

Chapter 12: Bacterial toxins

a.a. 2018-19

Bacterial toxins

Toxins: any organic microbial product or substance that is harmful or lethal to cells, tissue cultures, or organisms'.

Diphtheria toxin was isolated by Roux and Yersin in 1888, and has been recognized as the **first virulence factor(s)** for a variety of pathogenic bacteria.

Major symptoms associated with disease are all related to the activities of the toxins produced by pathogens.

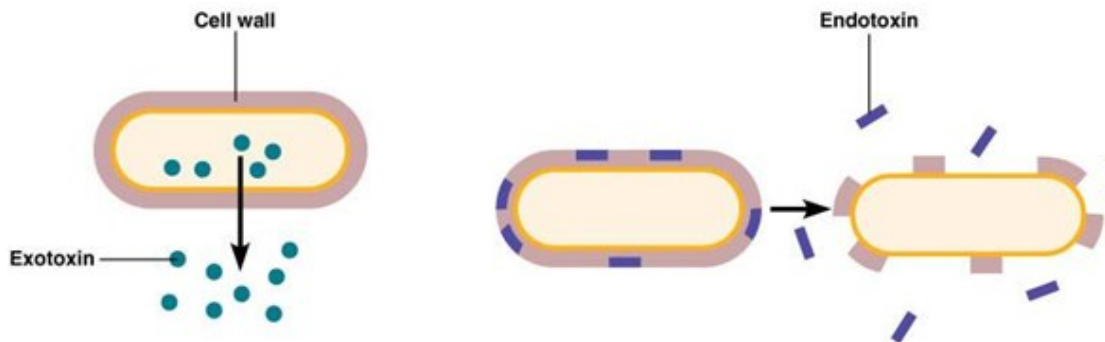
There are cases in which it has been difficult to discern benefits for the bacteria that product toxins and the role of toxins in the propagation of the bacteria is not so obvious.

With the recognition of the central role of toxin in these and other diseases has come the application of inactive toxins (toxoids) as **vaccines**. Such toxoid vaccines have had an important positive impact on public health.



Endotoxins and exotoxins

Endotoxins: Lipopolysaccharide (LPS) of Gram-negative outer membrane and LTA (lipoteichoic acid). They are cell-associated substances that are structural components of bacteria. They may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics. Their toxicity is due to **the promotion of the secretion of proinflammatory cytokines.**



Exotoxins: extracellular diffusible proteins. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However, some bacterial exotoxins act at the site of pathogen colonization.

Exotoxins are generally produced by a particular strain and show a specific cytotoxic activity. The target cell may be narrow (e.g. tetanus and botulinum toxins attack only neurons) or enlarged to several cell types (as produced by staphylococci, streptococci, clostridia, etc.)



Bacterial toxins are the most powerful human poisons known

Toxin	Toxic Dose LD50 (mg)	Host
Botulinum toxin	2×10^{-8}	Mouse
Tetanus toxin	2×10^{-8}	Mouse
Shiga toxin	4×10^{-4}	Rabbit
Diphtheria toxin	1×10^{-6}	Guinea pig

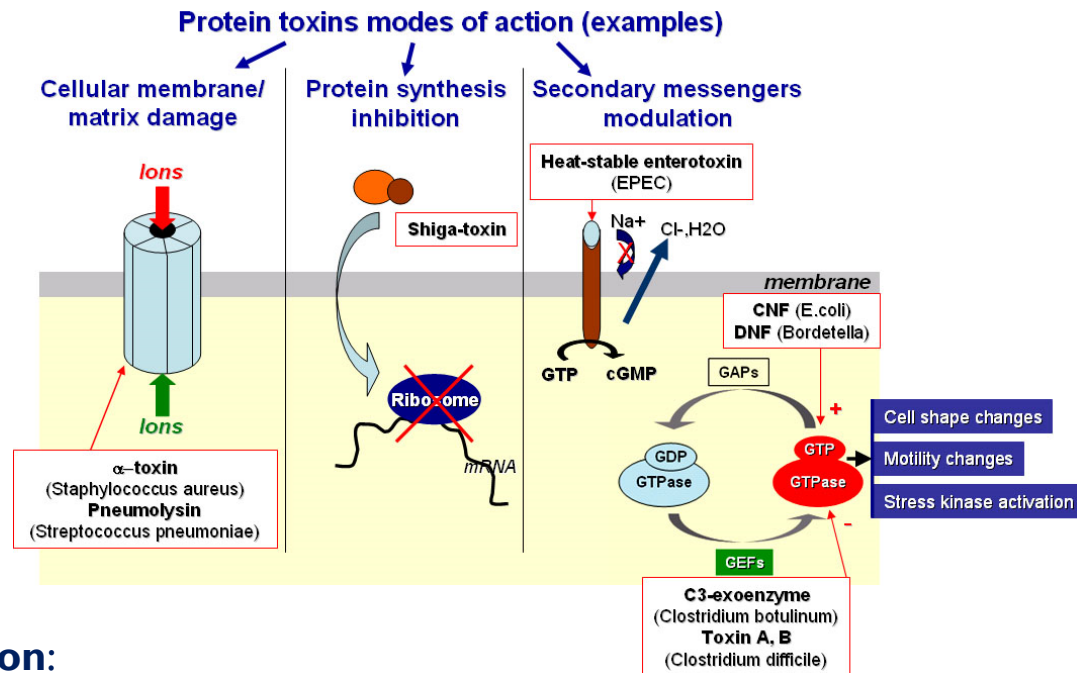
Botulinum is the most acutely lethal toxin known, : estimated human median lethal dose (LD50) of ≈ 2 ng/kg (intravenously or intramuscularly)



Classification of bacterial exotoxins

Bacterial toxins may be categorized according to **location of their target**:

1. Bind to the target cell surface (type I)
2. Membrane-acting toxins (type II)
3. Toxins with cytosolic targets (type III)



Or classified according their **mode of action**:

- Damage membranes – Forms pores
- Inhibit protein synthesis – N-glycosidase
- Modify signal transduction – ADP-ribosyltransferase
- Activate immune response – Superantigen
- Protease – Zinc-metalloprotease



Type I toxins: superantigens

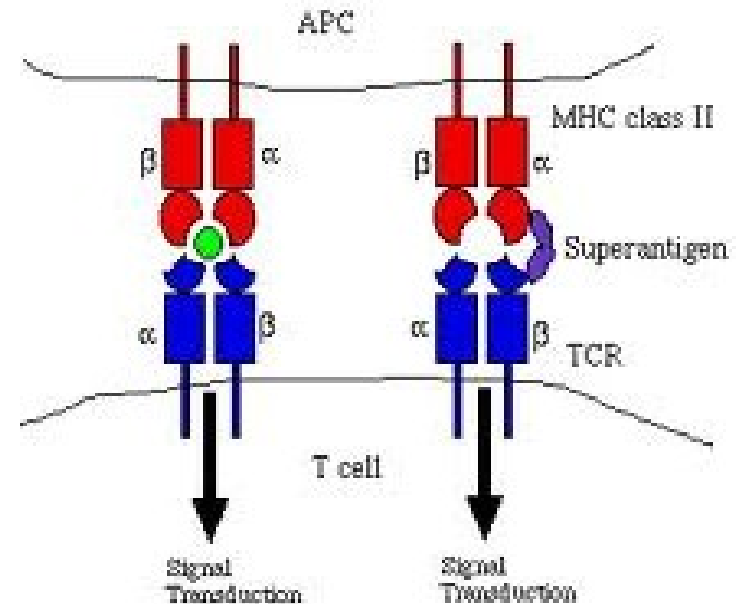
Type I: they do not enter the cell. Superantigens are toxins that cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. They produce a **toxic shock syndrome** similar to that induced by circulating LPS.

Toxic-shock syndrome toxin (TSST) is a superantigen of *Staphylococcus spp.* that have the ability to force unnatural association between macrophages (APC) and T cells causes an outpouring of cytokines that trigger the shock process.

TSST binds directly to major histocompatibility complex class II (MHC-II) on APC surface rather indiscriminately, and they bind also to T-cell receptors (TCR) again indiscriminately without the presence of a specific antigen.

They forms many more APC-T Helper cells pairs: 1/5 of T cells can be stimulated by the bridging action of superantigens instead of 1/10000 (normal response to an antigen).

One results is that most T-cells release cytokines especially IL-2.



Type II toxins: membrane-disrupting toxins

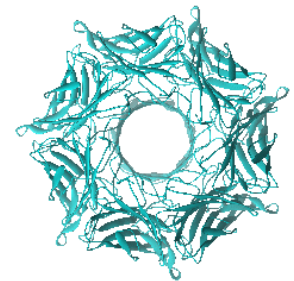
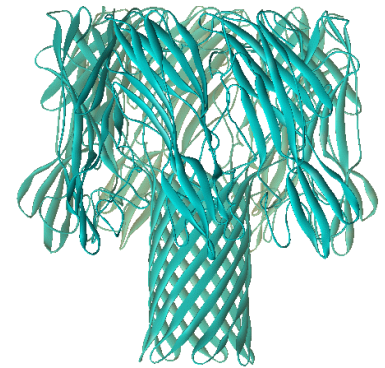
Pore-forming toxins are cytolytic to cells, disrupting the integrity of plasma membranes by inserting a **transmembrane pore**. Non selective influx and efflux of ions across the plasma membrane that eventually lead to the death of nucleated cells. They have broad spectrum targets.

2 roles in virulence: A) to kill host cells (especially phagocytes) and B) to escape from the phagosome and enter the cell's cytoplasm.

Pore forming toxins have different modes to disrupting the membranes.

Paradigmatic example: The **α -toxin** (α -haemolysin) of *S. aureus* is secreted as soluble single protomers, (33 kDa) which assemble on the plasma membrane to form a large mushroom-shaped heptameric structure comprising three distinct domains. The cap and rim domains of the α -toxin heptamer are situated at the surface of the plasma membrane, while the stem domain serves as the β -barrel transmembrane channel.

Pore forming is a widespread class of similar toxin molecules including aerolysin, leukocidin, the anthrax protective antigen, streptolysins produced by streptococci, and listeriolysin of *L. monocytogenes*, pneumolysin (cholesterol-dependent cytolysin), from *S.pneumoniae*,



homo-heptamer, composed of 7 identical subunits.



Type III toxins: A-B toxins

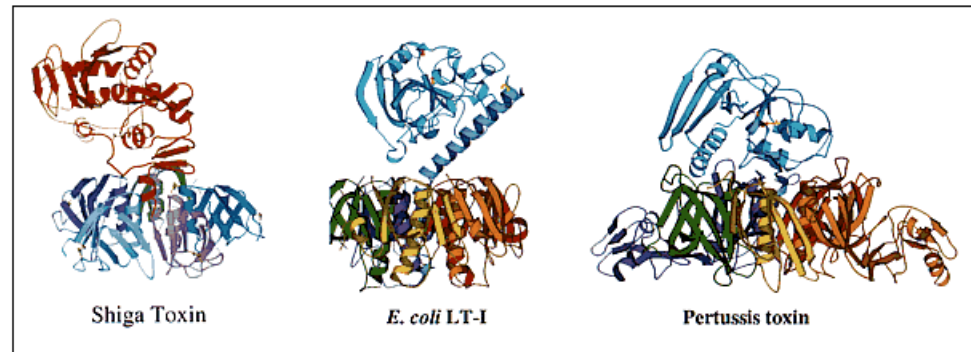
A-B toxins interfere with internal function of the cells.

They contain two functional domains (or subunits): the "A" component is usually the "**active**" toxic (or enzymatic) portion, and the "B" component is the "**binding**" portion which mediates the transport of the active **A**-domain into the target cell.

The A domain is proteolytically cleaved off from the B domain within the target cell or the toxin is composed of two separate subunits from the beginning.

AB stochiometry: the simplest model is the **A-B** toxin (examples, DT toxin). The **AB₅** (or **AB₅**) **toxins** are six-component protein complexes. All share a similar structure and mechanism for entering targeted host cells. **A** portion is a separate polypeptide from the **B** portion which is composed of multiple subunits that may be identical (cholera t) or not (pertussis t). Examples:

- Cholera toxin (*V. cholerae*)
- Heat-labile enterotoxins (LT-I) (*E. coli*)
- Pertussis toxin (*B. pertussis*)
- Shiga toxin (*S. dysenteriae*)
- Diphtheria toxin (*C. diphtheriae*)
- shiga-like toxin (or verotoxin) (EHEC)
- Exotoxin A (*P. aeruginosa*)



AB₅ toxins. B subunits are very similar in ShT LT-I and PT

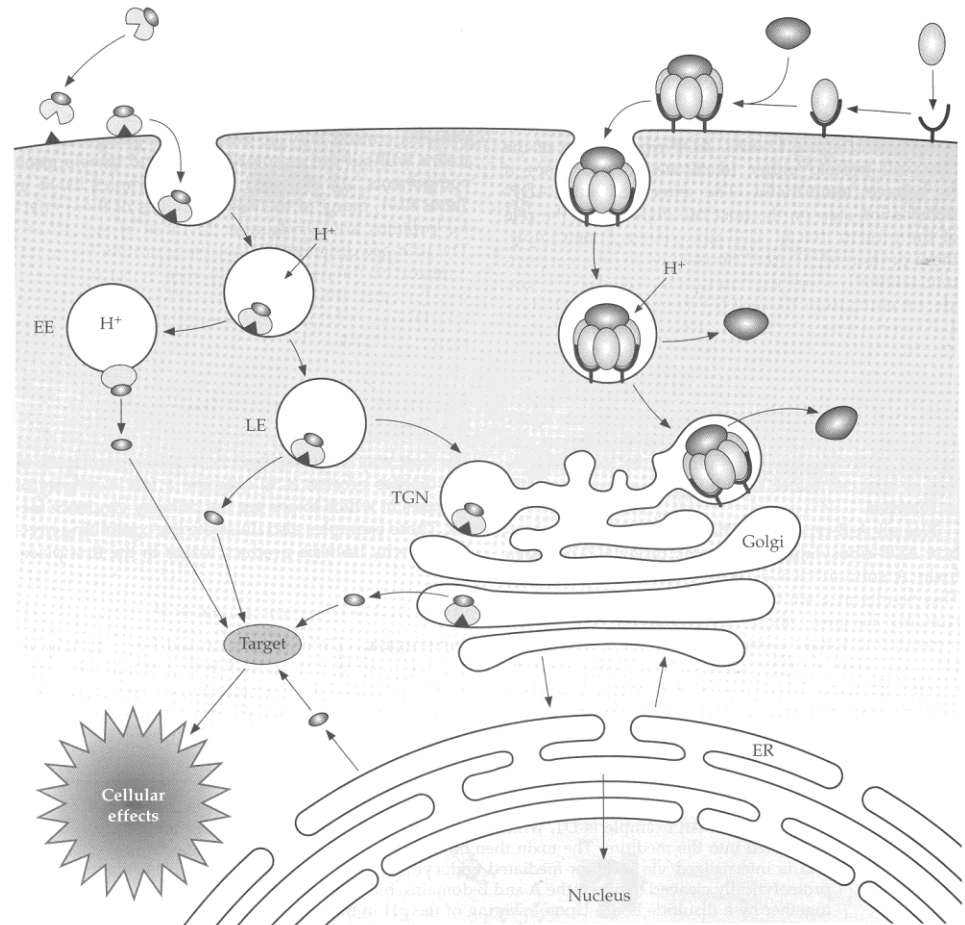
Binding and entry of A-B toxins into host cells

B subunit determines the cell specificity of the toxin. This portion binds to a specific cell surface receptor, often a carbohydrate part of glycoproteins, and the toxin is transported in an endocytic vacuole

Often a pH-dependent mechanism play a role in translocation for toxins by stimulating the separation of A part and B parts and insertion of the toxin into the membrane and internalization (**translocation**) of the A portion in the cytoplasm.

Some toxins have a more complex route involving the TGN and also ER (retrograde transport)

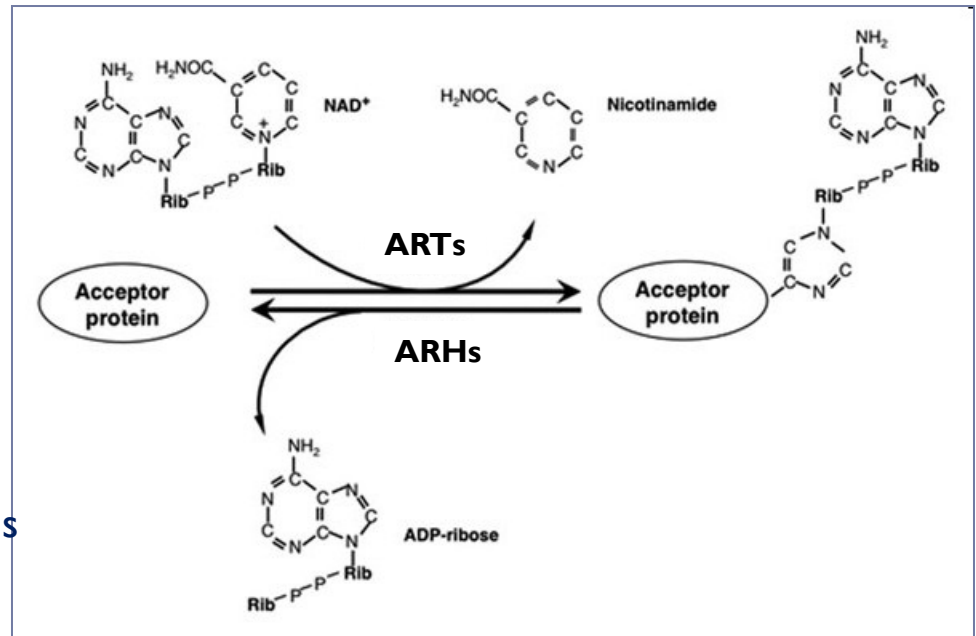
Once the A portion has entered the host cell cytoplasm it becomes enzymatically active and exerts its toxic effects.



Subunits A with ADP-ribosylation activity

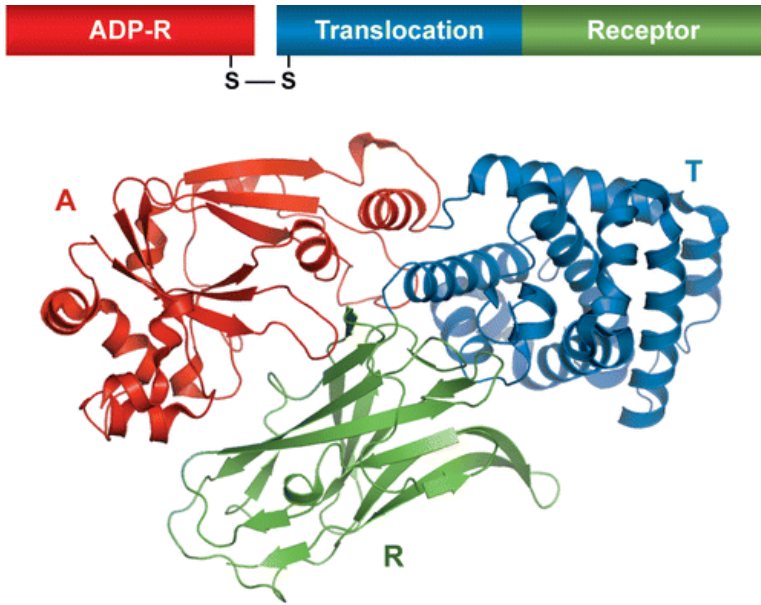
The different **A subunits** enter different cells but most of them catalyze the same reactions: they show **ADP-ribosyltransferase** activity. ADP-ribosylation of the target either inactivates it or causes it to behave abnormally. The final effect depends on the role of the protein that is ADP-ribosylated. ADP-ribosyltransferases (ARTs) and ADP-ribose-protein hydrolases (ARHs) that antagonize each other.

Toxin	Target
Diphtheria toxin	EF-2
Exotoxin A (<i>P aeruginosa</i>)	EF-2
Cholera toxin	Gs protein
Pertussis toxin	Gi protein
C3 toxin (<i>C botulinum</i>)	Rho-like proteins



Not all AB-type toxins have ADP-ribosylating activity: some AB type toxin (Shiga toxin of *Shigella dysenteriae* and verotoxin of some *E. coli* pathogen strains), **inactivate ribosomal RNA**; neurotoxin of *B. botulinum* is a zinc-dependent metalloprotease that cleave **neuron-specific proteins** (see below).

Diphtheria toxin



Diphtheria toxin (DT) is an A-B toxin produced by *Corynebacterium diphtheriae* as a 58 kDA mature protein.

The protein, after secretion is cleaved into two domains joined by a disulfide bond: the N-terminal catalytic domain **A** (ADP-R) and C-terminal B subunits (T+R) containing the **transmembrane domain (T)** for its translocation and the **receptor binding domain (R)** formed by a bent beta-sheet from antiparallel strands, mediates the first contact between the toxin and the eukaryotic membrane.

A Deng Q, Barbieri JT. 2008.
R Annu. Rev. Microbiol. 62:271–88

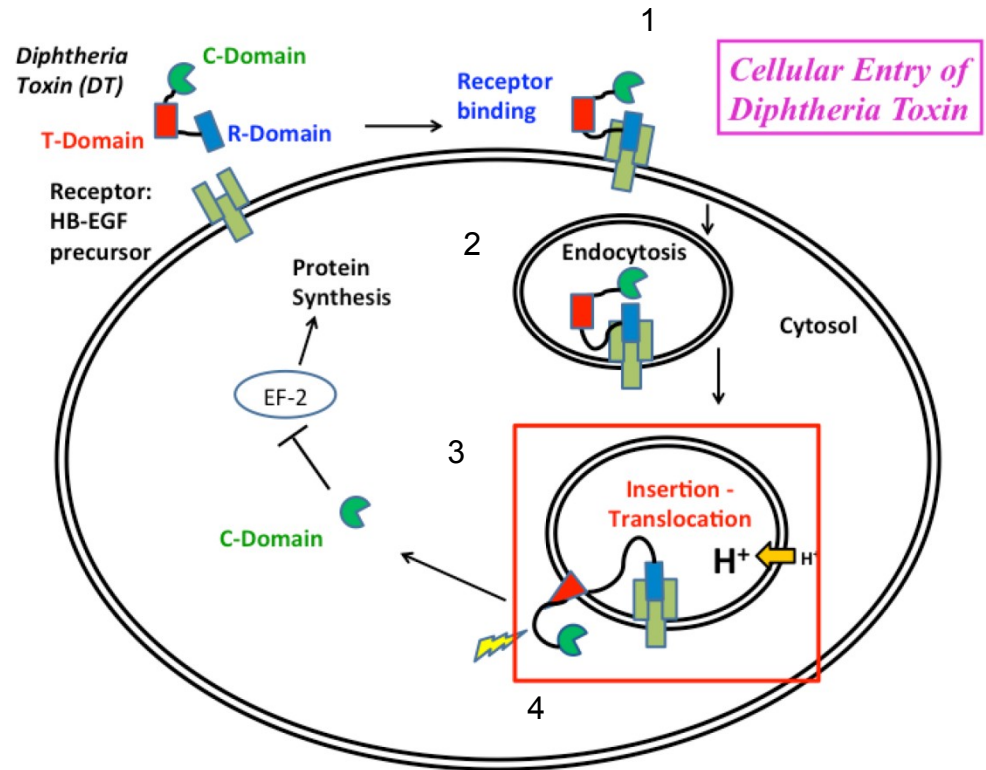
The gene encoding DT is carried by a group of lysogenic **corynebacteriophages**. A repressor regulates the DT gene which is induced by **low-iron** condition. Hypothesis: DT may kill host cells to capture iron for use by bacteria.

The unique cellular receptor for DT is a member of EGF family, important signals for growth and differentiation (the heparin-binding epidermal growth factor, HB-EGF).



Mechanism of diphtheria toxin entry into eukaryotic cell cytosol

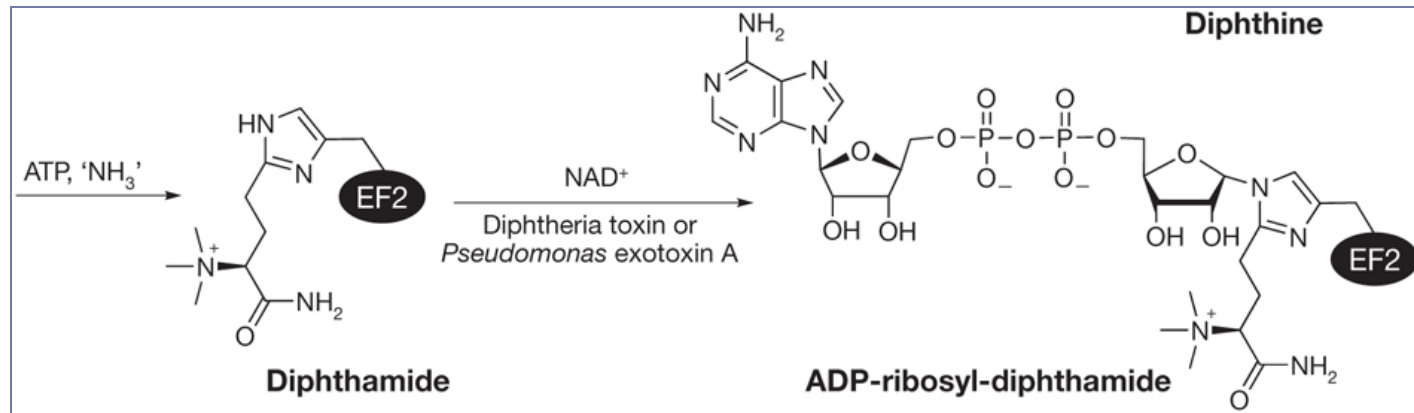
- (1) The binding of the toxin to its cell surface receptor.
- (2) Clustering of charged receptors into coated pits and internalization of the toxin by receptor-mediated endocytosis.
- (3) Acidification of endosomal compartment determined protonation of T subunit (blue) that becomes less hydrophilic causing insertion of the transmembrane domain into the membrane.
- (4) Insertion of domain A (green colour, C-domain in figure) through the membrane and expose A to the cytoplasmic side. Reduction of the disulfide bridge releases the toxin in the cytoplasm.



Toxins 2013, 5(8), 1362-1380; doi:[10.3390/toxins5081362](https://doi.org/10.3390/toxins5081362)

Inactivation of elongation factor-2

The catalytic domain (domain A) transfers the ADP-ribosyl group of NAD to a unusual derivative of histidine residue called **diphthamide** of the **elongation factor EF-2** of eukaryotic cells. This modification blocks protein synthesis of the cells and eventually kills the cells.



The role of **diphthamide** is unknown but it is essential for EF-2 activity and explains why DT is specific only for this protein.

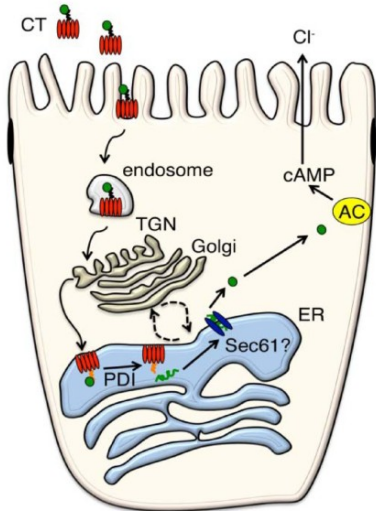


Other bacterial toxins have the same mechanism of action (*P. aeruginosa* exotoxin A)

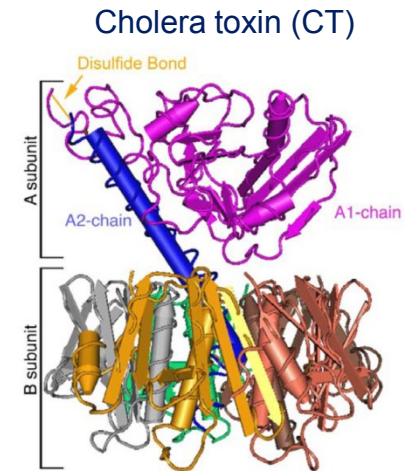
Cholera and pertussis toxins bind to protein G

A-B toxins may act at all level on signal transduction **modifying cell signaling events.**

Cholera toxin (CT) and **pertussin toxin (PT)** are **AB₅ toxins** that have a very similar mode of action: they lock specific **GTP-binding proteins** through an ADP-ribosylation.



CT binds enterocytes, through the interaction of the pentameric B subunit with a specific receptor triggering endocytosis of the toxin. CT traffics via the retrograde pathway through the Golgi to the endoplasmic reticulum (ER).



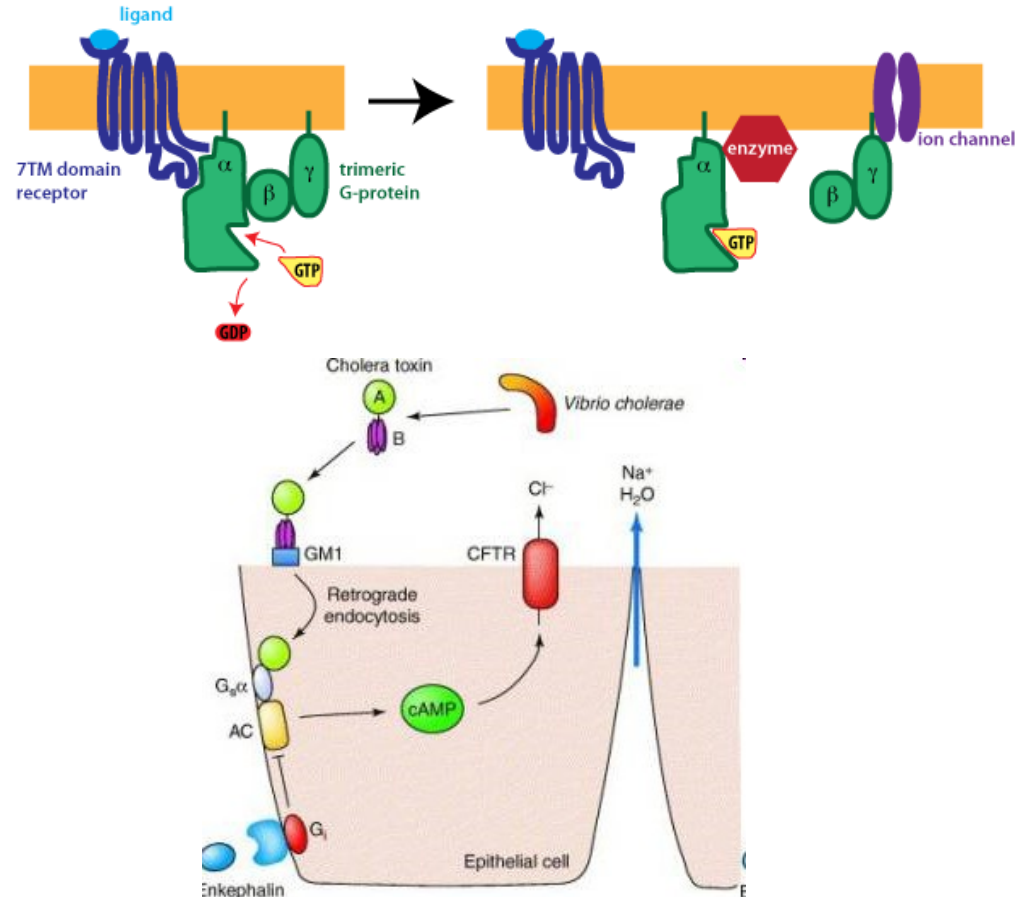
Toxins 2010, 2(3), 310-325;
doi:10.3390/toxins2030310

Once inside the ER, the A subunit (A₁ chain) is released by the B₅ subunit by protein disulfide- isomerase (PDI), recognized by the ER Hsp70 chaperone BiP and retro-translocated into the cytosol. Here, the A₁-chain refolds into its native conformation in the cytoplasm to become an active enzyme.

Cholera toxin activates protein G_s

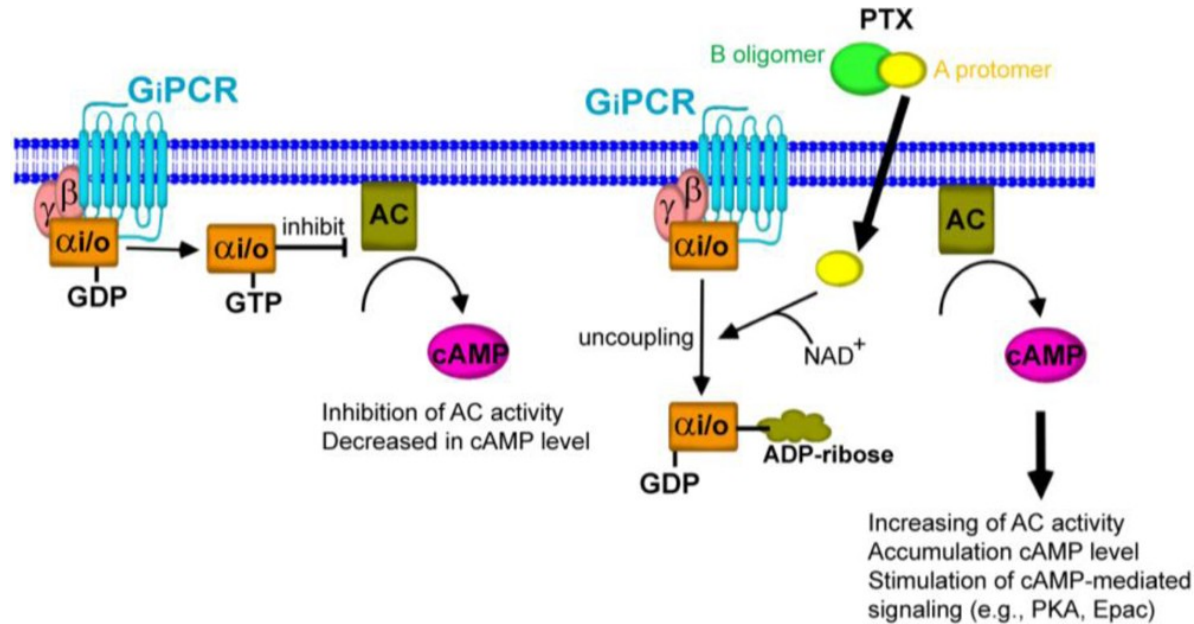
The subunit A₁ subunit enters the cytosol, where it activates the **GTPase α subunit of G proteins G_s** through an **ADP-ribosylation** reaction that acts to lock the G protein in its activated GTP-bound form, thereby continually stimulating adenylate cyclase to produce **cAMP**.

The high cAMP levels activate the cystic fibrosis transmembrane conductance regulator (CFTR), causing a dramatic efflux of ions and water from infected enterocytes, leading to watery diarrhoea.



Pertussis toxin inhibits protein G_i

PT is responsible of highly contagious bacterial disease caused by *Bordetella pertussis*.



Pertussis toxin A subunits transfer ADP ribose group to a Cys in a C-terminal motif of the α subunit of many type of G protein such as G_i .

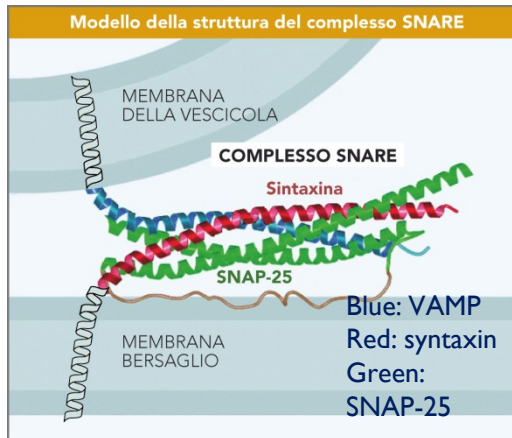
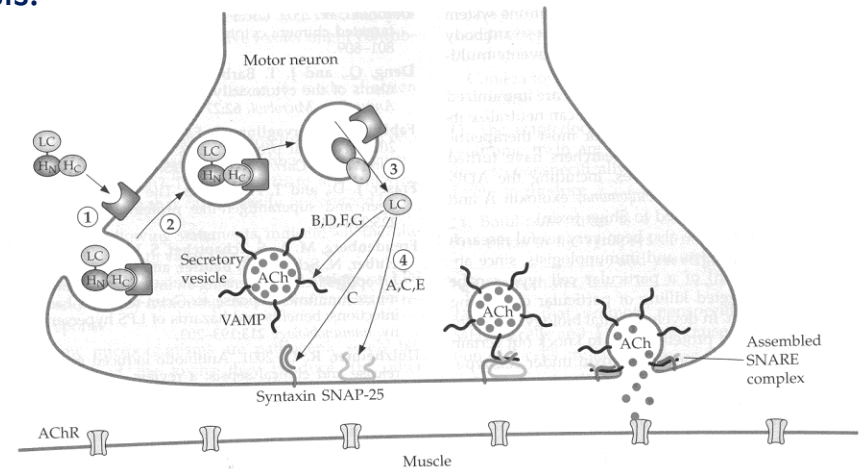
The consequence: the uncoupling of G protein from its receptor results in a G_i subunits in inactive state, always locked with a **GDP-bound**, thus unable to inhibit adenylyl cyclase activity. This leads to increased cellular concentrations of cAMP with systemic effects.

Botulinum and tetanus neurotoxins

Botulinum toxin (BT) produced by *Clostridium botulinum* is an A-B protein toxin, which binds to **presynaptic membranes of motor neurons** blocking release of **acetylcholine** and leading to inhibition of muscle contraction and flaccid paralysis.

BT: large protein containing 2 subunits (LC+HC). Mature toxin (LC) is released by endosomes into neuronal cells.

A chain (LC) is an **endoprotease** that possess a highly conserved zinc-binding motif. Its target are **SNARE proteins** involved in synaptic vesicle fusion at a neuromuscular junction which release the acetylcholine.



Different toxin serotypes cleave the t-SNARE protein synaptobrevin (VAMP), avoiding the vesicle fusion and the release of the neurotransmitter. Other serotypes inactivate SNAP25 or syntaxin. The net effect is that vesicle fusion does not occur and muscle stop contracting.

Tetanus toxin (*Clostridium tetani*); same mechanism to BT but different cell target: it binds to termini of **inhibitory interneurons** preventing the release of glycine; Its proteolytic activity causes a failure in the inhibitory pathway and spasm in which muscle is continuously contracted.

Enzymes for tissue penetration and dissemination

Many pathogenic bacteria produce factors that help them to disseminate from the site of entry into the body tissues. Not considered toxins because they act next to the producer bacteria.

The main factors include one or more of these:

DNAses: degrade DNA, thereby thinning the viscous material (pus) consisting of DNA and protein from dead phagocytes and other cells that may trap some bacteria

Enzymes similar to collagenases, elastases which degrade connective tissue matrix proteins, making it easier to spread outward from the area and into areas that contain healthy tissues.

Hyaluronidases: that degrade the charged polysaccharide hyaluronic acid

“Spreading factors”: other proteases helping the bacterial dissemination. Es. Streptococcal **streptokinase** protein: acts as a **plasminogen activator**, a serine proteases that cleaves plasminogen into plasmin which degrade fibrin clots. Bacteria escape from blood clots.

