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Review

# Bacterial genotoxins: The long journey to the nucleus of mammalian cells☆,☆☆

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ABSTRACT

Bacterial protein genotoxins target the DNA of eukaryotic cells, causing DNA single and double strand breaks. The final outcome of the intoxication is induction of DNA damage responses and activation of DNA repair pathways. When the damage is beyond repair, the target cell either undergoes apoptosis or enters a permanent quiescent stage, known as cellular senescence. In certain instances, intoxicated cells can survive and proliferate. This event leads to accumulation of genomic instability and acquisition of malignant traits, underlining the carcinogenic potential of these toxins. The toxicity is dependent on the toxins' internalization and trafficking from the extracellular environment to the nucleus, and requires a complex interaction with several cellular membrane compartments: the plasma membrane, the endosomes, the trans Golgi network and the endoplasmic reticulum, and finally the nucleus.
This review will discuss the current knowledge of the bacterial genotoxins internalization pathways and will highlight the issues that still remain unanswered. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.



**Contents**



☆ This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.
☆☆ Tribute to Gianfranco: This review is a tribute to Gianfranco Menestrina and to his deep interest in the biology of bacterial toxins, knowing that he would appreciate it.
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## 1. Introduction

Bacterial genotoxins are effectors that cause DNA breaks on mammalian cells [1]. The cellular response to the intoxication is the activation of DNA damage and repair responses. However, when the damage is too extensive and beyond repair, the target cells enter a non-replicative state known as senescence or die by apoptosis [1].

Although different in holotoxin composition and crystal structure, bacterial genotoxins need to access the nucleus in order to exert their activity.

Members of the genotoxin family, known as cytolethal distending toxin (CDT), are internalized from the plasma membrane, enter the endosomal compartment, and are retrogradely transported to the endoplasmic reticulum [1]. Conversely, the typhoid toxin, produced by the intracellular pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), is expressed after the bacterium has invaded the target cell, where it induces DNA damage. The toxin is further released into the extracellular environment, getting access to the nucleus of bystander non-infected cells [2].

The journey to the cellular nucleus requires dynamic and specialized interactions with membranes of several subcellular compartments, extensive membrane remodelling, involving budding of a vesicle from a donor membrane followed by the delivery to the correct acceptor membrane [3] and proper sorting of the cargo [4–6], revealing a complex interaction between bacterial genotoxins and biological membranes.

Currently, several key steps of the internalization pathways of these effectors have been identified. However, many questions, such as the identity of the cellular molecules associated with membrane remodelling and cargo sorting, remain open.

This review will discuss the relevant issues in internalization and interaction of these bacterial toxins with the subcellular membrane compartments and will complement recently published reviews [7] by highlighting aspects such as the role of secretion and internalization of genotoxins *via* OMVs. The present work will further propose possible explanations to reconcile discrepancies present in the literature, contributing to the discussion of the still open issues.

## 2. Bacterial genotoxins

The cytolethal distending toxins (CDTs) and the typhoid toxin belong to a relatively new family of bacterial AB protein toxins, where "A" stands for active and "B" for binding component, respectively.

CDTs are produced by Gram-negative mostly extracellular pathogens, such as *Escherichia coli*, *Aggregatibacter actinomycetemcomitans*, *Haemophilus ducreyi*, *Shigella dysenteriae*, *Campylobacter sp.* and *Helicobacter sp.*, (*reviewed in*[1]). To distinguish between CDTs produced by the different bacterial species or even within a single species, Thelestam et al. and Jinadasa el al. have introduced a nomenclature based on the first three letters of the species name in lower case, placed after the capitalized first letter of the bacterial genus (*e.g.* HducCDT for the *H. ducreyi* CDT). When necessary, the strain number or other common designations are specified after CDT [8,9].

The typhoid toxin is produced by the intracellular pathogen *S. enterica* serovar Typhi, upon bacterial internalization within the host cell [10].

Although the crystal structure of the typhoid toxin is vey different from that of the *H. ducreyi* CDT (Fig. 1), both are heterotrimers and share a common "A" subunit, encoded by the *cdtB* gene [11,12]. This subunit is functionally and structurally homologous to mammalian DNase I, and causes DNA strand breaks once internalized within the nuclear compartment [13–16].

The crystal structure of *H. ducreyi* and *A. actinomycetemcomitans* CDTs shows that the CdtB subunit is non-covalently linked to two accessory subunits, named CdtA and CdtC, which share structural homology with the B-chain repeats of the plant toxin ricin [11,17], thus forming an $AB_2$ toxin. The "B" function of CdtA and CdtC has been inferred from mutagenesis experiments. Mutations introduced within the large aromatic patch and an adjacent deep groove at the interface between CdtA and CdtC do not compromise the stability of the ternary complex, but completely or partially abolish the ability of the toxin to bind and cause cell cycle arrest [11,18] (Fig. 1). The typhoid toxin is an $A_2B_5$ toxin, where CdtB is connected to the second "A" subunit, known as PltA, which possesses ADP ribosyl transferase activity. Currently, the cellular target(s) for PltA has not been identified [12]. The PltB subunit represents the "B" component of the holotoxin, organized into a pentameric disc, directly interacting with PltA [12] (Fig. 1).

## 3. Interaction with the plasma membrane

### 3.1. CDT binding and lipid rafts

The requirement of lipid rafts (cholesterol- and sphingolipid-rich domains) for CDT binding to the plasma membrane has been shown for several members of the family (Fig. 2). Cholesterol depletion by methyl β-cyclodextrin reduces the ability of *H. ducreyi* (HducCDT), *A. actinomycetemcomitans* (AactCDT) and *Campylobacter jejuni* (CjejCDT) to associate to the surface of HeLa, Jurkat or CHO cell lines, respectively, thus preventing the toxic activity [19–22]. Confocal microscopy analysis has further demonstrated that AactCDT and CjejCDT co-localise with the ganglioside GM1, a known lipid raft marker [20,21]. Inactivation of the gene SGMS1, coding for sphingomyelin synthase 1, an enzyme essential in the sphingomyelin biogenesis, strongly reduces intoxication with CDTs produced by *H. ducreyi*, *A. actinomycetemcomitans*, *E. coli* and *C. jejuni*[23] (Fig. 2). This biochemical evidence is supported by bioinformatics and molecular simulation data, which show that the CdtC subunit from *A. actinomycetemcomitans*, *C. jejuni* and *Haemophilus parasuis* contains a cholesterol recognition/interaction amino acid consensus (CRAC) region. Mutation within the putative CRAC site results in decreased binding of the holotoxin to cholesterol-containing model membranes as well as to the surface of Jurkat and CHO cells, respectively [24–26].

In contrast, other reports have failed to inhibit binding and toxicity of AactCDT, HducCDT and EcolCDT-III in CHO cells upon depletion of cholesterol [22,27]. This discrepancy may be due to the different cell lines used in the experimental set ups: HeLa *versus* CHO for HducCDT [19,22], or Jurkat *versus* CHO for AactCDT [20,27]. A relevant issue to discuss in the context of such divergent results is the use of chemicals or inhibitors, such as methyl β-cyclodextrin, to study toxin internalization. It may be possible that these chemicals may alter additional cellular pathways/functions in a cell type-dependent manner, influencing the experimental outcome. The use of alternative strategies may help to resolve these discrepancies. However, we cannot rule out that other factors, such as toxin concentration and experimental design may influence the results.

No data are yet available regarding the requirement of the lipid raft microdomains for the binding of the typhoid toxin.

### 3.2. Bacterial genotoxins and their receptors

#### 3.2.1. CDT receptor: a glycoprotein or a glycolipid?

The identity of the CDT receptor is still unknown, and discordant results have been published. The structure(s) recognized by CDTs on the cell surface should be ubiquitously expressed, since a broad panel of cell types have been shown to be sensitive to the activity of this toxin family within a define species [1].

The *E. coli* CDT-II (EcolCDT-II) binds to fucose and fucose-containing glycoproteins in *in vitro* assays [28]. Competition experiments demonstrated that fucose-specific lectins block the toxin-mediated cell cycle arrest, and removal of N- but not O-linked surface sugars moieties prevents CDT-intoxication of HeLa cells [28]. Also the purified CdtA subunit from AactCDT has been shown to bind fucosylated thyroglobulin,