Entry of toxins into the cells

Toxins are poisonous substances produced by certain microbes. And the ability to produce toxins by which many pathogens produce disease is known as "toxigenesis". Toxins may be carried far from the site of invasion by the blood or lymph. Various toxins may cause fever, cardiovascular disturbances, diarrhea, and shock. They can inhibit protein synthesis, destroy blood vessels, and disrupt the nervous system. Most of the toxins are bacterial origin whereas some toxins are also produced by some fungi as a competitive resource. The toxins, named mycotoxins, deter other organisms from consuming the food colonised by the fungi.

In response to the presence of a toxin, the body produces antibodies called antitoxins, which will combine with the toxin and make it harmless. The active toxins are treated by heat or expose to chemicals such as formaldehyde. This makes them harmless but still able to trigger the immune response that causes the production of antibodies. The inactivated toxins are called toxoids, and are used for vaccinations. Diphtheria and tetanus vaccines are prepared this way.

Bacterial Toxins:

There are two main types of bacterial toxins, lipopolysaccharides, which are associated with the cell wall of Gram-negative bacteria, and proteins, which are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. The cell-associated toxins are referred to as endotoxins and the extracellular diffusible toxins that are secreted by bacteria are referred to as exotoxins. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides that act enzymatically or through direct action with host cells and stimulate a variety of host responses.

The production of the toxin is specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria).

Usually the site of damage caused by a toxin indicates the location for activity of that toxin. Some protein toxins have very specific cytotoxic activity. For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Bacterial protein toxins are strongly antigenic. Protein exotoxins are inherently unstable. In time they lose their toxic properties but retain their antigenic ones.

A plus B Subunit Arrangement

Many toxins act intracellularly and consist of two components: subunit A which is responsible for the enzymatic activity of the toxin and subunit B is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

Pore forming toxins:

Lipids are hydrophobic molecules which are essential constituents of membranes in the cells, whereas bacterial toxins are mainly hydrophilic proteins. All bacterial toxins interact first with their target cells by recognizing a surface receptor, which is either a lipid or a lipid derivative, or another compound but in a lipid environment. When bound to the receptor, some toxins act locally at the cell membrane, triggering pore formation across the lipid bilayer to release cell nutrients or kill target by disturbing their membrane. In contrast, other toxins enter cells and modify an intracellular target. These active toxins are trapped into endocytotic vesicles and follow different steps to access into the cytosol. One of the example is Staphylococcus which secretes pore forming toxins, to alter the host cells and trigger a release of nutrients useful for their growth.

Pore forming toxins use two mechanisms to form pores in the cell membrane, according to the structural domain building the channel:

- Insertion of amphipathic α-helices or α-PFT

Represented by colicins and staphylococcus δ-toxins

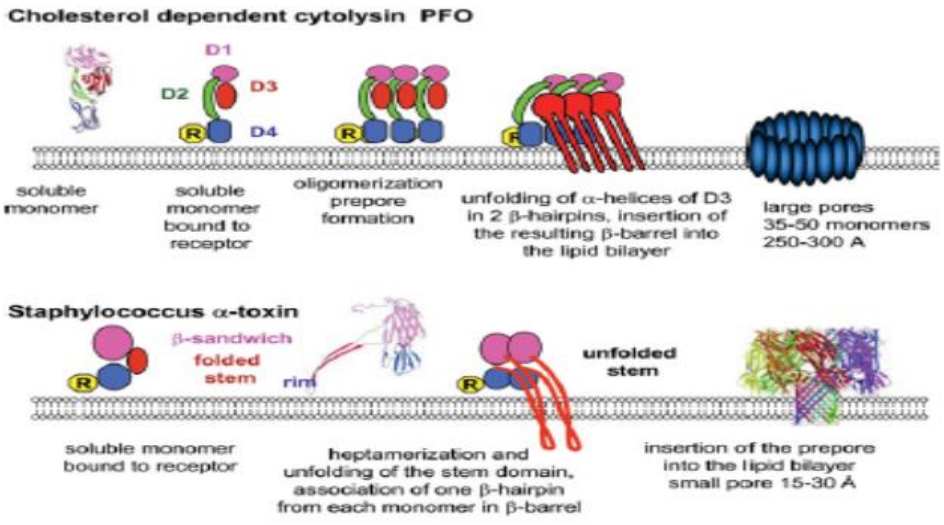
- Insertion of amphopathic β-hairpins organized in β-barrels or β-PFT

These are hydrophilic proteins rich in β-sheets and most of the bacterial PFT belong to this class. β-PFTs bind to a cell surface receptor, oligomerize and one or two β-hairpins of individual monomers associate into a β-barrel structure which inserts into the lipid bilayer and creates a channel.

Example: β-PFTs forming large pores: cholesterol dependent cytolysins (CDCs)

CDCs, such as *Clostridium perfringens* PFO (perfringolysin), recognize cholesterol as a receptor. The molecule is rich in β-sheets and is hydrophilic.

PFO pore formation includes the binding of water-soluble PFO monomers to cholesterol in lipid bilayers, which is mediated by the short hydrophobic undecapeptide loop at the tip of the domain 4. Domains 1, 2, and 4 fit into L-shaped forming a cylindrical structure. Interaction of domain 4 with cholesterol induces a conformational change of domains 3, which are rotated from domains 2 and form a belt in the outside face of the cylinder which promotes in the exposure of hydrophobic residues and the insertion of a transmembrane β-barrel into the lipid bilayer. And hence larger pores are formed which contain 35-50 monomers.



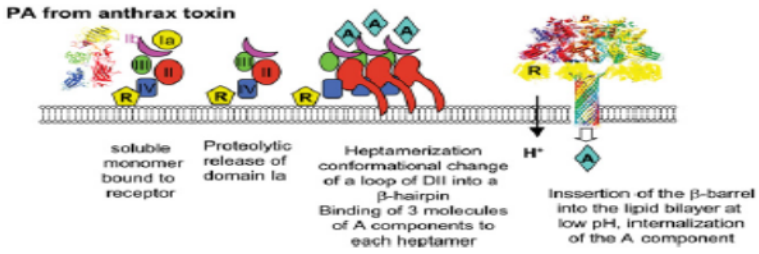


Figure 1: Pore formation of three β-PFTs. Pore formation can occur in the following ways: large pores induced by PFO, an example of a CDC; small pores resulting from heptamerization of *Staphylococcus* α-toxin; and pores formed by the binding component of anthrax toxin (PA) through endosome membrane, permitting the internalization of the corresponding enzymatic components. All the three types of β-PFTs show a common mode of β-barrel formation and subsequent insertion into lipid bilayers. Please draw this figure

Receptor mediated endocytosis:

The bacterial toxin consist of two components: one component (subunit A) which is responsible for the enzymatic activity of the toxin and the other component (subunit B) which is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are two mechanisms of toxin entry into target cells:

1. Direct entry:

The B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

2. Receptor mediated endocytosis:

Here the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of receptor-mediated endocytosis. The toxin is further internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit

affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

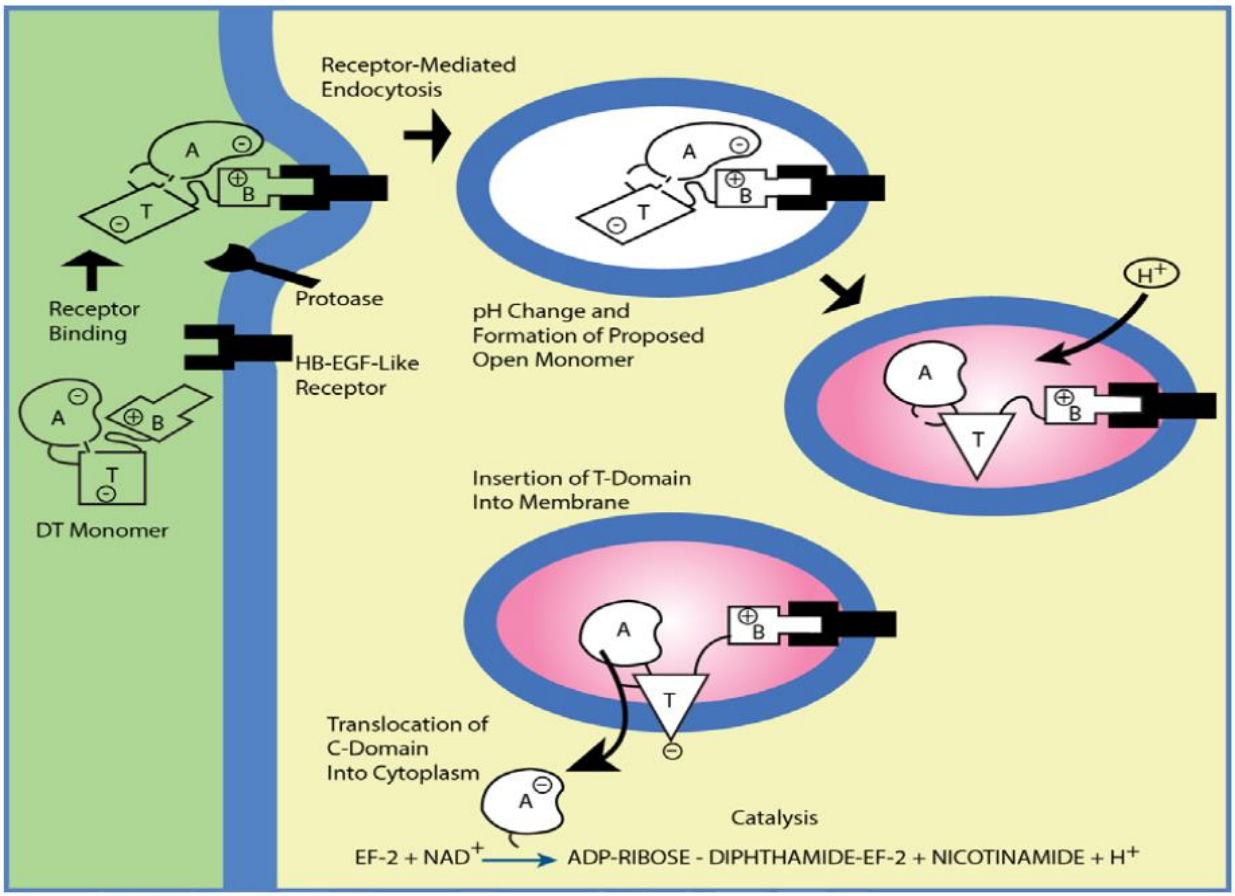
In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is based in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Example: Diphtheria Toxin

The diphtheria toxin is produced by *Corynebacterium diphtheriae*. It is a bacterial exotoxin of the A/B prototype. It has two parts: subunit A, contains the enzymatic activity for inhibition of elongation factor-2 involved in host protein synthesis and subunit B, is responsible for binding to the membrane of a susceptible host cell. The B subunit possesses a region T (translocation) domain which inserts into the endosome membrane thus releasing the enzymatic component into the cytoplasm. In vitro, the native toxin is produced in an inactive form which is activated by the proteolytic enzyme

trypsin in the presence of thiol. The diphtheria toxin enters its target cells by either direct entry or receptor mediated endocytosis.

Figure 2: Entry and activity of diphtheria toxin (Dtx) in susceptible cells. The B domain of the toxin binds to a cognate receptor on a susceptible cell. The toxin is taken up in an endosome by receptor mediated encocytosis. Acidification of the endocytic vesicle allows unfolding of the A and B chains exposing the hydrophobic T domain of the toxin. The T domain inserts into the endosome membrane translocating the A fragment into the cytoplasm where it regains its enzymatic configuration. The enzymatic A component utilizes NAD as a substrate. It catalyzes the attachment of the ADP-ribose portion of NAD to elongation factor (EF-2) which inactivates its function in protein synthesis. Redraw this figure



Clathrin independent endocytosis of ricin and Shoga toxin:

Plant toxin ricin binds to both glycolipids and glycoproteins with terminal galactose all over the cell surface and is therefore localized to all types of membrane invaginations and the toxin is internalized by all endocytic mechanisms. Ricin has been localized in clathrin-coated pits, but is still endocytosed when this pathway is blocked.

Clathrin-independent endocytosis is different from uptake by caveolae and macropinocytosis. For instance, clathrin-independent endocytosis occurs on the apical side of polarized cells, whereas caveolae are localized in the basolateral domain. Clathrin-independent endocytosis of ricin occurs when uptake from caveolae and clathrin-dependent endocytosis are inhibited by extraction of membrane cholesterol. Removal of cholesterol leads to the disappearance of caveolar and inhibits formation of invaginated clathrin-coated pits (**Sandvig et al.,2000).**

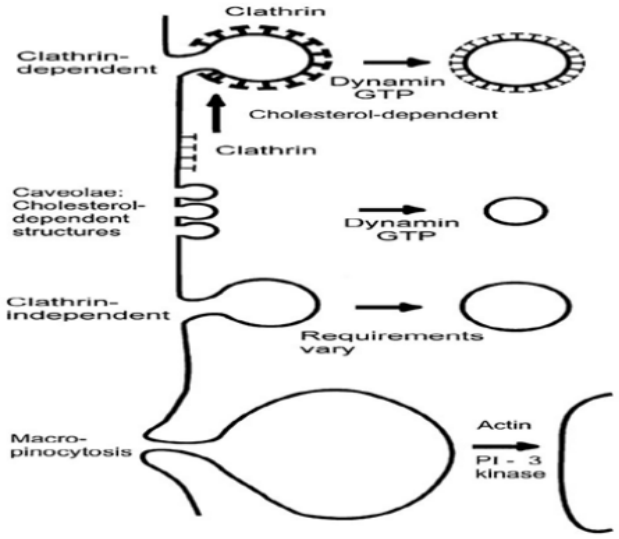


Figure 3: Structures proposed to be involved in endocytosis.

On the other hand, Shiga toxin is endocytosed preferentially by the clathrin-coated pathway, although it is bound to a glycolipid receptor (globotriasylceramide; Gb3).

Bacterial toxin transport using macromolecular syringes:

The pathogens inject their toxins into the cytosol of host cells through bacterial transport machines that function as macromolecular syringes are either bacterial flagella or conjugative pili and facilitate the direct passage of toxin effectors from bacterial donor cells into eukaryotic cells by processes of Type III or Type IV secretion mechanisms or by constructing large pores within the plasma membrane of target cells that function as for direct effectors delivery.

As the first step in cellular entry, AB toxins bind to one or more plasma membrane surface receptors. They incorporate two discrete and essential functional components that vary in physical arrangement but are generally conserved in terms of function. Thus, toxins "A fragments" are the active moiety that can modify intracellular target molecules by one of the enzymatic activities, including ADP-ribosylation, UDP-glucosylation, or proteolysis. Meanwhile, the "B fragments" serve as delivery vehicles for their A components by binding to plasma membrane surface receptors and facilitating translocation of the A components into the cytosol through available portals.

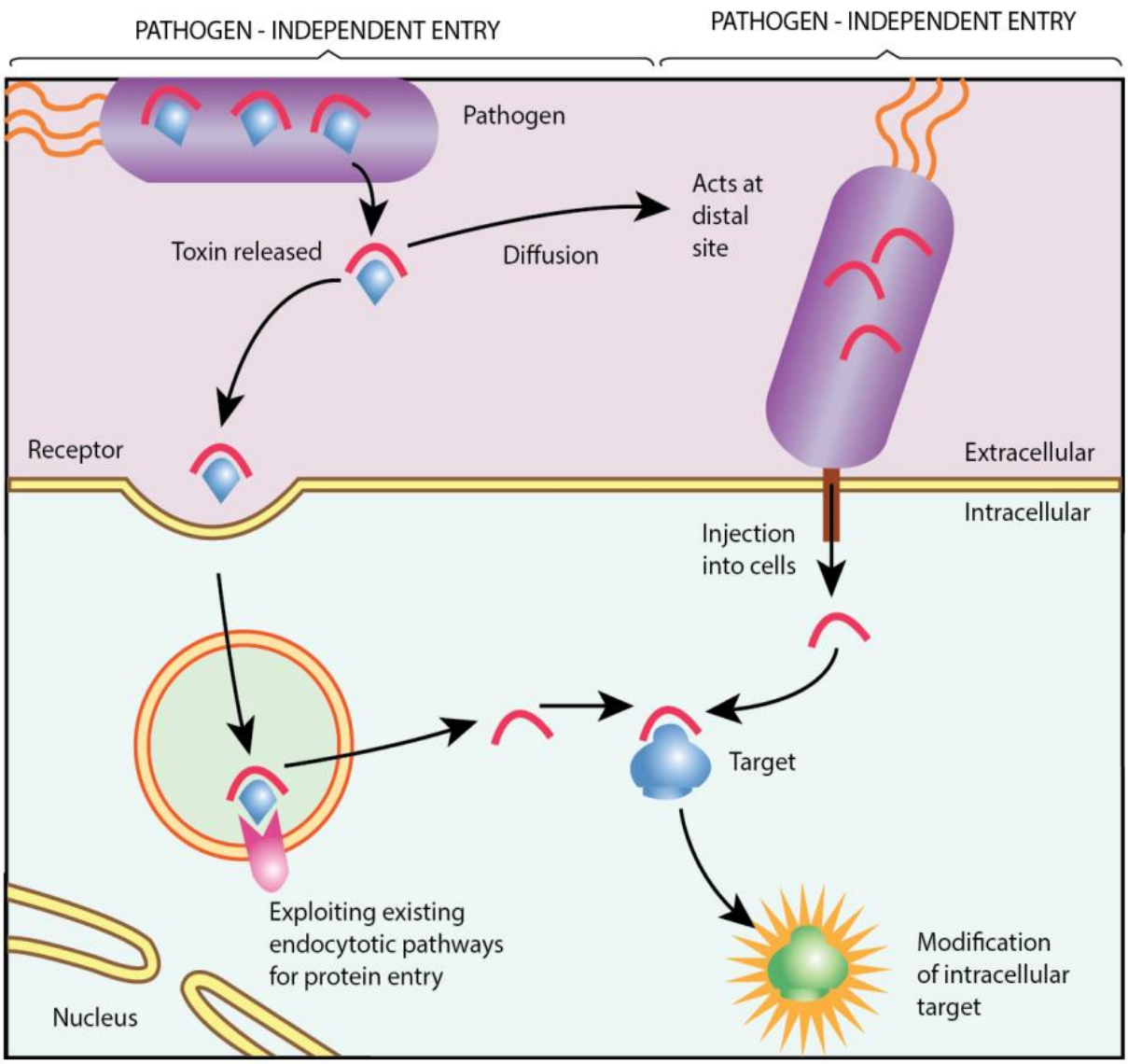


Figure 4: Intracellularly acting bacterial toxins access their substrates within host cells by one of several mechanisms. Some gram-negative pathogens directly inject toxin effectors through flagellar- or pilus-adapted transport machines into eukaryotic cells by Type III or IV secretion systems, respectively. Alternatively, bacteria release intracellular-acting toxins, also called AB toxins, into the host environment where they act locally or diffuse to act distally to the site of colonization. AB toxins commonly exploit endocytic pathways that eukaryotic cells use for importing proteins. Finally, *Bordetella* adenylate cyclise toxins directly enter the cytosol from the plasma membrane. Redraw this

Toxins Exploit the Acidic Environment of Endosomal Compartments

Some AB toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH to between 5.0 and 6.0 as endocytic vesicles are trafficked from the plasma membrane into the cell. This acidification results in the insertion of B fragments into the membrane and the formation of ion-conducting channels. Partially unfolded A fragments use these B fragment-derived channels as conduits into the cytosol.

Toxins Exploit the Sec61 Retro-Translocon in the Endoplasmic Reticulum

The second group of AB toxins includes cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A and exploit the degradation pathway for misfolded proteins. The secretion pathway is at least partially reversible to the fate of nascent proteins for secretion, enabling several bacterial toxins to travel this "retrograde" pathway from the plasma membrane to the ER lumen through pores to the cytosol. Within the lumen of the ER, the A fragments are transported through an existing membrane complex whose primary protein is Sec61. The B fragments of these toxins bind to receptors to facilitate the trafficking of catalytic A fragments to the ER.

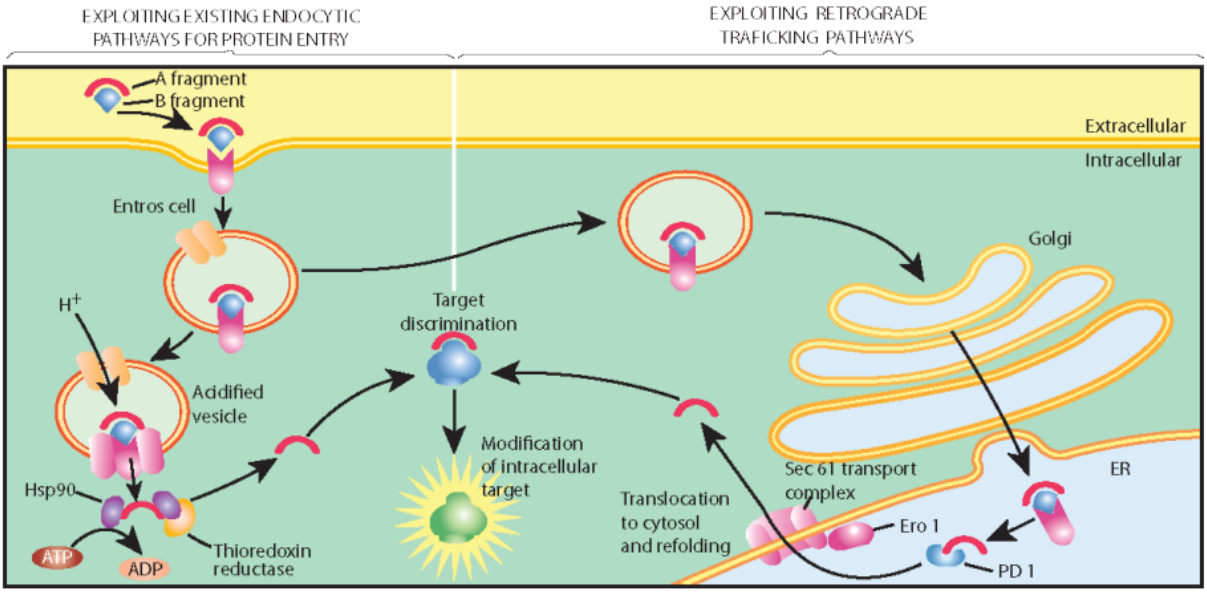


Figure 5: Entry of AB toxins into target cells. Some toxins use host endocytic pathways that cells use for degrading exogenous proteins within lysosomes. Redraw this

Entry of toxin with influx of Ca^{2+}:

In the absence of $Ca^{2+,}$ cells were not sensitive to the toxic proteins like abrin and modeccin and also the sensitivity to ricin and diphtheria toxin was reduced. Calcium deprivation leads to negative effect on the binding and endocytosis of these toxins. Some studies indicate that Ca^{2+} is involved in the entry mechanism for abrin, modeccin, and ricin, possibly as a Ca^{2+} flux together with the toxin.

The plant toxins abrin, modeccin, and ricin consist of two polypeptide chains connected by a disulfide bond. B-chain binds the toxins to cell surface receptors while A-chain enters the cell and inhibits protein synthesis.

Diphtheria toxin is synthesized as one polypeptide chain which is cleaved by proteolytic enzymes into an A- and a B-fragment. "A fragment" possesses enzymatic activity and inactivates components of the protein-synthesizing machinery. Recently it was shown that low pH is required for entry of diphtheria toxin into the cytosol before transport of the A-fragment across the membrane. The low pH induces exposure of a hydrophobic domain in the B fragment that forms ion permeable channels across the membrane and interacts with the membrane lipids and hence facilitates entry of the A-fragment (**Sandvig et al., 1982**).

Interesting facts:

- "A" denotes to the active protein and "B" dentotes to the binding protein in AB toxin.
- Some toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH for its entry as endocytic vesicles are trafficked from the plasma membrane into the cell.
- Some toxins like cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A exploit the sec61 retro-translocon in the endoplasmic reticulum for its entry.
- Calcium deprivation leads to negative effect on the binding and endocytosis of these abrin, modeccin, ricin and diphtheria toxin. The entry mechanism for these toxins depends on Ca^{2+} flux together with the toxin.

Questions:

1. Which of the following is not an A-B exotoxin?
   a. Streptolysin O
   b. Staphylococcus aureus enterotoxin
   c. Cholera toxin
   d. Diphtheria toxin
   e. Tetanus toxin
2. All of the following are true of A-B exotoxins except:
   a. The B portion of the toxin binds to surface receptors on host cells.
   b. They consist of two polypeptide components.
   c. They are only produced by gram-negative bacteria.
   d. The A portion of the toxin is the active component.
   e. Many exotoxins are A-B toxins.
3. Which of the following bacterial toxins binds to nerve cells, preventing chemical communication between nerve and muscle cells?
   a. Diphtheria toxin
   b. Erythrogenic toxin
   c. Staphylococcal enterotoxin
   d. E. coli endotoxin
   e. Botulinum toxin
4. Which is true of endotoxins?
   a. They increase blood pressure.
   b. They are produced by gram-positive bacteria.
   c. They are disease-specific.
   d. They are released upon cell lysis.
   e. They are proteins.

5. In a bacterial exotoxin:
   a. The A subunit allows the toxin to bind to the surface of specific host cells.
   b. The A subunit is part of the outer bacterial membrane, released when the bacterialcell dies.
   c. The A subunit is able to interfere with a specific host cell activity, once it has beentaken into the host cell.
   d. We expect the A subunit for all exotoxins to operate in the same manner.
6. What effect does botulin toxin have on the body?
   a. It causes excitation of neurons.
   b. It stimulates the removal of fluid from the tissues.
   c. It stimulates increased intestinal motility.
   d. It paralyzes the muscles of the respiratory tract.
   e. It prevents gastrointestinal motility.
7. Where are the target cells of diphtherotoxin located?
   a. The throat
   b. The skin
   c. The skeletal muscles
   d. The lungs
   e. The heart and nervous system
8. What does the A in AB toxin stand for?
   a. Active
   b. Agglutination
   c. Adhesion
   d. Accumulation
   e. None of these is correct.
9. Which bacterium produces the hemolysin streptolysin?
   a. *S. pyogenes*
   b. *S. aureus*

c. *C. diphteriae*
d. *C. botulinum*
e. *C. tetani*

10. Which toxin contains LPS and triggers fever?
    a. Endotoxin
    b. Exotoxin
    c. Both
    d. None
11. CDCs regonize:
    a. Cholesterol as a receptor
    b. Carbohydrates moieties as a receptor
    c. Glycolipids receptor
    d. Glycoproteins
12. How the toxins enter into the cell?
13. What are macromolecular syringes?
14. What are the functions of A- and B-fragments in A/B toxin for the entry of toxin into the cell?
15. What are the two mechanisms that are used by pore forming toxins for the formation of pores in the cell membrane? Explain with an example.