177, inhibit actin polymerization finally leading to depolymerization of all intracellular actin. Thus, impaired cells will undergo programmed cell death or apoptosis and the cell remnants might be used as nutrients for the multiplication of the bacterium or impair immune cells. In contrast, the TccC3 toxin from *P. luminescens* ADP-ribosylates actin at Thr148 leading to polymerization/ aggregation of the intracellular actin. *Photorhabdus* bacteria live in the gut of nematodes, which after invading insect larvae release these bacteria into their open circulatory system, where they infect insect hemocytes and subsequently evade their elimination by inhibiting phagocytosis. Since this actin modification reduces also its interaction with F-actin depolymerizing ABPs, Thr148-ADP-ribosylation appears to be practically irreversible. In PTC5-intoxicated cells the Rho GTPases are additionally activated after ADP-ribosylation by the TccC5 component inducing a signaling cascade that will further stimulate actin polymerization. Irreversible polymerization of most intracellular actin will lead to an arrest of most cellular functions and inevitably to cell death.

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