Bacterial Channel Forming Protein Toxins

Channel-forming proteins (CFPs) can be:

- Integral components of membranes
- Water soluble proteins which have the ability to form channels
Toxin nomenclature

- Many toxins are given names associated with the cells they killed in the assay first used to identify them
  - E.g., hemolysin (erythrocytes); identified by screening bacteria producing hemolysis on blood agar plates
  - *Note: This doesn’t mean that erythrocytes are the favored target of the bacteria secreting hemolysins!*

- Pores, channels and holes (terminology is often used indiscriminately)
  - **Pore:** hole of defined size with little or no selectivity (e.g., aerolysin, \( \alpha \)-toxin)
  - **Channel:** formation of defined ion-selective channels (e.g., colicins)
  - **Hole** (usually of variable size): pore or channel which is less uniform in its properties (e.g., cholesterol-dependent toxins)
Toxin Complexity

• Bacterial protein toxins are usually large molecules
  – E.g., $\alpha$-toxin (S. aureus): 33 kDa
  anthemx protective antigen: 90 kDa
  RTX HlyA (E. coli): > 100 kDa

Why are these proteins so large?

Bacterial protein toxins have several properties that require the complexity of a larger structure
1. Protein toxins do not normally kill the secreting bacteria
   CFPs can exist in inactive conformations which are activated by proteolysis, acylation, pH change, etc.

2. CFPs are polymorphic (i.e., they exist in a soluble “pro”form and in an insoluble membrane-inserted form)
   Transition can be achieved by change in protein conformation or by oligomerization of monomers

3. Many CFPs are primarily active against specific types of cells
   CFPs contain domains that recognize and bind to unique receptors on the cell surface

4. Many CFPs display enzymatic activity
Classification of CFPs

- CFPs are very diverse proteins
- Two subgroups (based on how these proteins generate the channel):

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BETA-BARREL TOXINS          ALPHA-HELIX TOXINS

  BINDING
     |   
  Oligomerization
     |   
  Insertion
     |   
Channel Formation

  BINDING
     |   
  Insertion
     |   
Oligomerization?
     |   
Channel Formation
```
β-barrel toxins

- Largest group
- Establish relatively non-specific pores
- Somewhat analogous to outer membrane porins (common ancestor?)
- Some display anion or cation selectivity
- None of these proteins are enzymes
- Toxins do not need to be internalized

**Two subgroups:**

A) toxins forming oligomers consisting of up to 7-8 monomers (channel diameter of ca. 1-2 nm); e.g. α-toxin, aerolysin, protective antigen

B) toxins producing large channels (e.g., cholesterol-dependent toxins, streptolysin O, perfringolysin O)
α-helix toxins

• similar to TM proteins of the bacterial cytoplasmic membrane

• Most α-helix toxins insert into the membrane as monomers

• Many α-helix toxins have enzymatic activity

• Many α-helix toxins are internalized (e.g., by receptor-mediated endocytosis – change in pH can trigger channel formation)
Critical steps in channel formation

1. Binding to target cells

   1. Many CFPs bind to specific receptors on target cells (concentration of the toxin leading to an increase in the rate of channel formation)

   2. CFPs lacking specific receptors:
      
      1. Most of them are active only at high concentrations; no discrimination between different types of membranes
      2. Some selectivity may result since some CFPs only form channels in membranes that contain specific lipid components (e.g., cholesterol-dependent toxins)
2. Activation

1. Some CFPs require activation to proceed to channel formation

2. Activation can be achieved by:

   1. Proteolytic “nicking” by proteases (proaerolysin and protective antigen are activated by furin or furin-like proteases after binding, whereas Cry toxins are activated before binding)

   2. Exposure to low pH environment (e.g., protective antigen)

   3. Acylation of one or two specific lysine residues (e.g. in RTX toxins)
3. **Oligomerization**

1. Predominantly found with β-barrel toxins
   They lack hydrophobic α-helices that can span the membrane!

2. **Formation of prepore complexes:**
   
   1. Oligomerization of monomers on the membrane surface
      (not yet inserted)
   
   2. Some toxins (e.g. Cry and colicins) may form oligomers
      after insertion

4. **Insertion**

   Molecular details on how prepore complexes are inserted are known in only a few instances
Consequences of channel formation

• **Erythrocytes are lysed**
  - They are unable to repair channels and are susceptible to osmotic imbalance

• **At high concentrations of the toxin:**
  - If many channels in the membrane: most cell types are lysed
  - If smaller number of channels in the membrane: cells remain intact until they die by apoptosis
Toxins forming small β-barrel channels

A. Aerolysin from *Aeromonas hydrophila*

- Secreted from bacteria as highly water-soluble proaerolysin (dimeric)

- Proaerolysin is proteolytically activated to water-soluble aerolysin (also dimeric) by nicking near the C terminus (*furin is likely to be involved in vivo*)

- Both pro- and activated forms bind specifically to receptors bearing glycosylphosphatidylinositol (GPI) moieties

- Cells with GPI receptors are extremely sensitive to the action of aerolysin (e.g., T-lymphocytes can be killed by the toxin at concentrations as low as 10 pM)

- Depending on the number of aerolysin channels in the membrane of a target cell, death can occur rapidly (due to osmotic imbalance) or more slowly (by triggering apoptosis)
Channel formation by aerolysin

Bilobal structure of the proaerolysin dimer

- Small, globular lobe consists of domain 1
- Large elongated lobe consists of domains 2-4
- Large lobe contains very long β-sheets (presumably to form the β-barrel structure after oligomerization)

- **Domain 1**: involved in dimerization and receptor binding
- **Domain 2**: involved in receptor binding and oligomerization
- **Domain 3**: Neck/spacer region
- **Domain 4**: involved in activation (from proaerolysin to aerolysin) and membrane insertion

Taken from: Rossjohn et al. (1998) *J. Struct. Biol.* 121, 92-100.
Activation by proteolysis results in the excision of ca. 40 aa from the C terminus.

Cleavage at the activation site would lead to exposure of a hydrophobic surface, probably triggering a rearrangement in the β-sheets.

Rearrangement of the β-sheets may result in the loss of dimer contacts as a prelude to oligomerization.

FIG. 6. Consequences of activation in Domain 4. The contacting residues between Domain 2 of one monomer and Domain 4 of the other monomer in the dimer are denoted in ball-and-stick. Only polar interactions are shown for simplicity. Loss of the propeptide (shown in dark shade) in Domain 4 would result in reorganization of the beta-strands leading to likely disruption of the contacts in the dimer interface.

Images of the heptameric aerolysin channel derived from electron microscopy

Staphylococcus aureus α-toxin

- 33 kDa water-soluble monomer
- secreted in an active form (in contrast to aerolysin)
- no specific high-affinity receptors (toxin associates non-specifically with lipid bilayer, preferably containing phosphatidylcholine and cholesterol)
- lyses a variety of cells (in 1 nM to 1_μM range)
- forms a heptameric (β-barrel) prepore (hexameric forms have also been observed)
- no crystal structure available for the monomer, but a crystal structure is available for the heptamer (this is in contrast to the situation with aerolysin!)
Crystal structure of the α-toxin heptamer from *S. aureus*

**Fig. 2.** Ribbon representations of the αHL heptamer with each protomer in a different color. (A) View perpendicular to the sevenfold axis and approximately parallel to the putative membrane plane. The mushroom-shaped complex is approximately 100 Å tall and up to 100 Å in diameter, and the stem domain measures about 52 Å in height and 26 Å in diameter from Cα to Cα. Approximate locations of the cap, rim, and stem domains are shown. Thr^{129} is located at the base of the stem domain. (B) View from the top of the structure and parallel to the sevenfold axis. The amino latch of one protomer makes extensive interactions with its clockwise-related immediate neighbor and residues in each glycine-rich region wrap around the sevenfold axis approximately 180°. Protomer-protomer contacts consist almost exclusively of side chain–side chain interactions in the cap domain while in the stem domain main chain–main chain contacts predominate as the β strands form a continuous β sheet.

Taken from: Song et al. (1996) *Science* 274, 1859-1866.
Fig. 6. A stereo view of a model for the interaction of the heptamer with phospholipid head groups. Difference electron density from $\alpha_7$ crystals soaked in a solution containing DiC$_7$PC shows 5$\sigma$ peaks near the guanidinium group of Arg$^{200}$. We used the difference density in conjunction with chemical and biochemical information to guide the construction of a model for the interactions between $\alpha_7$ and seven DiC$_7$PC molecules. Residues Tyr$^{112}$, Lys$^{116}$, Tyr$^{118}$, His$^{144}$, and Tyr$^{148}$ project from the surface of the stem, and Trp$^{179}$, Tyr$^{182}$, Trp$^{187}$, Arg$^{200}$, and Met$^{204}$ define the rim side of the crevice, together forming an attractive binding site for phospholipid molecules.

Taken from: Song et al. (1996) Science 274, 1859-1866.