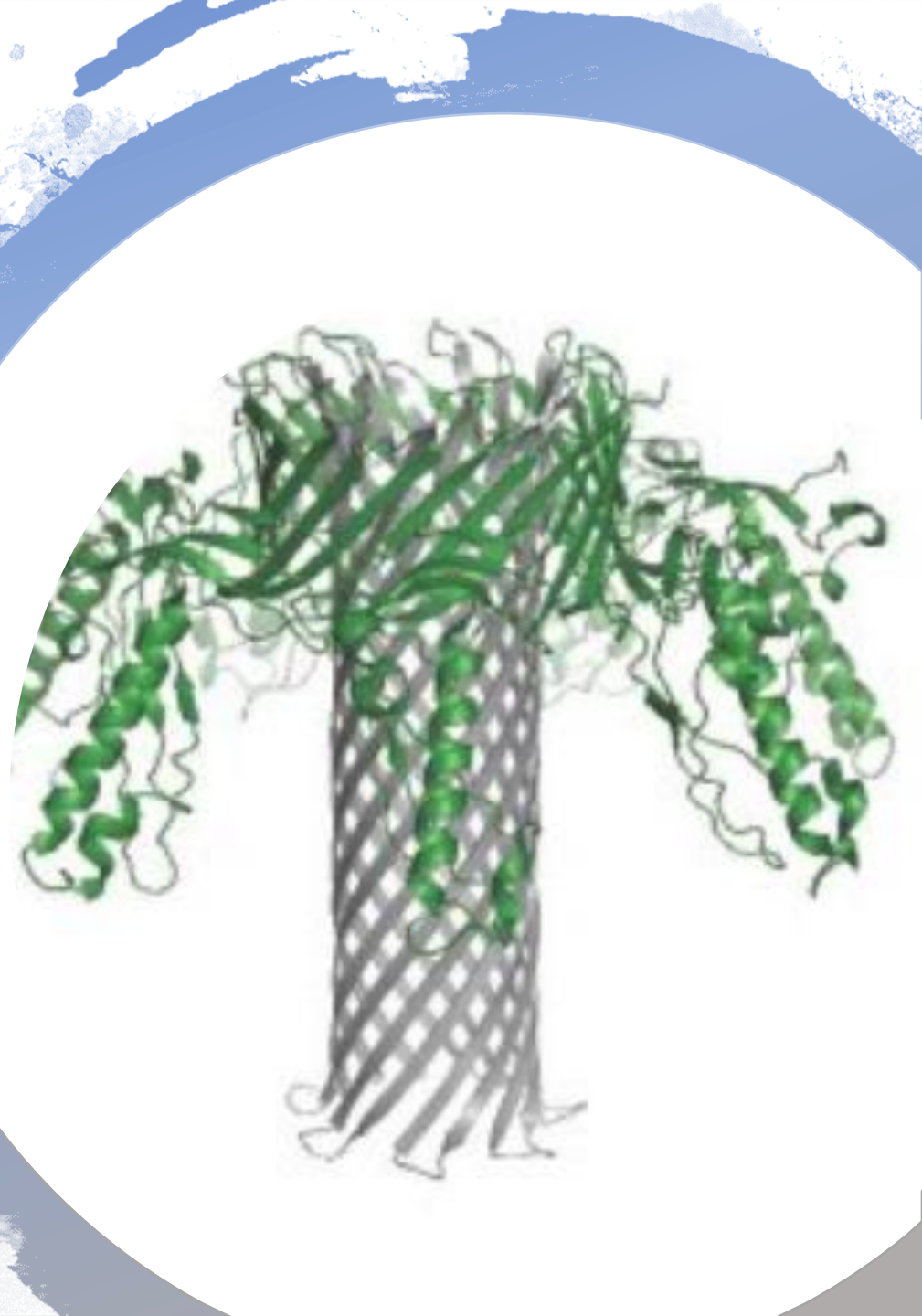
Bacterial toxins



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Introduction

- Bacterial toxins are powerful poison
  - Tetanus and botulinum neurotoxins
- Bacterial toxins are numerous
- Bacterial toxins act in different ways

What is a toxin?

- Poisonous substance produced within microorganisms or other living organisms.
- Name used first by an organic chemist Ludwig Brieger from the word “toxic”.
- Two kinds of toxins produce by bacteria:
  - lipopolysaccharides (LPS) are cell-associated toxins released after disruption of the cell : endotoxins
  - protein toxins synthesized inside the cells and then released to the target cells : exotoxins
- Various species: Gram positive / Gram negative

Toxin production by bacteria likely serves to:

(1) protect against phagocytosis by predatory cells

(2) aid in penetrating tissue barriers

(3) promote nutrient release

(4) alter cellular architecture and metabolism in ways that facilitate the establishment of a niche for colonization and replication.

Toxins act in vicinity of their production or at distance

- At distance : Secretion by bacteria in a soluble form and diffusion in the aqueous environment of body fluids (for examples: respiratory secretions, interstitial fluid surrounding infected tissues, lymph, and blood)
- In vicinity: injected directly inside the eukaryotic cells or produced by intracellular bacteria inside the eukaryotic cells

Where are the toxin genes located?

- On Chromosome
  - Ex : botulinum toxin
- On Plasmids
  - toxin of intestinal *Escherichia coli* pathotypes (enterotoxigenic *E. coli*)
  - Anthrax toxin
  - Tetanus neurotoxin
  - ….
- On Bacteriophages
  - toxin of *Vibrio cholerae* on phage CTX
  - Toxin of *Corynebacterium diphtheriae* on phage B
  - …

Strains of the same species could be toxigenic or not

Toxin genes are sometimes located on Pathogenicity island

- Definition of pathogenicity island:

Cluster of genes conferring virulence and acquired by microorganisms through horizontal gene transfer

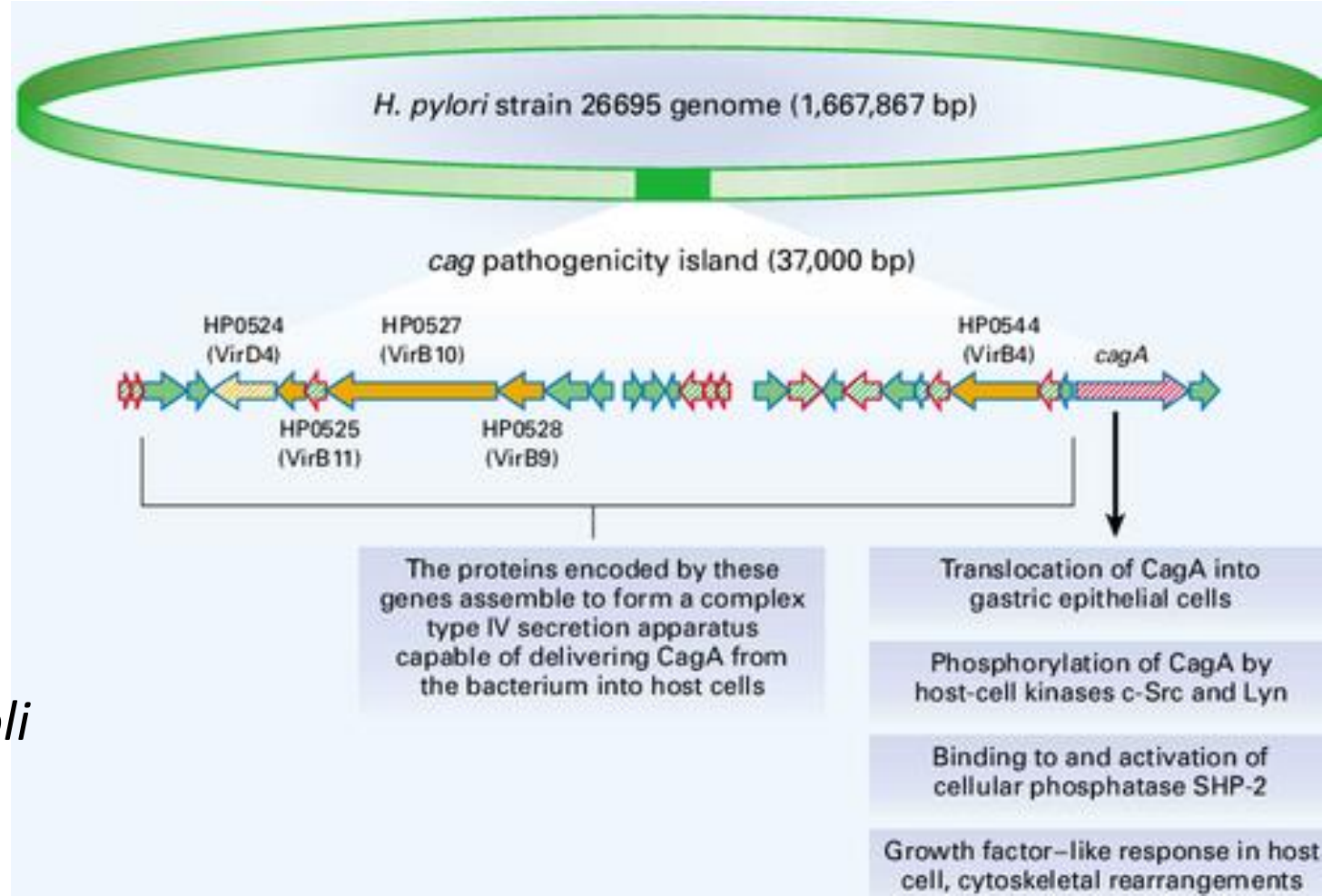
- Pathogenicity island are flanked by direct repeats, insertion sequences or tRNA genes, which act as sites for recombination into the DNA

- Examples:

- the cag pathogenicity island in *Helicobacter pylori*

- PAI I539 of Uropathogenic *Escherichia coli*

- …



3 Classes of bacterial toxins

- **Pore forming toxins**

- **Toxins with extracellular targets**

  Membrane-damaging and cytotoxic sphingomyelinases and phospholipases

- **Toxins with intracellular targets**

One genius presents a lot of toxin producers : genius clostridium

Pore forming toxins

Membrane-damaging and cytotoxic sphingomyelinases and phospholipases

Espèces	Toxine
Cytotoxines se liant au cholesterol	
C. bifermentans	bifermentolysin
C. botulinum	botunolysin
C. chauvei	chauveolysin
C. histolyticum	histolycolysin
C. novyi	novyilysin
C. perfringens	perfringolysin
C. septicum	septicolysin
C. sordellii	sordellilysin
C. tetani	tetanolysin
Autres toxines formant des pores	
C. septicum	Alpha-toxine
C. perfringens	Beta1-, beta2-toxine
C. perfringens	delta-toxine
C. perfringens	epsilon-toxine
C. perfringens	enterotoxine
Activité phospholipase	
C. absonum	PLC
C.baratii	PLC
C. bifermentans	PLC
C. novyi	Beta-toxine
C. perfringens	alpha-toxine
C. sordellii	PLC
Protéases	
C. histolyticum	Collagénases
C. perfringens	Collagénases

From Popoff M.

Toxins with intracellular target

Espèces	Toxine	Activité enzymatique	Substrat
Toxines binaires			
C. botulinum C et D	C2 Toxine	ADP-ribosylation	Actin G cellulaire
C. difficile	Transferase	"	Actin G cellulaire et musculaire
C. perfringens	Iota toxine	"	Actin G cellulaire et musculaire
C. spiroforme	C. spiroforme toxine	"	Actin G cellulaire et musculaire
Toxines de haut poids moléculaires			
C. difficile	Tox A	Glucosylation	Rho, Rac, Cdc42
	Tox B	"	Rho, Rac, Cdc42
C. novyi	Alpha-toxine	"	Rho, Rac, Cdc42
C. sordellii	LT	"	Rho, Ras, Cdc42
	HT	"	Rho, Rac, Cdc42
Neurotoxine			
C. botulinum A	BoNT/A	Protease zinc-dépendente	SNAP25
C. botulinum B	BoNT/B	"	VAMP
C. botulinum C	BoNT/C	"	Syntaxine, SNAP25
C. botulinum D	BoNT/D	"	VAMP
C. botulinum E	BoNT/E	"	SNAP25
C. botulinum F	BoNT/F	"	VAMP
C. argentinense	BoNT/G	"	VAMP
C. tetani	TeTx	"	VAMP

I. Pore forming toxins (PFTs)

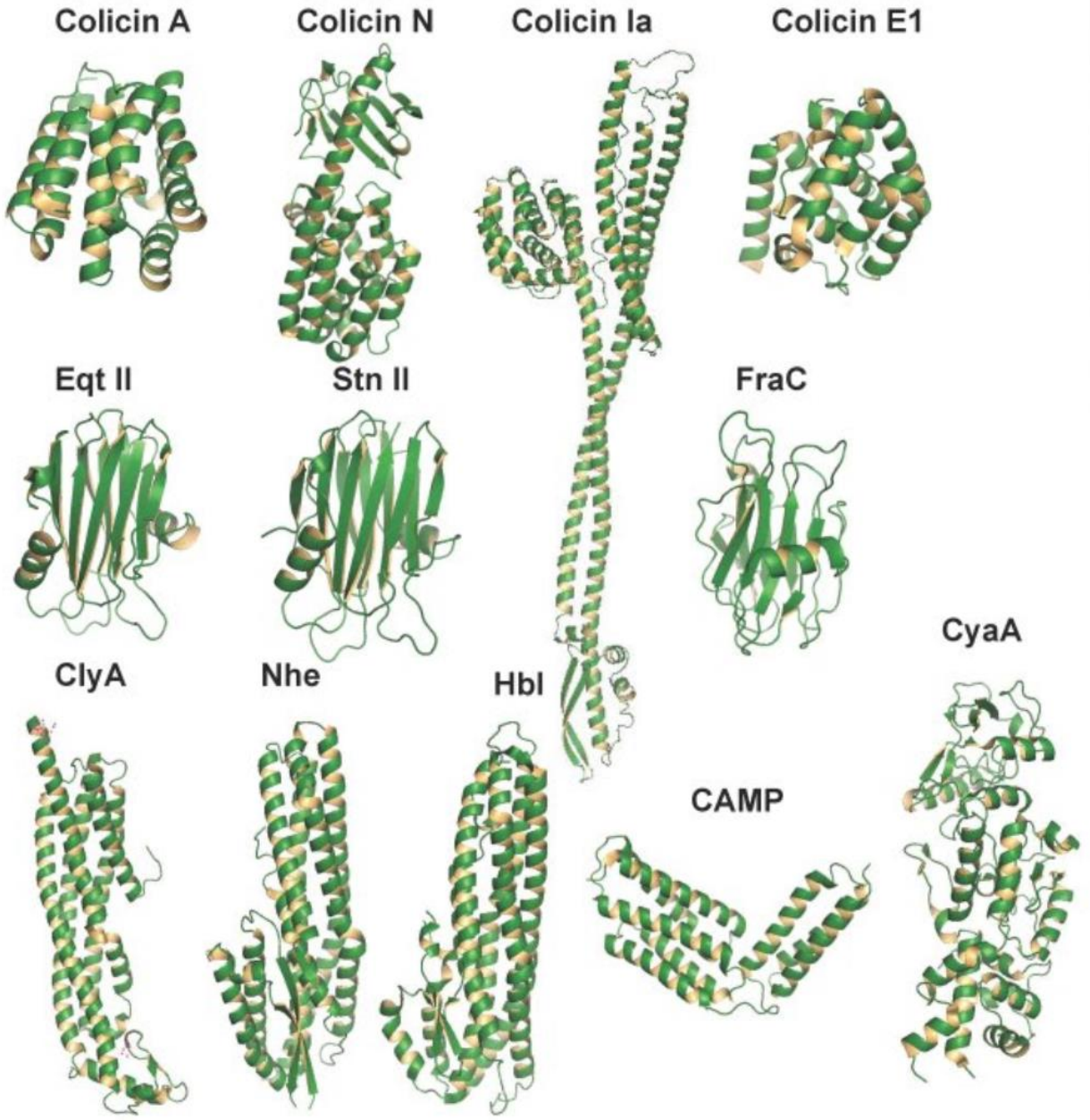
- Largest class of bacterial toxins
- Common virulence mechanism in a wide range of pathogenic bacteria (gram positive or negative)
- The pore produced by PFTs alters membrane permeabilization which allows small molecules such as ATP, specific ions, or large molecules proteins to pass through.

  -> one of the ancient cell killing mechanisms by which bacteria invade human organism

Structures of the PFTs

Based on the secondary structure of the transmembrane motif, PFTs can be categorized in:

- α-PFTs:
  - upon binding to the membrane, α-helices undergo a conformational change to insert into the membrane and form membrane pore



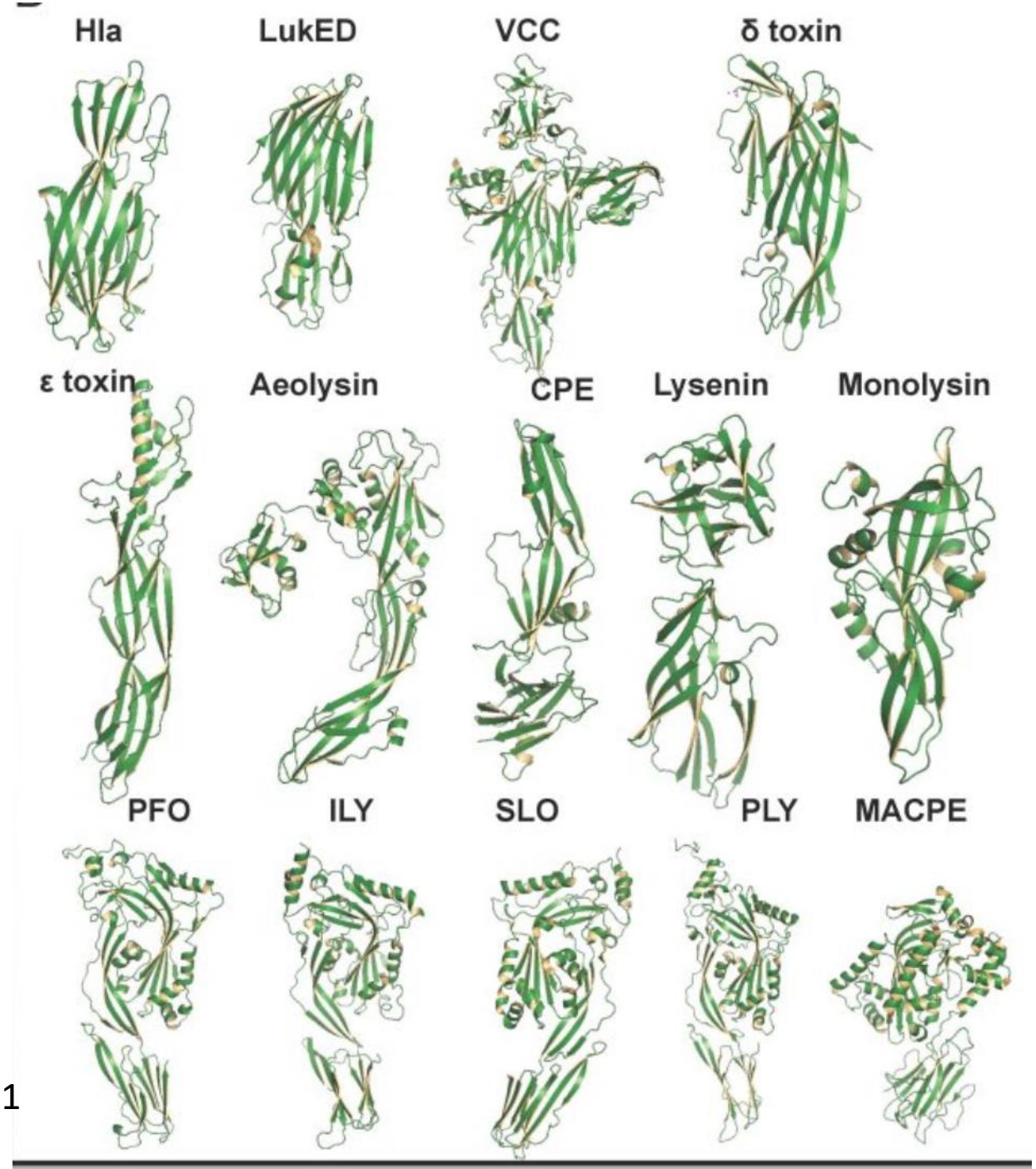
Li et al, Toxin 2021

Structures of the PFTs

- β-PFTs:

- composed mostly of β-strand-based domains

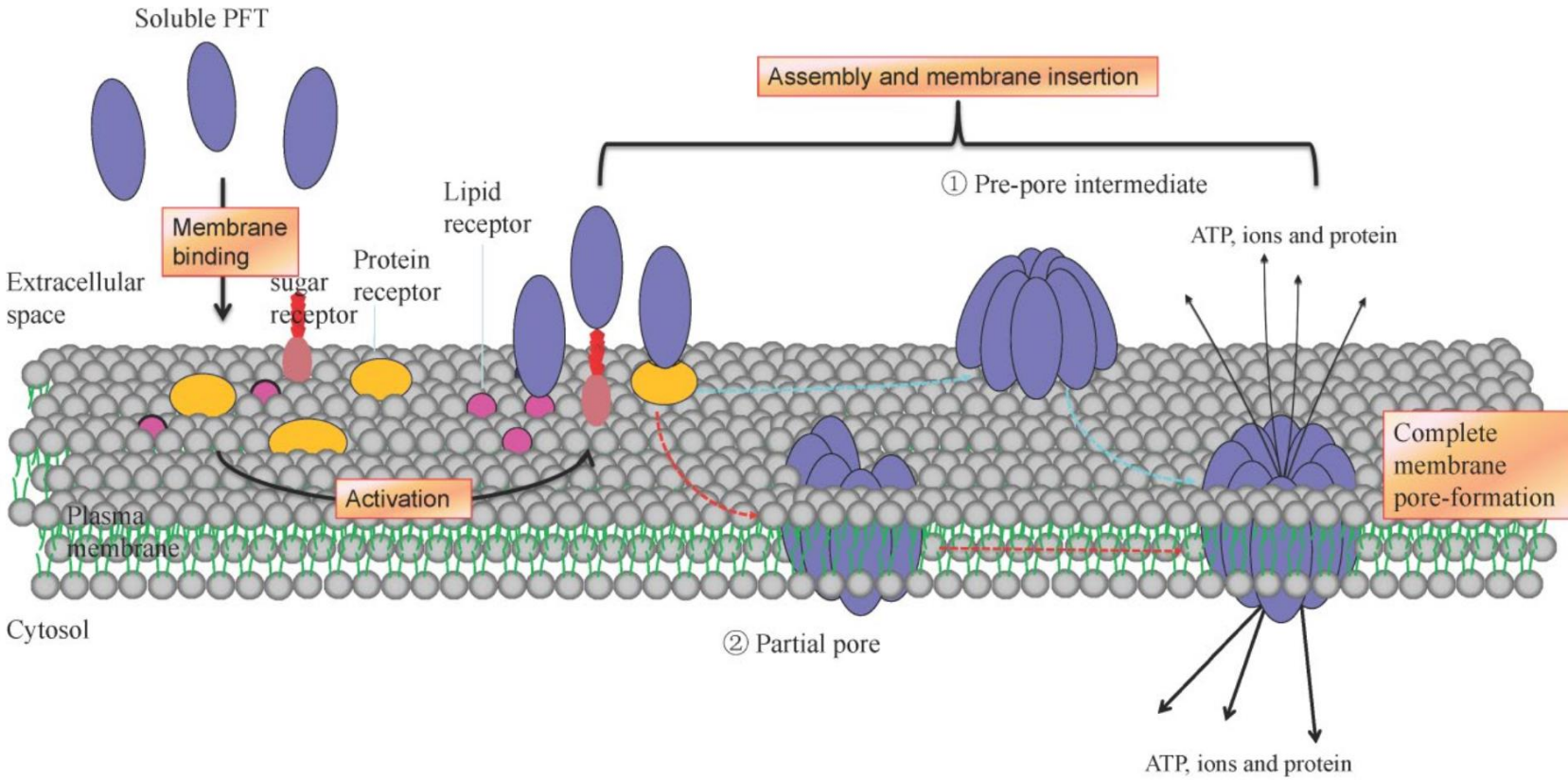
- monomers come together to create a mushroom-shaped pore



Li et al, Toxin 2021

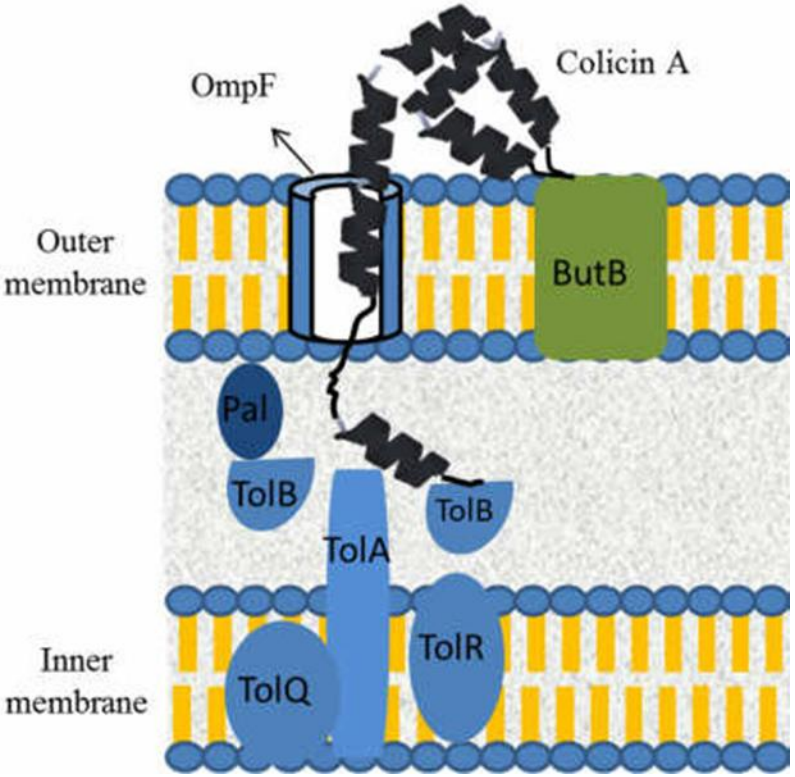
General mechanism of membrane pore formation by PFTs

- 1) Folding into a water-soluble, monomeric structure
- 2) Binding to specific receptors (sugars, lipid, or proteins) in the membrane
- 3) Oligomerization to form transmembrane pores with refined architecture (2 ways)
  - sequential oligomerization (α-PFT)
  - pre-pore intermediate structure (β-PFT)
- 4) pore alters membrane permeability and allows small molecules to pass through

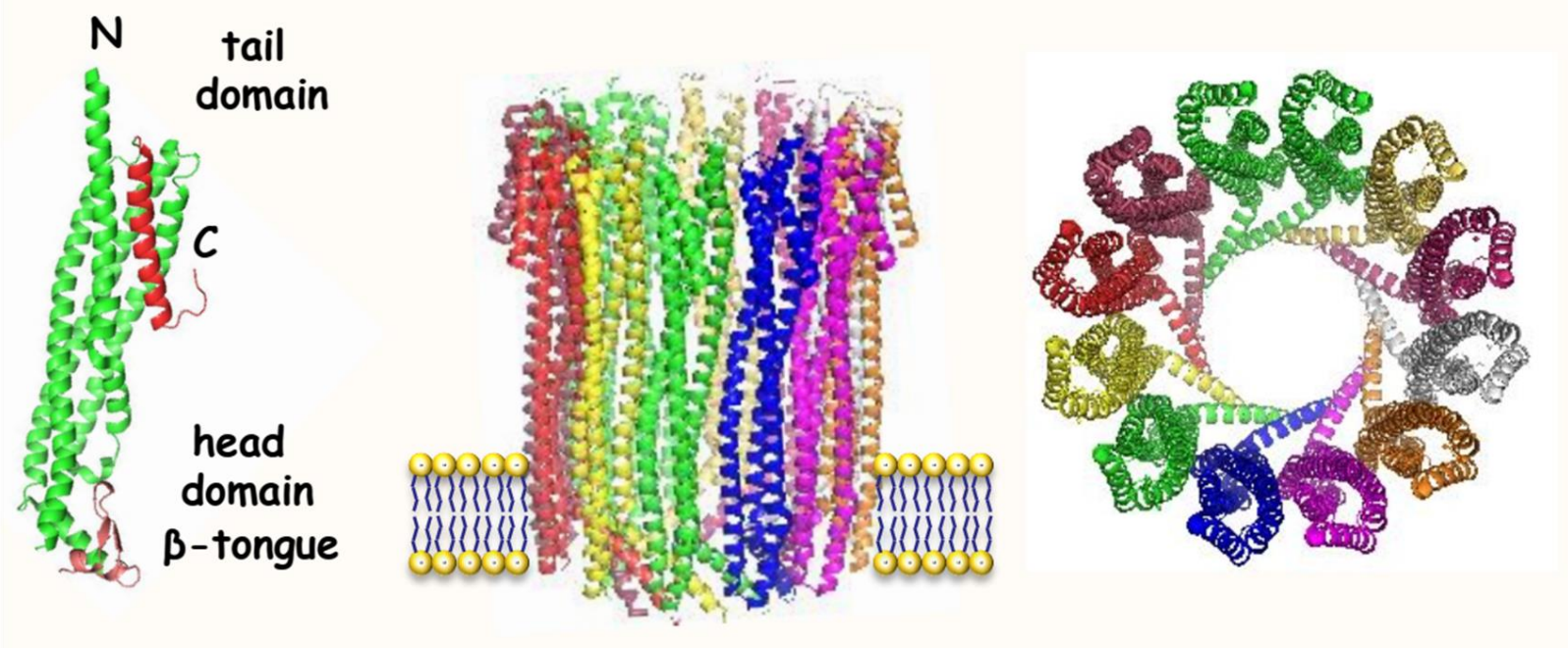


Examples by family

- α-PFTs
- A) Colicins: typical α-PFTs generally produced by *Escherichia coli*
  - 25 different colicin members have been identified
  - Toxic to other bacteria (bacteriocin)
  - production induced during stress conditions
  - Forms ion-permeable channels in the cytoplasmic membrane with its C-terminal part



- B) The Cytolysin A Subfamily
  - produced by *E. coli, Salmonella enterica* and *Shigella flexneri.*
    - High concentration : lysis of cells. Help bacteria to penetrate tissue.
    - Low concentration : affect intracellular signaling processes regulating physiological response. Help the bacteria to penetrate cells.



• β-PFTs

- β-PFTs are secreted by a wide variety of pathogenic bacteria
  - toxins of commercial interest for the control of pest insects

1) Hemolysins/Leukocidines: lysing red blood cells or leukocytes

Examples:

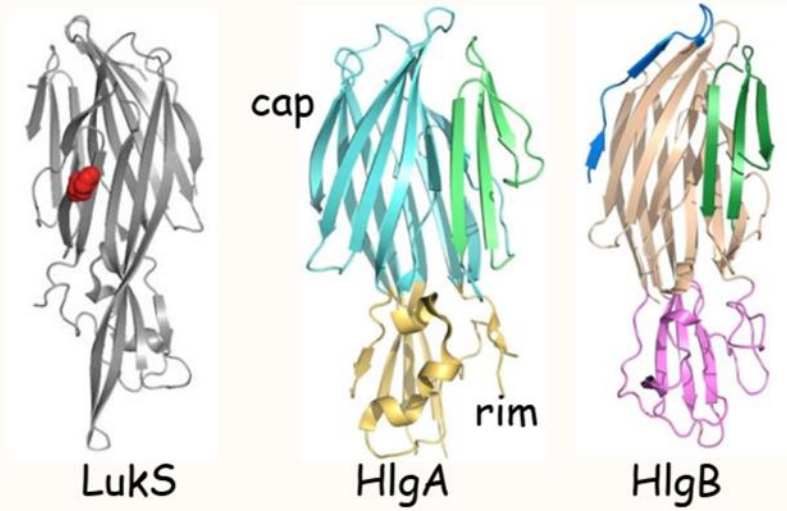
- *S. aureus* α-hemolysin (Hla) with a single component assembling into heptameric pores

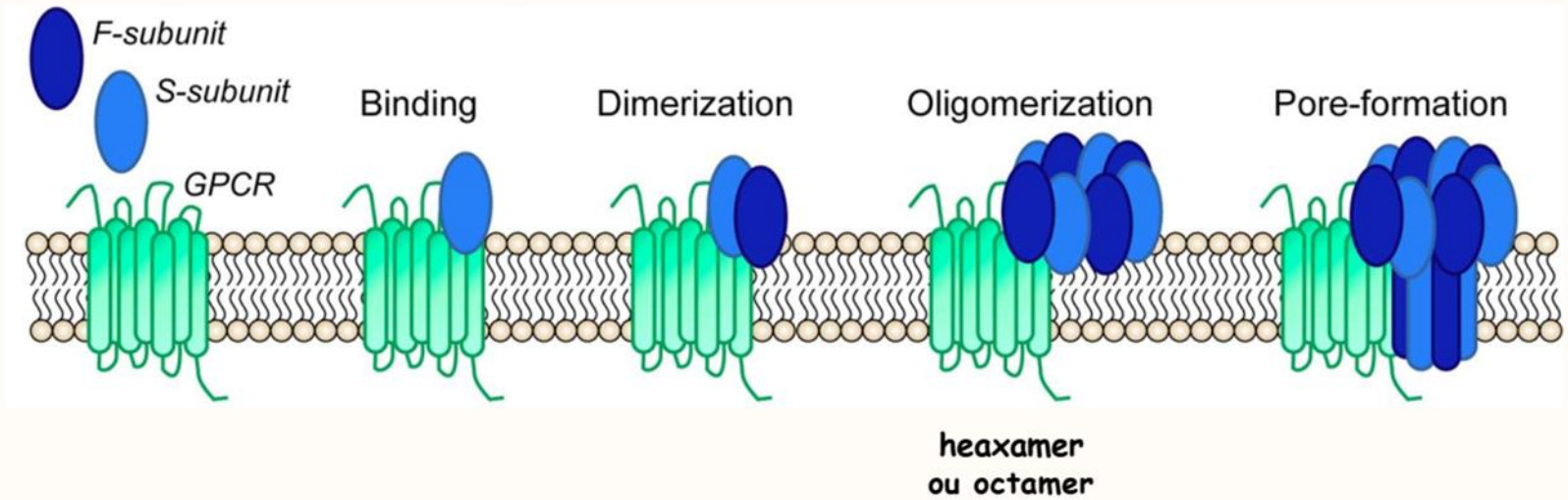
- *S. aureus* Panton-Valentine leukocidin (PVL) and leukocidin ED (LukED) with two components assembling into octameric pores with four copies of each subunit

Hla

LukED

LukS-LukF Panton-Valentine toxin
LukE-LukD
LukA-LukB (or LukH-LukG)
LukM-LukF
LukP-LukQ
γ-hemolysins HlgA-HlgB
HlgB-HlgC





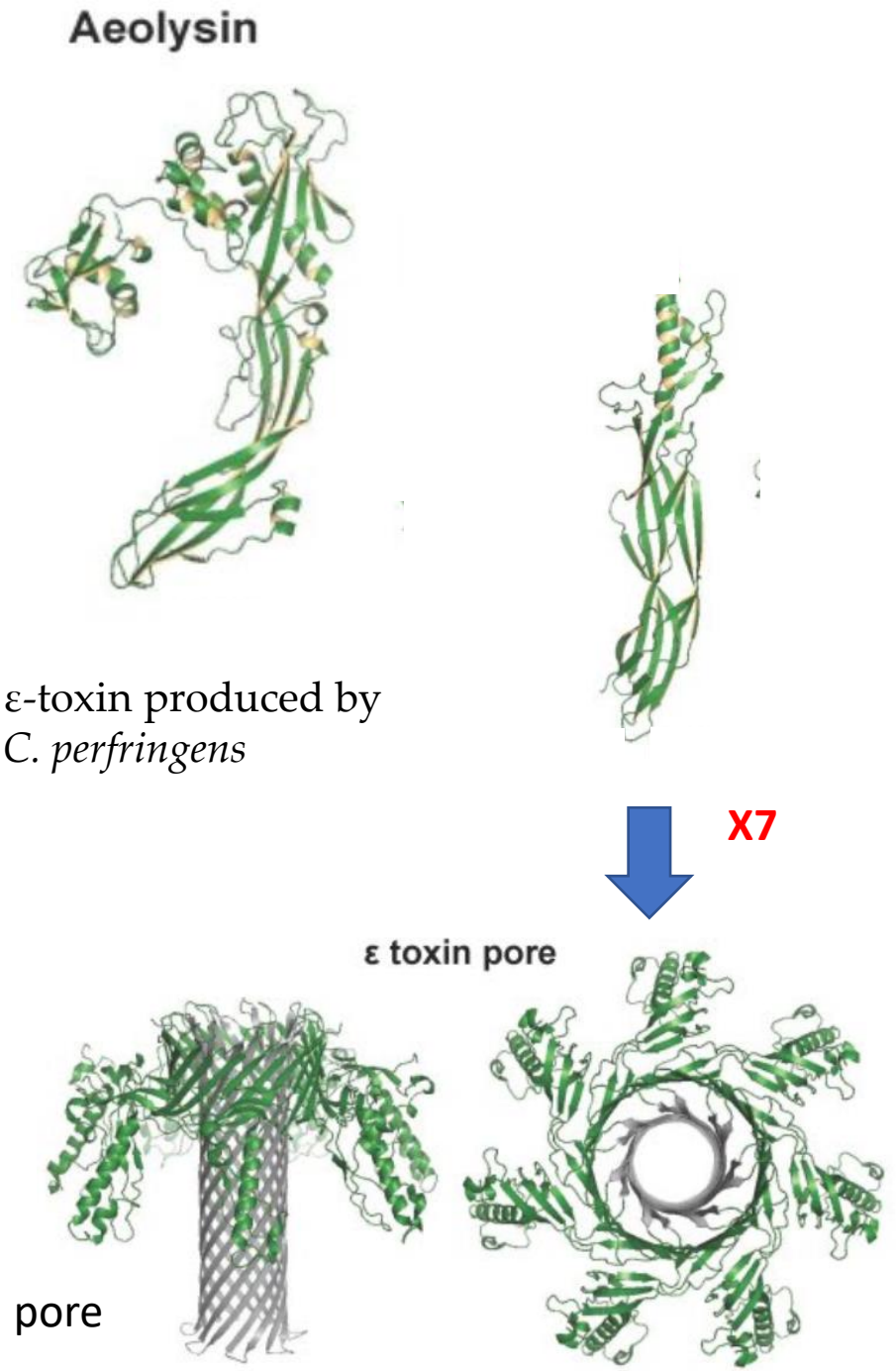
- β-PFTs

2) Aerolysins:

- *Clostridium perfringens* ε-toxin, *Clostridium septicum* α-toxin, *Pseudomonas entomophila* monalysin, *B. thuringiensis* parasporin …

- The monomers assemble into a pre-pore structure with heptameric oligomer docking on the membrane surface.

- Aerolysin pores are relatively small, with the diameter ranging from 1–4 nm.



II. Membrane-damaging and cytotoxic sphingomyelinases and phospholipases

- Bacterial sphingomyelinases (SMases) and phospholipases (PLases) are esterases
  - hydrolysis of ester bonds (between an alcohol or phenol and an acid)

- Target : glycerophospholipids, sphingolipids, and cholesterol of the cellular membrane

- Contribute to the development of disease in different ways:
  - tissue destruction that contributes to bacterial colonization and dissemination,
  - plasma membrane integrity loss and cell lysis, providing some essential nutrients (iron).
  - generating bioactive lipid products that activate endogenous mediators of cell death
  - cleave phospholipids from the pulmonary surfactant releasing products to be used as a carbon source.
  - membrane disruption in vacuoles, allowing bacterial escape from phagosomes (for intracellular pathogens)

TABLE 1 Distribution of SMases and PLases which play a role in virulence among bacterial phyla

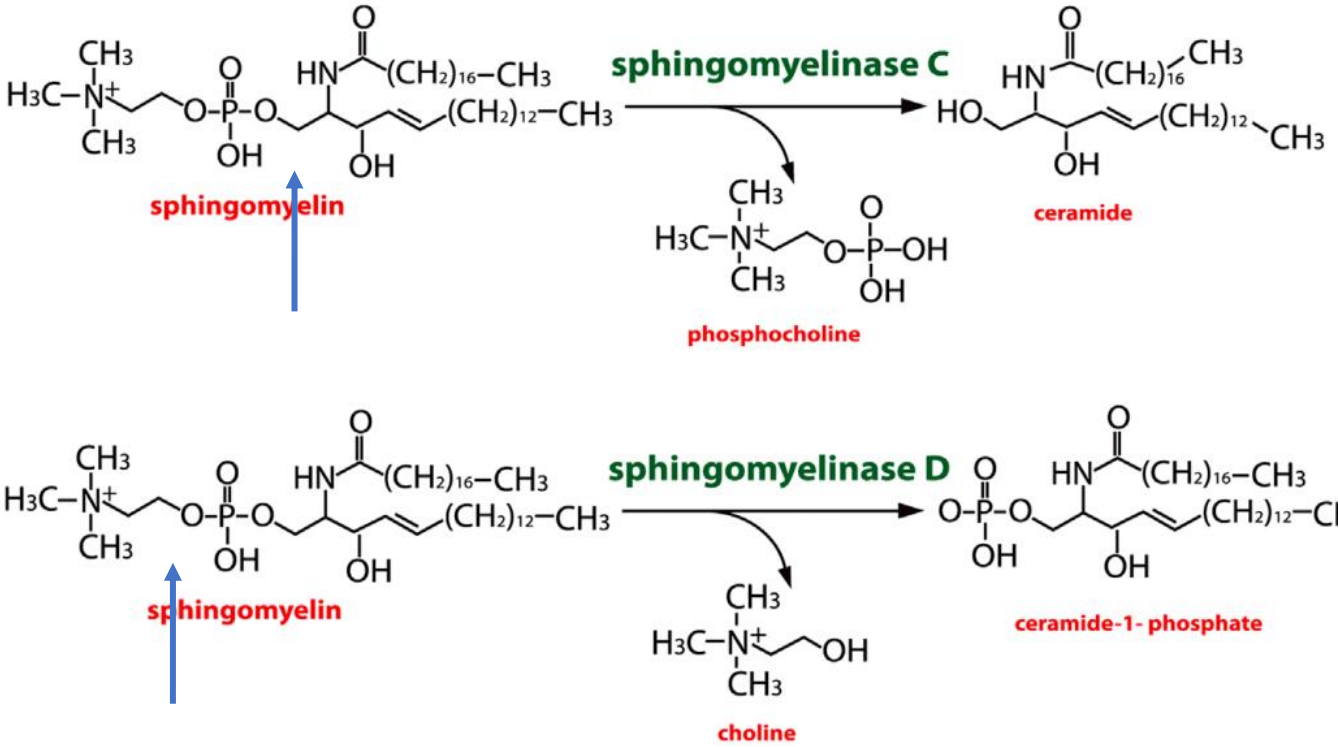
Enzyme type	Bacterial phylum (class) with a role in virulence
Sphingomyelinase Cs	*Proteobacteria* (*Gammaproteobacteria*, *Epsilonproteobacteria*), *Spirochaetes*, *Actinobacteria*, *Firmicutes* (*Bacilli*), *Chlamydiae*
Sphingomyelinase Ds which adopt a TIM barrel structure	*Proteobacteria* (*Betaproteobacteria*), *Actinobacteria*
Other sphingomyelinase Ds	*Proteobacteria* (*Gammaproteobacteria*)
Acyl hydrolases which adopt an antiparallel β-barrel structure	*Proteobacteria* (*Betaproteobacteria*, *Gammaproteobacteria*, *Epsilonproteobacteria*)
Surface-associated PLA_2s	*Proteobacteria* (*Gammaproteobacteria*)
Acyl hydrolases from the SGNH esterase family	*Proteobacteria* (*Gammaproteobacteria*, *Betaproteobacteria*), *Actinobacteria*
Acyl hydrolases which adopt the α/β hydrolase fold	*Proteobacteria* (*Gammaproteobacteria*, *Alphaproteobacteria*), *Actinobacteria*
Secreted patatin-like acyl hydrolases	*Proteobacteria* (*Gammaproteobacteria*, *Alphaproteobacteria*), *Actinobacteria*
Class XIB phospholipase A_2s	*Proteobacteria* (*Alphaproteobacteria*), *Firmicutes* (*Bacilli*, *Clostridia*)
PI-specific phospholipase C	*Firmicutes* (*Clostridia*, *Bacilli*), *Spirochaetes*, *Actinobacteria*
Zn^{2+} metallophospholipase C with SMase C activity	*Proteobacteria* (*Gammaproteobacteria*), *Firmicutes* (*Clostridia*, *Bacilli*)
Phospholipase C from the acid phosphatase superfamily	*Proteobacteria* (*Gammaproteobacteria*, *Betaproteobacteria*), *Actinobacteria*
Other phospholipase Cs	*Proteobacteria* (*Gammaproteobacteria*)
Phospholipase D	*Proteobacteria* (*Betaproteobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Epsilonproteobacteria*), *Actinobacteria*, *Chlamydiae*

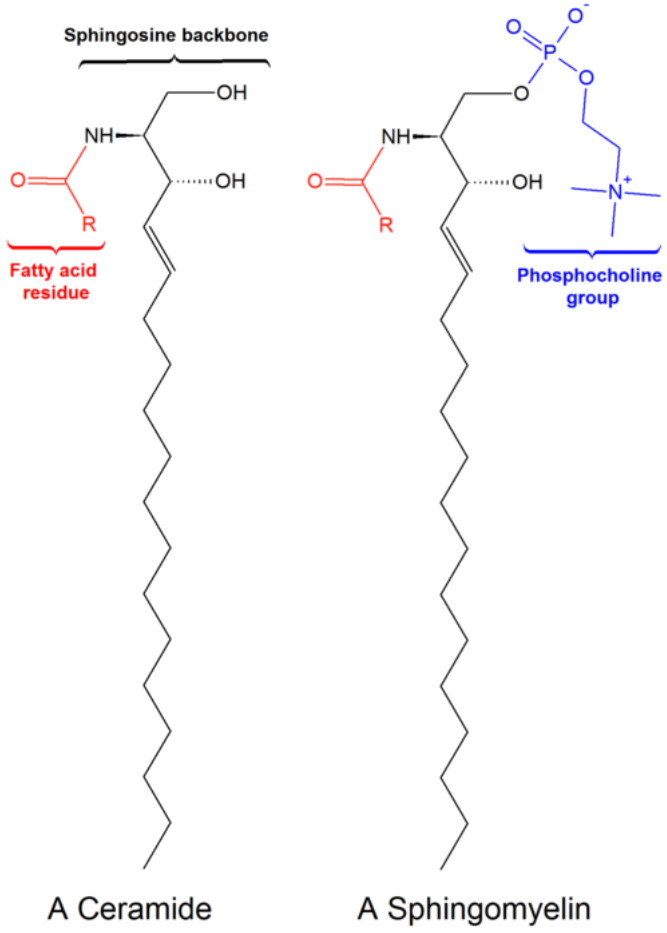
Flores-Díaz M, Monturiol-Gross L, Naylor C, Alape-Girón A, Flieger A. 2016.
Bacterial sphingomyelinases and phospholipases as virulence factors. Microbiol Mol Biol Rev 80:597–628

A) Sphingomyelinases (SMases)

- Cleave sphingomyelins (eukaryotic membrane sphingolipids).
- Sphingomyelins have a phosphocholine moiety attached to ceramide (sphingosine derivative with an N-linked fatty acid of variable length and saturation).

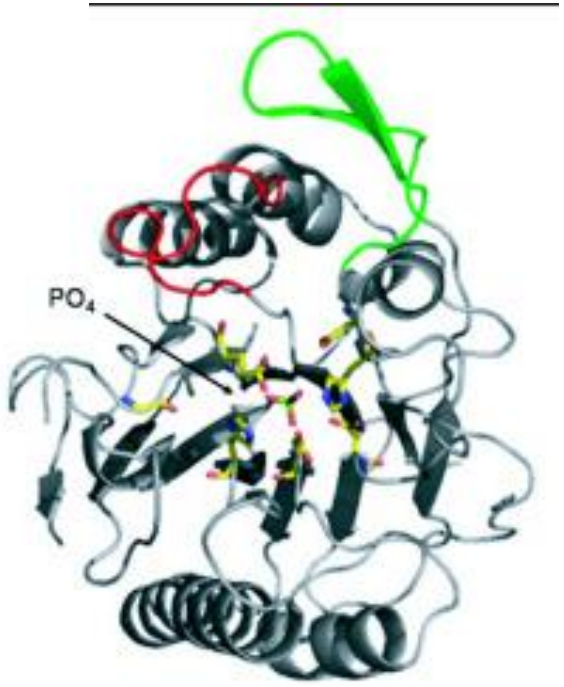
- 2 types of SMases :
  - SMase Cs hydrolyze the ester bond between ceramide and phosphorylcholine,
  - SMase Ds hydrolyze the phosphodiester bond between Cer-1-phosphate and choline.





1) SMases C

- Bind and lyse red blood cells : aid iron acquisition from heme groups
- Cytotoxicity is mediated by:
  - changes in the physical properties of the membrane induced by the increase in the ceramide content = Cer rich domain causing lysis
  - apoptotic effect induced by the activation of Cer-dependent pathways.



- Example of SMaseC in *Bacillus cereus*:

-
  -> play a crucial role in the evasion from the host innate immune system during early stages of infection decreasing macrophage activities.

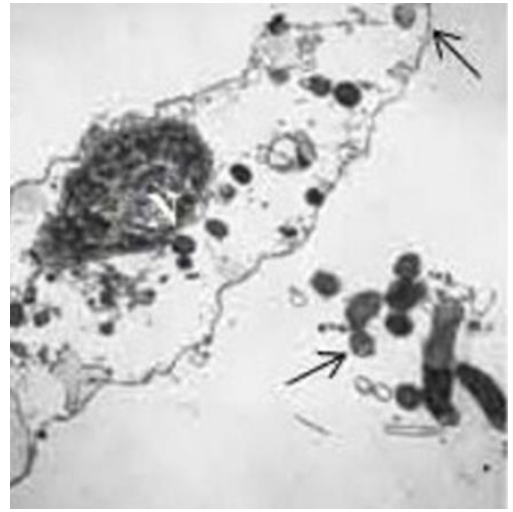
2) SMases D

- The first bacterial SMase D was isolated from supernatants of a pathogenic strain of *Corynebacterium pseudotuberculosis*

- 8 helices lying on the protein exterior and alternating with 8 parallel - strands that form the central barrel

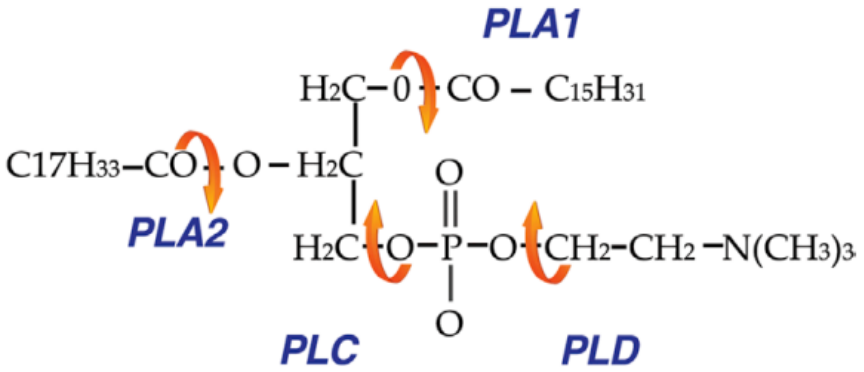
- Example: the SMase D of *Arcanobacterium haemolyticum*

SMase D generates Cer-1-phosphate within the plasma membrane, inducing the rearrangement of lipid rafts, which could enhance bacterial adhesion to the membranes of the host cells, favoring bacterial invasion.



PLD induces host cell damage by necrosis (Transmission electron micrographs of HeLa cells). Arrows indicate bacteria.

B) Phospholipases



- secreted or membrane-bound enzymes

- 4 major groups (A to D) based on their site of cleavage within the phospholipids.

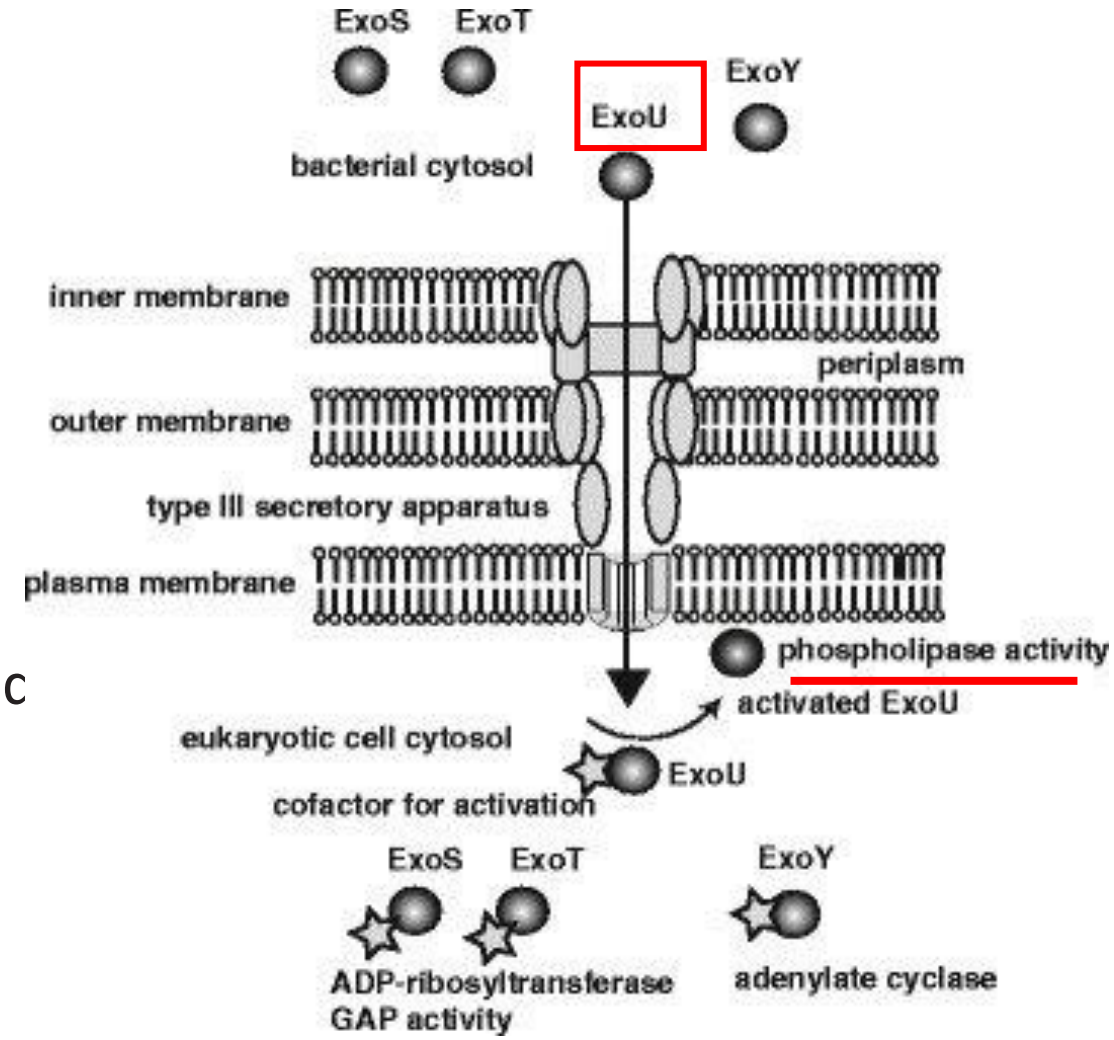
  The polar head group attached to the diacylglycerol (DAG) categorizes the different glycerophospholipids.

  4 types: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), or phosphatidylinositol (PI)

- Several bacterial pathogens utilize phospholipases for invasion of host cells, virulence, and initiation of a proinflammatory response
  - *Listeria monocytogenes, Pseudomonas aeruginosa, Legionella pneumophilia….*

Example1: the patatin-like PLases A2 ExoU of *P. aeruginosa*

- structure similar to that of the potato tuber storage protein and lipid acyl hydrolase patatin
- identified in lung pathogens *P. aeruginosa*, *L. pneumophila*, and *M. tuberculosis*
- *P. aeruginosa* exotoxin U (ExoU) is translocated into the cytosol of the host cell by a T3SS
- ExoU requires activation by three cofactors: the superoxide dismutase, ubiquitin or ubiquitin-modifiec proteins, and Phosphatidylinositol 4,5-bisphosphate
- Targets a broad range of substrates, including PC, PE, ….

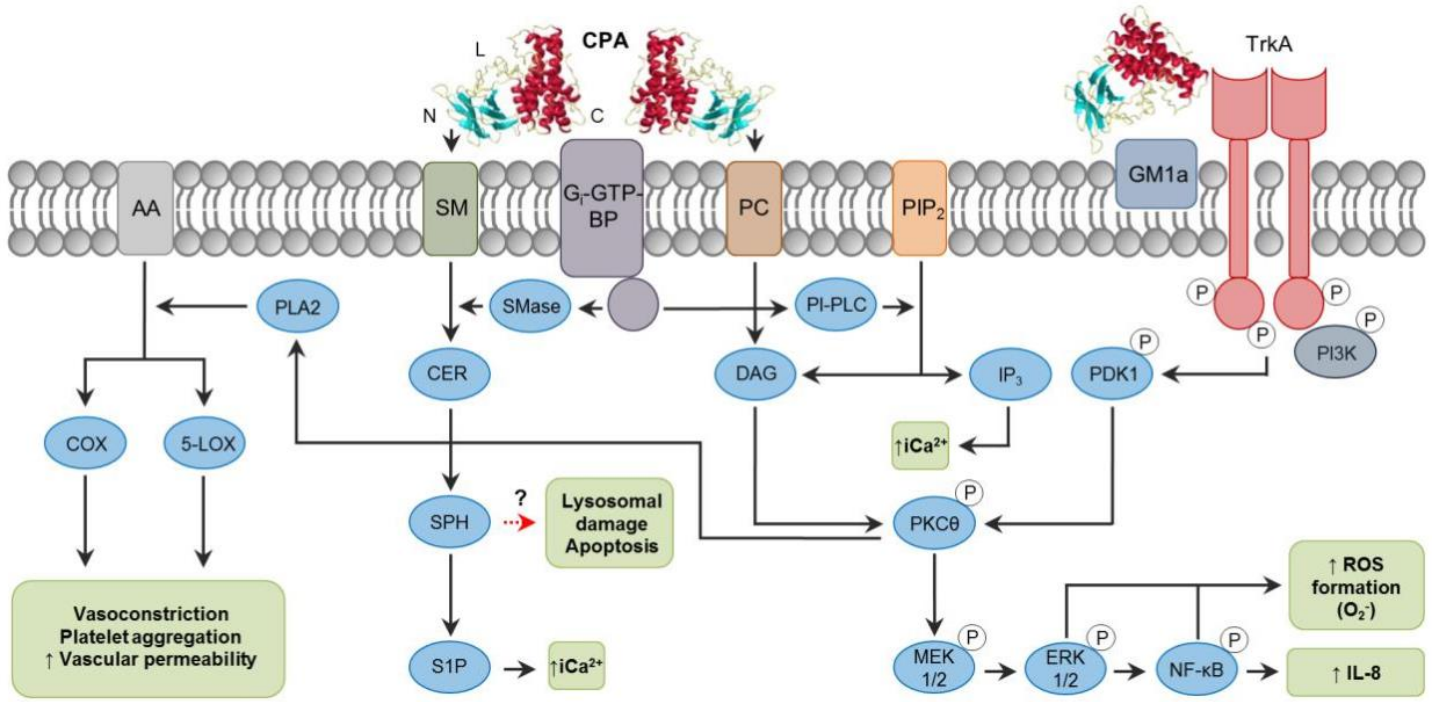


Example2: the α-toxin or CPA of *Clostridium perfringens (PLC)*

C. perfringens is the causative agent of gas gangrene characterized by severe myonecrosis, intravascular leukocyte accumulation, and significant thrombosis

Mechanism of action :

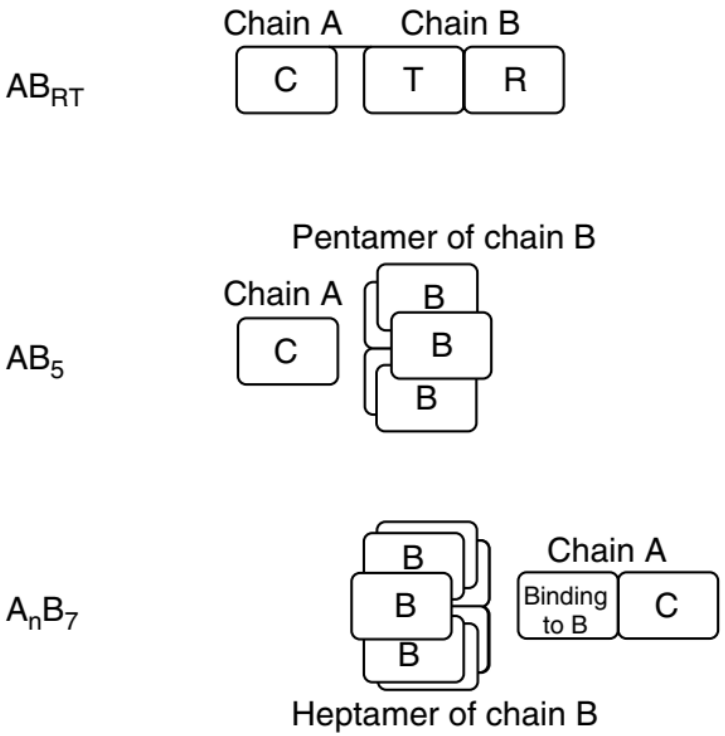
- Membrane disruption : CPA hydrolyzes PC and SM in the plasma membrane, producing diacylglycerol (DAG) and ceramide (CER)
- DAG activates protein kinase C (PKC) and leads to superoxide production, oxidative stress and cytokines production (MEK/ERK and NFKB pathways)
- The interaction of the ganglioside-binding site of the toxin with tropomyosin receptor kinase A (TrkA) on cell membranes results in activation of the MEK/ERK and NFKB pathways.



- High inflammation

III. Toxins with intracellular targets

- Structure of the toxins:
  - AB_{RT}
    - Chain A : formed of a catalytic domain
    - Chain B : formed of a receptor-binding domain (R) responsible for binding to the cell surface, receptor-mediated internalization, and intracellular trafficking and a translocation domain (T) responsible for passage of the C domain inside the cytoplasm
  - AB_5
    - Chain A : formed of a catalytic domain
    - Chain B : receptor-binding domain (R) responsible for binding to the cell surface, receptor-mediated internalization, and intracellular trafficking
  - A_nB_7
    - Chain A : formed of a catalytic domain and a binding domain to chain B
    - Chain B : idem AB_5 type toxin

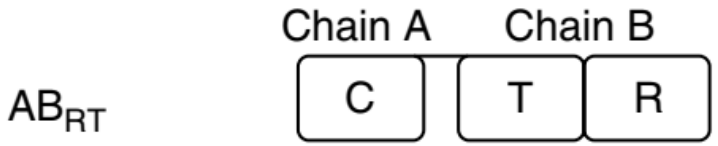


Structure/function organization of toxins with intracellular targets (Perrier et al, 2006)

Process in commun

- 1) Binding to a receptor
- 2) Internalization and directed to given intracellular compartments depending on their receptors
- 3) translocation of their catalytic domain through the membrane of given cell compartments into the cell cytoplasm.
- 4) enzymatic activity

A) The AB_{RT} toxins

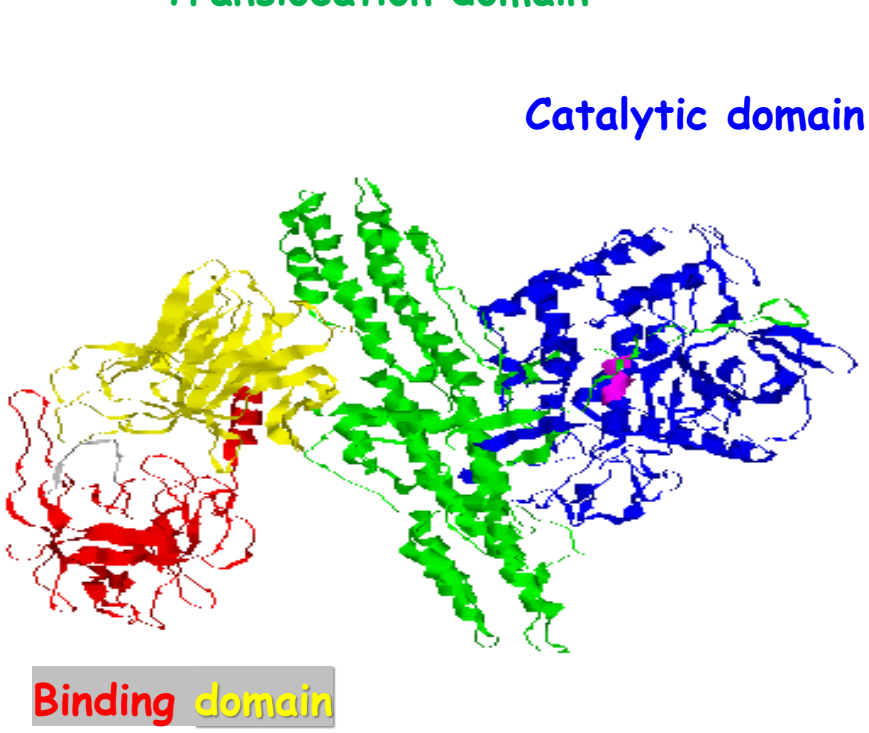


Example1: Botulinum toxins and tetanus toxin

- Botulinum toxins (7 types) prevent the release of neurotransmitter from axon endings at the neuromuscular junction, thus causing flaccid paralysis

- Tetanus toxin blocks the inhibitory interneurons in the CNS producing rigidity, unopposed muscle contraction and spasm

- Same structure. One protein cleaved into two parts: a 100 kDa heavy or B-chain (binding domain and translocation domain) and a 50 kDa light or A-chain (catalytic domain).



Mechanism of action

Botulinum toxins

Food

transcytosis

extracellular fluid
blood circulation

blockade of neuromuscular junctions: flaccid paralysis

Tetanus toxins

growth of *C. tetani* in wound

extracellular fluid

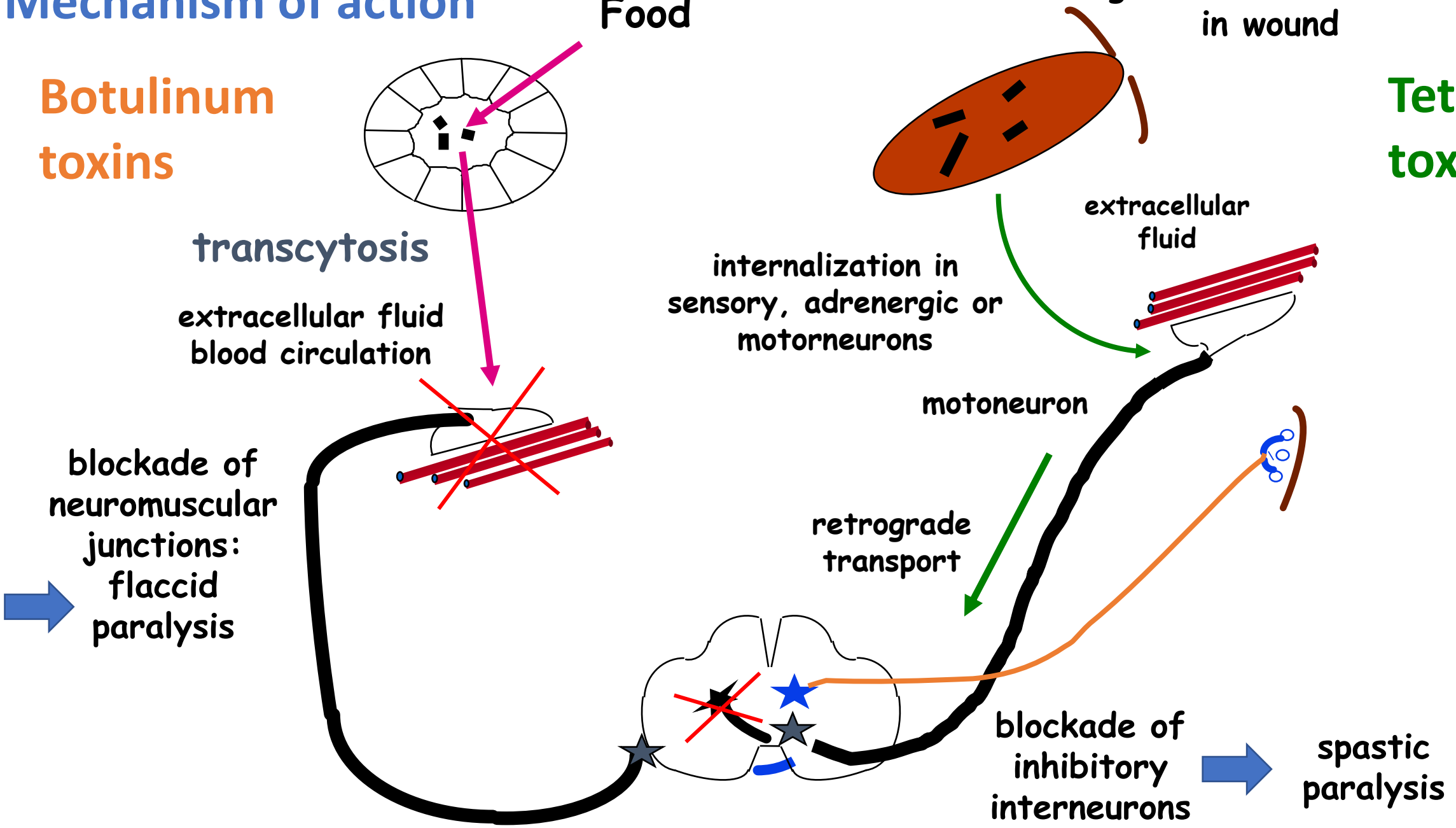
internalization in sensory, adrenergic or motorneurons

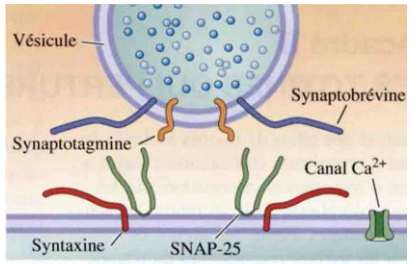
motoneuron

retrograde transport

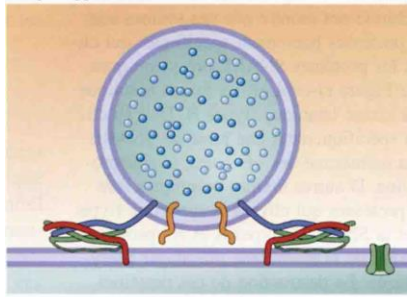
blockade of inhibitory interneurons

spastic paralysis

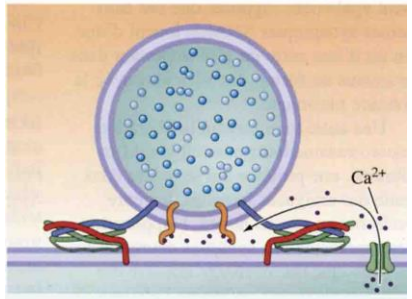




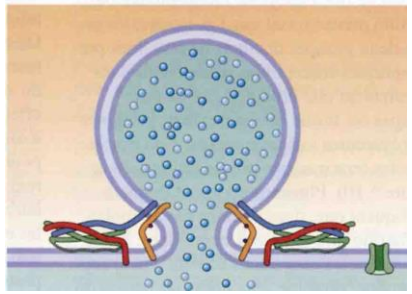
(2) Formation de complexes SNARE qui rapprochent les membranes



(3) Entrée de Ca^{2+} qui se lie à la synaptotagmine



(4) Catalyse de la fusion des membranes par la synaptotagmine liée au Ca^{2+}



Synaptic Vesicle

BoNT/G

TeTx
BoNT/B

BoNT/D

BoNT/F

C

Synaptobrevin

++

V2

V1

BoNT/E

BoNT/A

S4

C

SNAP25

BoNT/C

S3

S2

S1

X2

X1

BoNT/C

Syntaxin

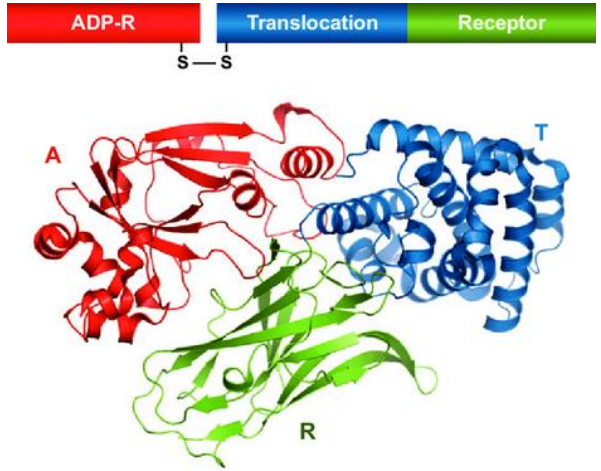
C

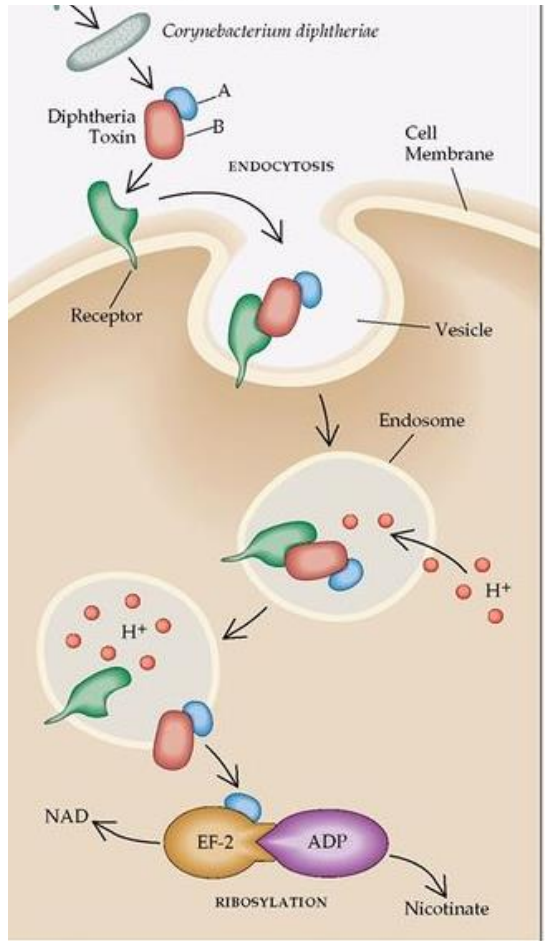
presynaptic
Membrane

Example2: The diphtheria toxin

- *Corynebacterium diphtheria* : Sore throat, gray membrane covering the throat. Death occurs through necrosis of the heart and liver

- Mechanism of action
  - Binding of the toxin to a receptor : heparin_binding epidermal growth factor
  - Endocytosis
  - Endosome vesicle acidifies
  - The transmembrane chain facilitates passage of the chain A through the endosome membrane
  - Chain A transfers a NAD^+ to a diphthamide residue in elongation factor 2 (EF2)
  - Protein synthesis is stopped.

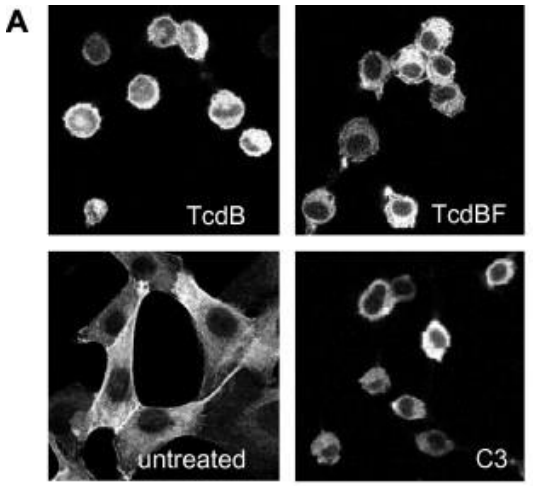




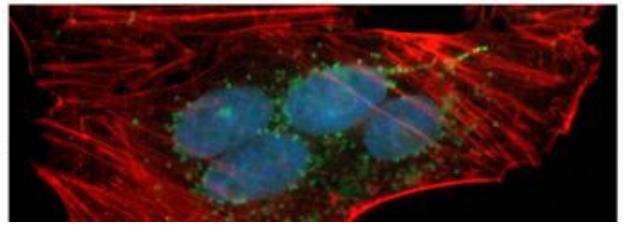
Example3: Other AB_{RT} Toxins

- Large clostridial toxins (toxin>250 kDa from *C. difficile, C. sordellii, C. novyi*)
  - inactivation of Rho family proteins of the small GTPases of the Rho family (Rho, Ras and p38)

alterations of the actin cytoskeleton

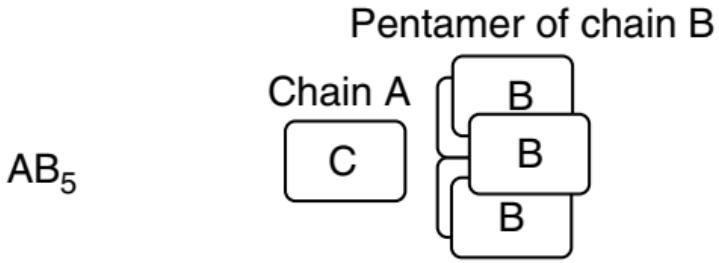


- Cytotoxic necrotizing factors (CNF from *Escherichia coli*, *Yersinia pseudotuberculosis*, *Shigella* species, *Salmonella enterica)*
  - deamidate a glutamine (Q61 or Q63) in the active site of the small Rho GTPase family (RhoA, Rac1, and Cdc42)



Multinucleation due to CNF on Hep2 cells. Chaoprasid, Cell 2021)

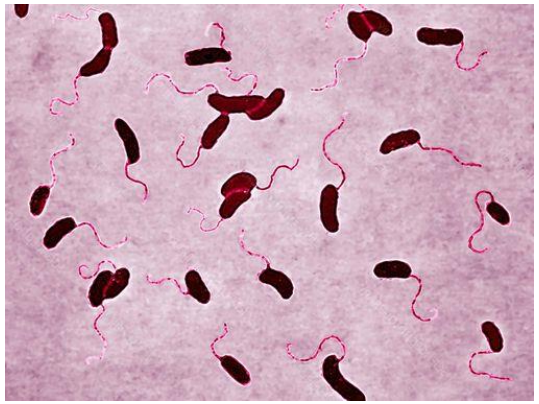
B) AB_5 toxins



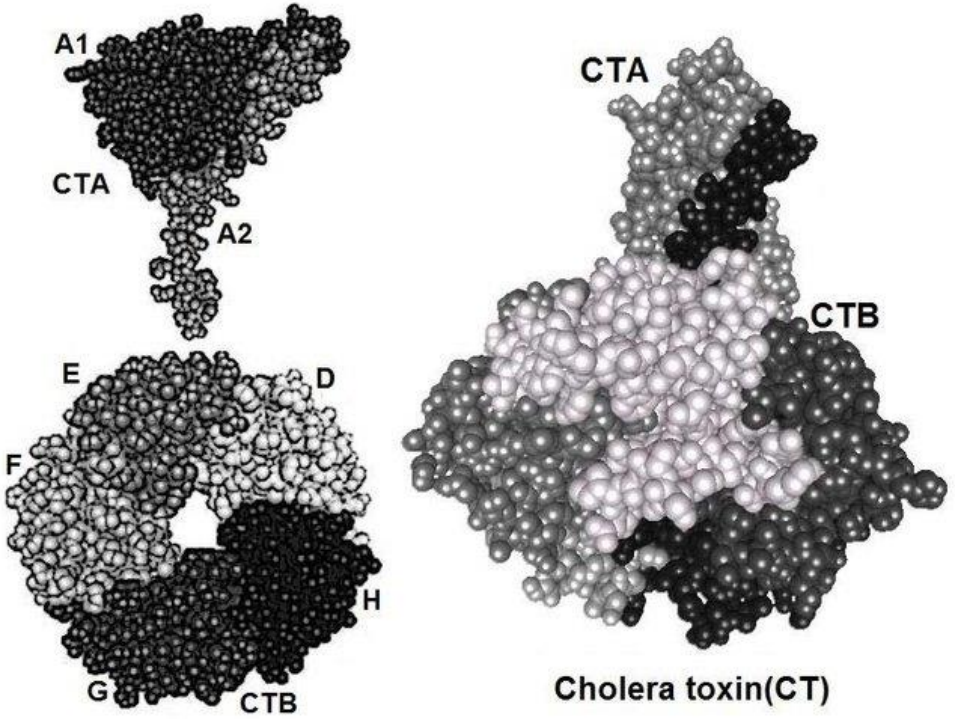
- Act as individual molecules (monomers or preassembled oligomers
- Among AB_5 toxins are:
  - Cholera toxin (*Vibrio cholerae*): CT
  - Heat-labile toxins (*E. coli*): LT
    - LT is very similar to cholera toxin; 80% homologous in amino acid sequences
  - Pertussis toxin (*Bordetella pertussis*): PT
  - Shiga toxin (*Shigella dysenteriae*): STX
  - Shiga-like toxins (Enterohemorrhagic *E. coli*)

Example: CT of *Vibrio cholerae*

- Symptoms include chronic and widespread watery diarrhea and dehydration (death).
- The bacteria are ingested, and after colonization of the small intestine, secrete the toxin CT
- Structural model: CTA (A1 chain and A2 chain) and CTB (pentamer D, E, F, G and H chains).

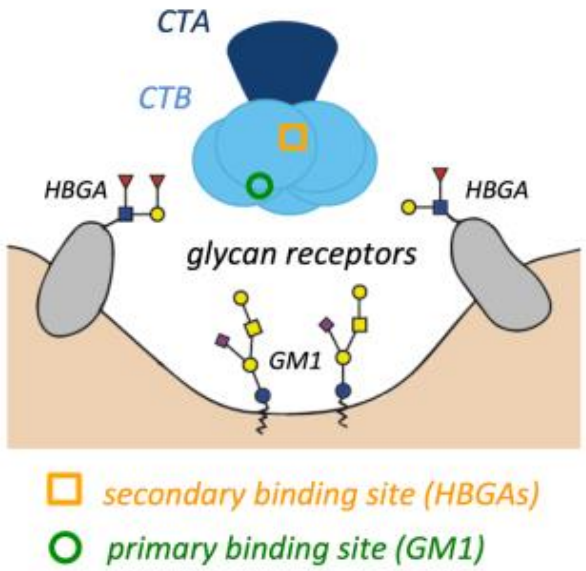


Vibrio cholerae

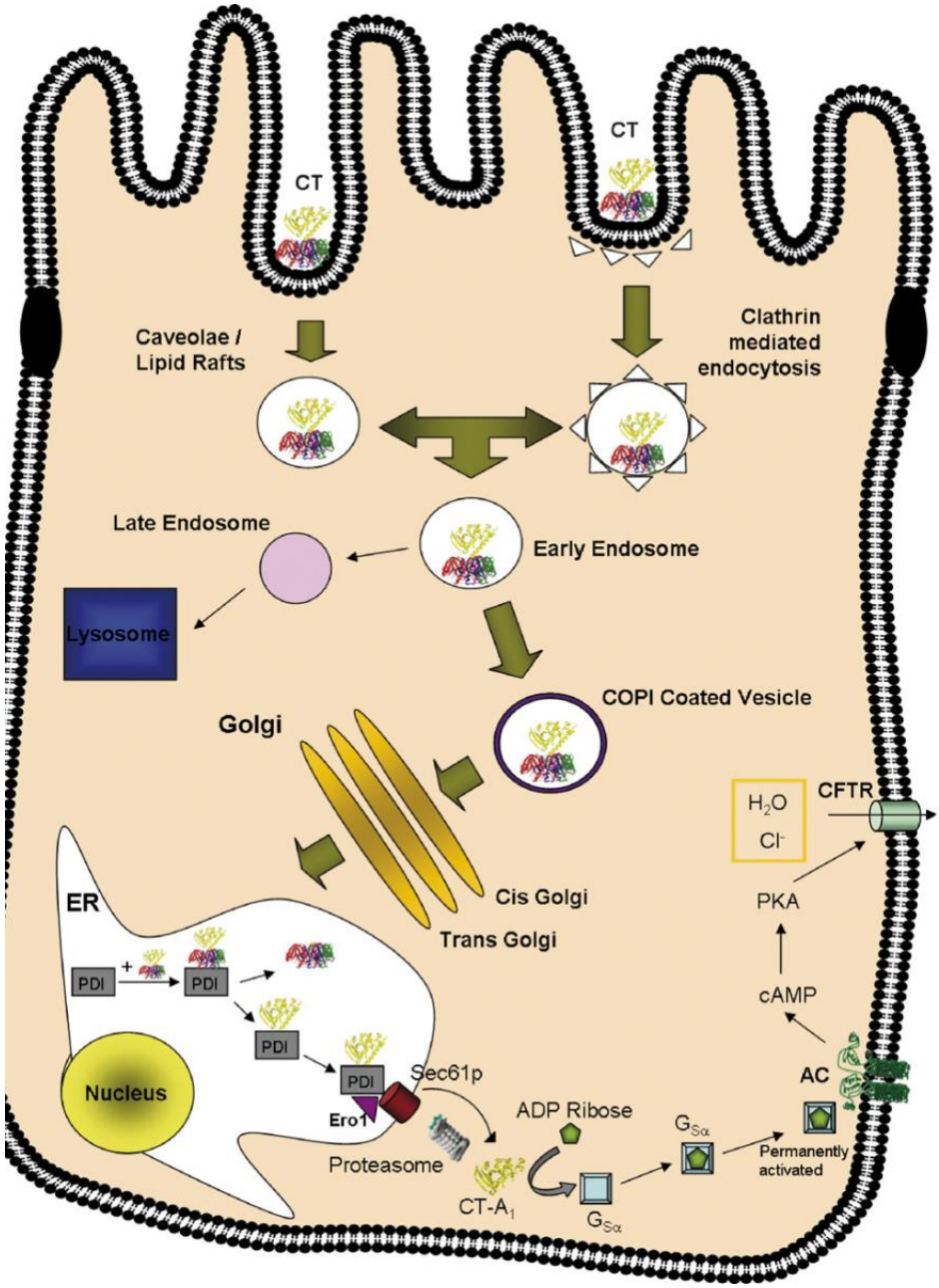


Mechanism of action of the toxin

- **Binding of the toxin to their receptors**
  - GM1 ganglioside is considered the primary receptor of CT
    - strongest protein-carbohydrate interactions known
  - A secondary binding site on the lateral side of the B-pentamer is histo-blood group antigens (HBGAs)
    - Antigen Lewis (X or Y) with the fucose residue implicated
- **Endocytosed by the cell (caveolae, clathrin..)**



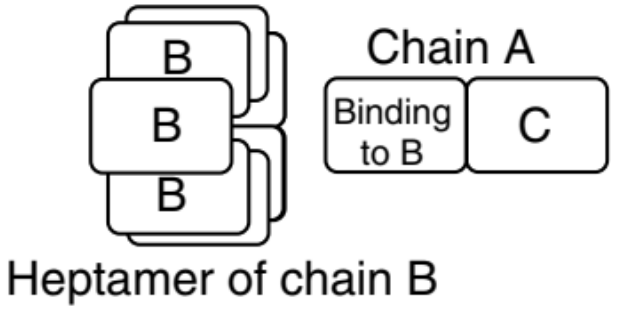
- CT moved to the Golgi apparatus, then to the endoplasmic reticulum,
- the A1 chain (CT-A1) is released by the reduction of a disulfide bridge, and Ero1 triggers the release of CT-A1 into the cytoplasm by the Sec61 channel,
- CT-A1 catalyzes the ADP-ribosylation of the Gs alpha subunit (Gαs) proteins, thus maintaining Gαs in its activated state,
- Increased Gαs activation leads to increased adenylate cyclase activity, which increases the concentration of cyclic AMP and over-activates cytosolic cAMP-dependent protein kinase A (PKA),
- Active PKA phosphorylate the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel proteins, which leads to efflux of chloride ions and secretion of H2O, into the intestinal lumen.



D. Vanden et al. The International Journal of Biochemistry & Cell Biology 39 (2007)

C) A_nB_7 toxins

A_nB_7



- Assemble as oligomers following binding to the cell surface
- Among A_nB_7 toxins are:
  - Anthrax toxin (*Bacillus anthracis*)
  - VIP toxin (*Bacillus cereus*)
  - actin-ADP-ribosylating toxins:
    - C2 toxin (*C. botulinum*),
    - Iota toxin (*C. perfringens*),
    - CDT from *C. spiroforme* and *C. difficile*

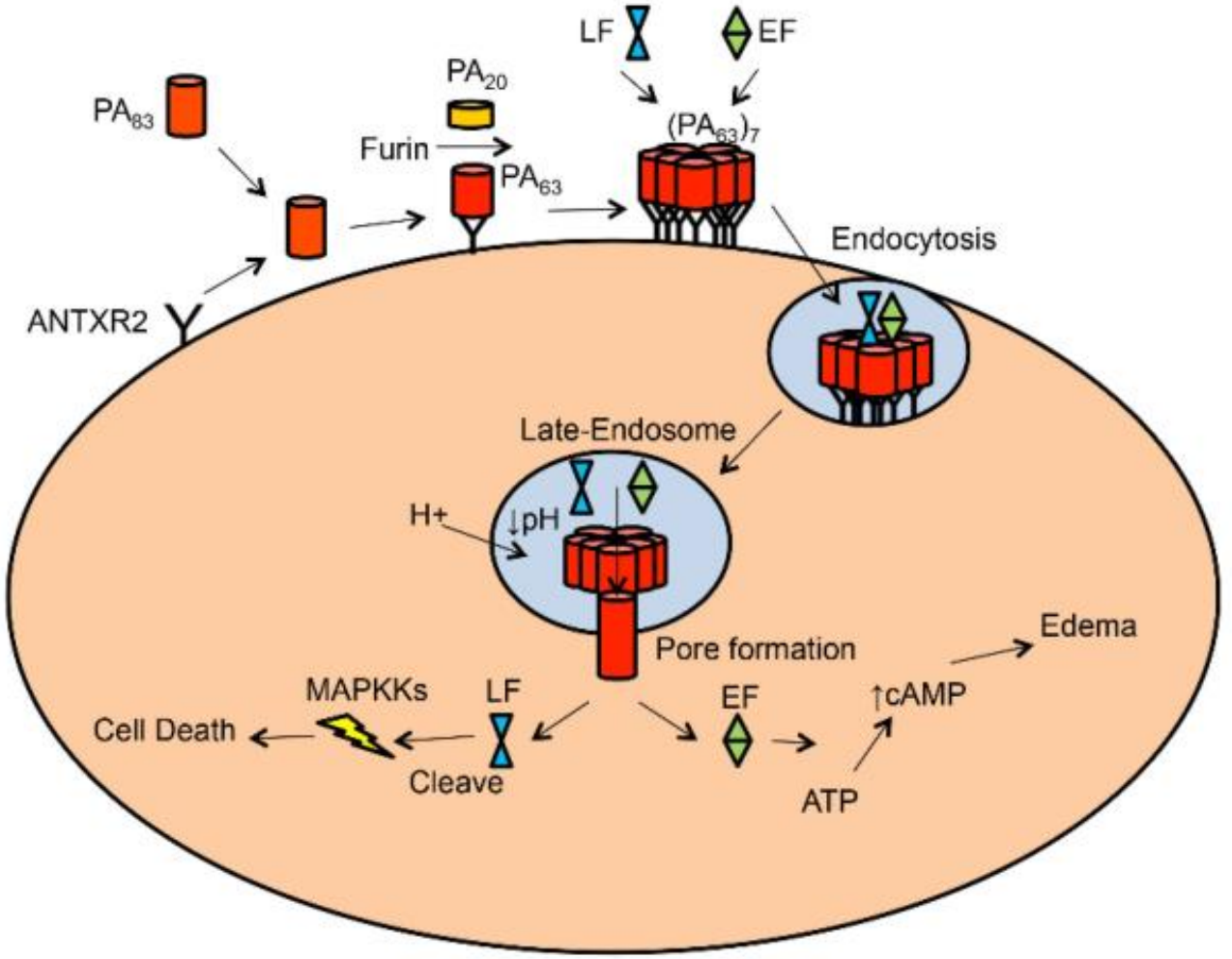
Example1: the anthrax toxin

- Anthrax is a disease caused by the spore-forming bacteria *Bacillus anthracis*.
- Anthrax spores are extremely resistant and can survive in the environment for decades
- Causes high mortality, primarily in domestic and wild herbivores as well as most mammals and several bird species.

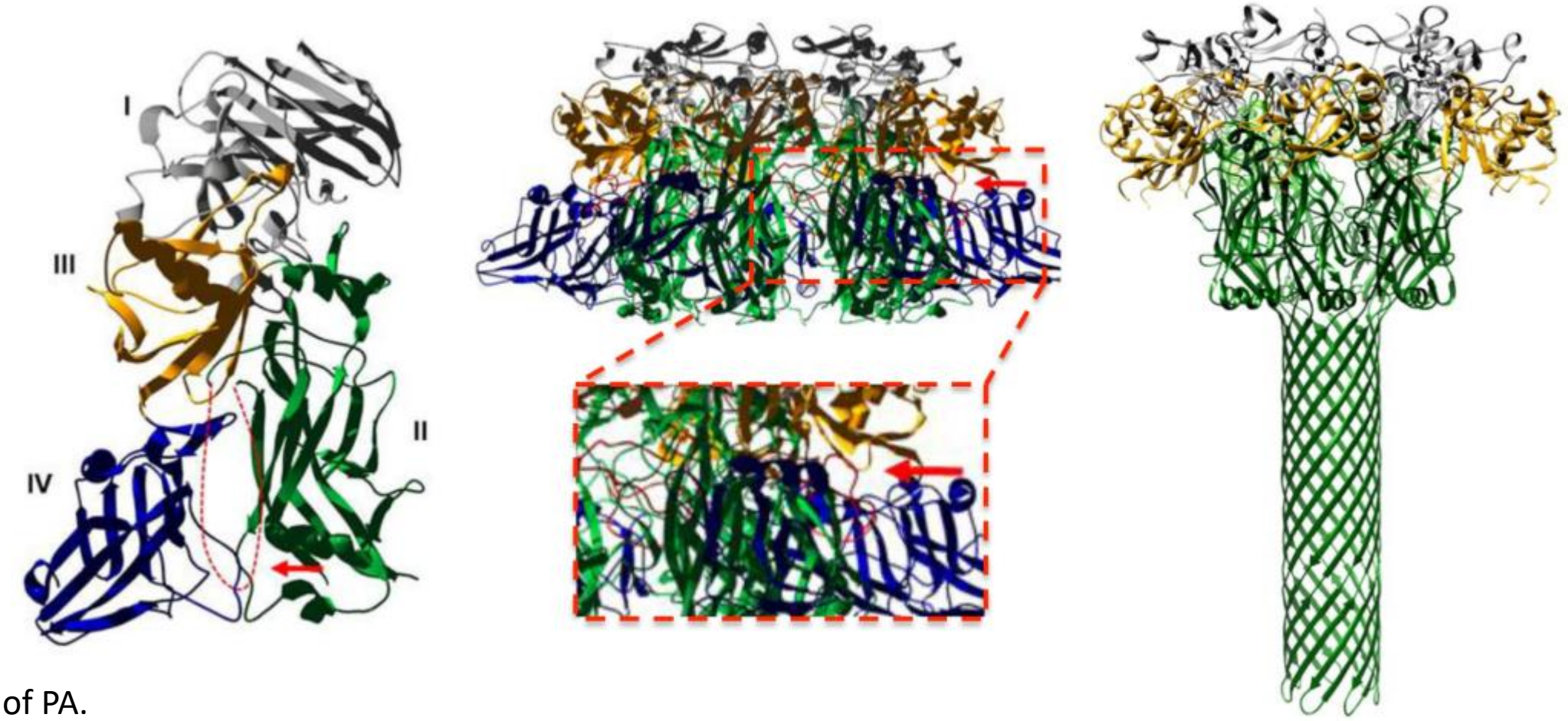
- Anthrax toxin is a tripartite toxin: two catalytic A moieties and one receptor-binding/pore-forming B moiety.
- The A moieties are edema factor (EF), a calmodulin-dependent adenylate cyclase, and lethal factor (LF), a zinc protease.
- The B moiety is called protective antigen (PA). Size 83kDa.

Mechanism of action of the toxin

- PA83 binds to cell surface receptor and is cleaved by furin into PA63 and PA20.
- PA63 assembles into a heptameric prepore $(PA63)_7$, to which LF/EF binds.
- The complex is internalized by the receptor-mediated endocytosis and travels to the late endosome, where acidification triggers conversion of PA prepore to pore.
- EF/LF is translocated into the cytosol through the PA pore.
- In the cytosol, LF cleaves MAPKK (apoptose) and EF elevates cAMP (edema).



Toxins **2016**, *8*, 34; doi:10.3390/toxins8020034



Structures of PA.

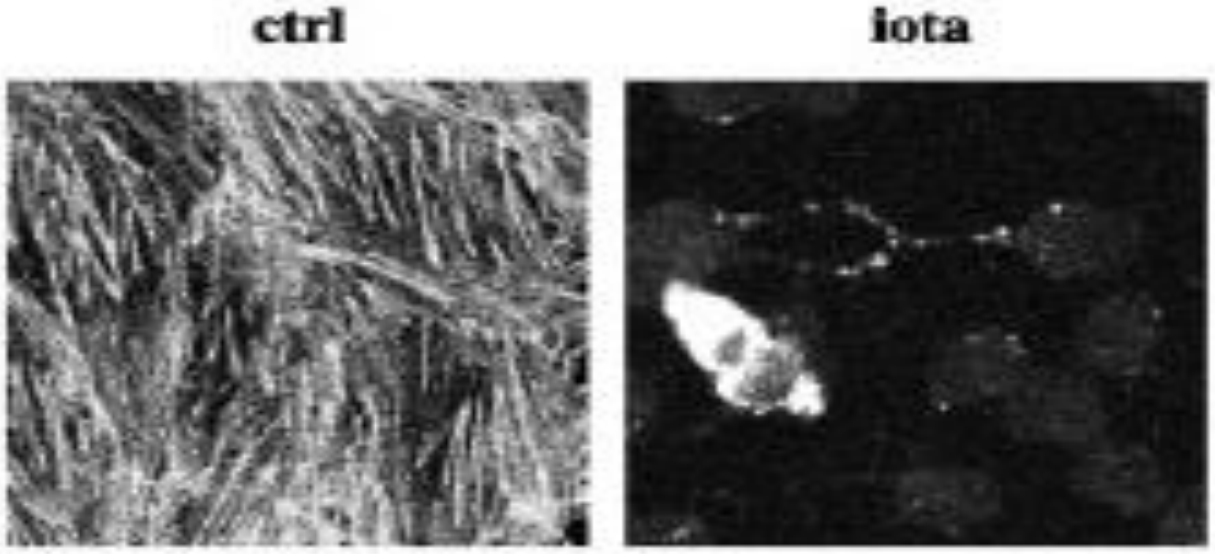
(A) Full-length PA_{83}. Four domains are shown in different colors and labeled I–IV. The disordered 2β2–2β3 loop is shown as a red dotted line;

(B) Upper panel: PA heptameric prepore. Lower panel: a subset of heptameric prepore is zoomed to show the 2β2–2β3 loops indicated by a red arrow;

(**C**) Single particle reconstruction of the PA pore from cryo-EM. The 2β2–2β3 loops convert to a 14-strand transmembrane β-barrel (shown in green).

Example2: the Iota toxin of *C. perfringens*

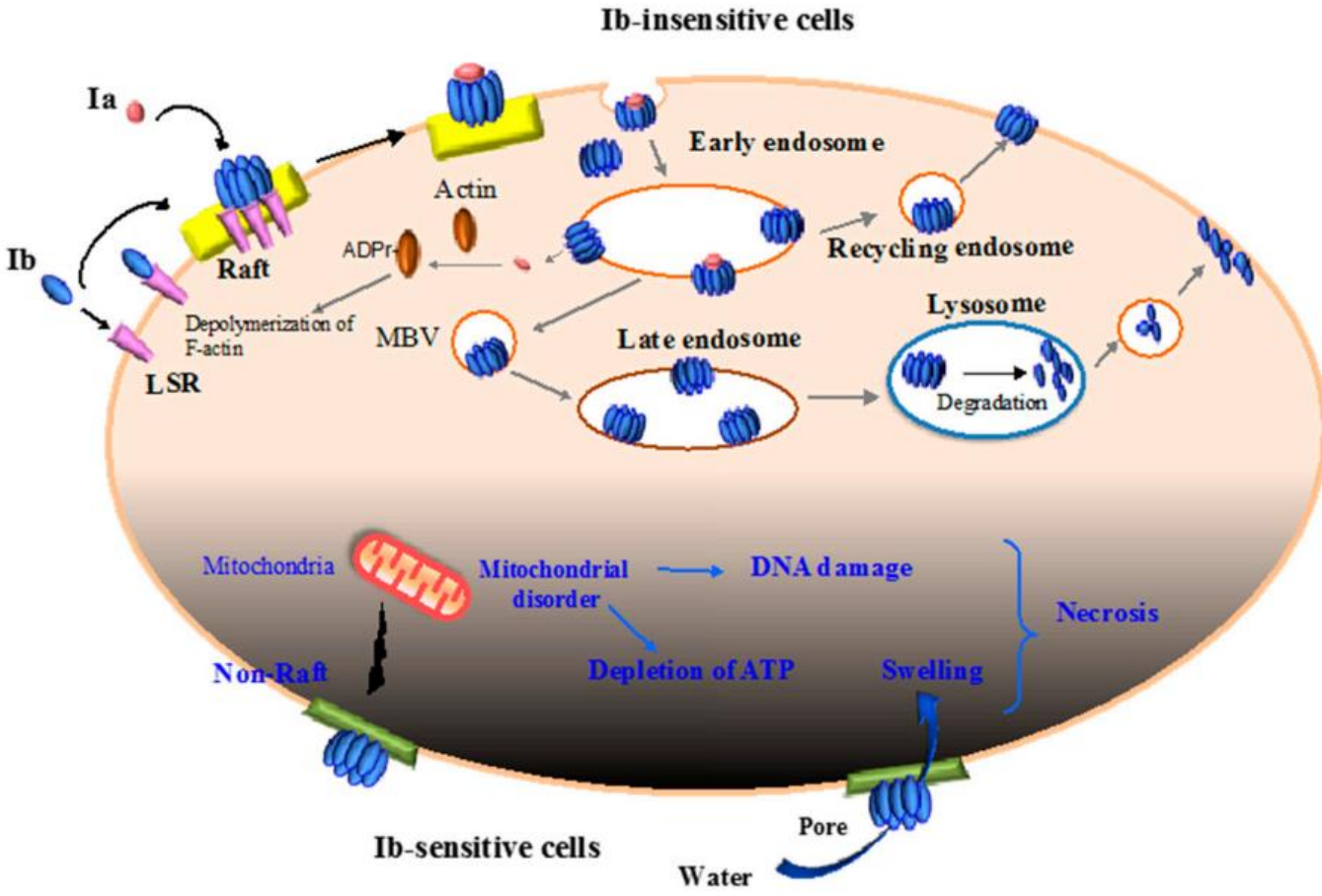
- Produced by *C. perfringens* type E
- Responsible for a variety of veterinary illnesses
- Causes lethal, dermonecrotic, and cytotoxic activities
- Called binary toxin with the enzymatic component Ia and the binding component Ib
- Ia ADP-ribosylates G-actin in the cytoplasm causing its depolymerization



- sensitive cells round-up as a result

Mechanism of action of the toxin

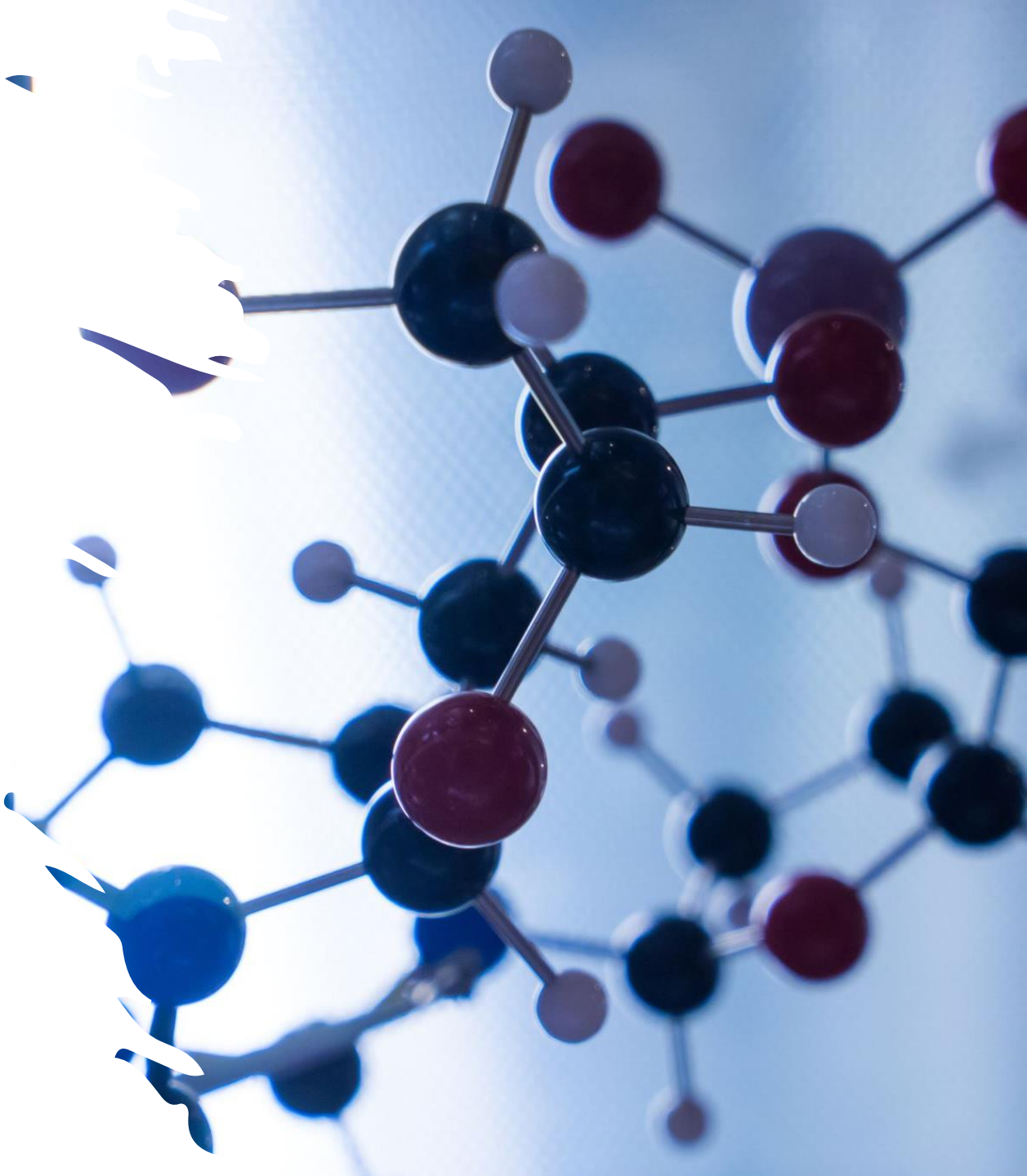
- Ib associates with lipolysis-stimulated lipoprotein receptor (LSR) on the plasma membrane and migrates to membrane lipid raft
- Ia bound to Ib oligomers forms on the rafts.
- The Ia and Ib complex enters the cell.
- The complex is trafficked to the early endosome
- Acidification facilitates the cytosolic release of Ia
- Ia ADP-ribosylates G-actin in the cytoplasm
- Ib alone is able to cause cytotoxicity by forming pore on sensitive cells.



Takehara et al Toxins, 2017

Conclusions

- Autonomous molecular devices
- Key virulence factors that target different functions of host cells
  - Facilitates survival of the microorganisms
- Useful for research
  - Deciphering cellular process
- Medical purposes
  - Ex: botulinum toxin in multiple diseases



THANK YOU FOR YOUR ATTENTION