**Abstract**

Bacterial toxins can be used as tools to help us understand how mammalian cells function. The Whitefish Bay SMART (Students Modeling A Research Topic) Team is working with Dr. Frank from the Medical College of Wisconsin to model the bacterial toxin ExoU using MSOE’s 3D printer. ExoU is a phospholipase that destroys both organelle and plasma membranes by cleaving the phospholipids. The toxin is delivered into host cells by a special injection apparatus localized in the membrane of Pseudomonas aeruginosa, a highly resistant strain of bacteria. Injected proteins are unfolded to fit through the bacterial needle for threading into the host cell. Once inside of the cell, ExoU binds to ubiquitin or ubiquitylated proteins, becomes active, and destroys cellular membranes through some still to be determined method. The interaction of ExoU with ubiquitin is postulated to change the enzyme’s conformation. In patients with cystic fibrosis, a genetic disorder which causes symptoms of pneumonia and destruction of the airways, *P. aeruginosa* aggravates the immune system by causing chronic inflammation, resulting in mucus buildup. In other cases unrelated to cystic fibrosis, *P. aeruginosa* infects wounds, the urinary tract and can cause corneal ulcers in individuals who wear contact lens. If we understand the process of activation, we can potentially discover new ways to treat infections caused by *P. aeruginosa* and prevent future infections.

**The Role of ExoU**

Bacteria have mechanisms to kill off host cells. *P. aeruginosa* encodes a number of toxins including ExoU. Each is important for the biology of the organism in the environment and in humans. The protein ExoU has the ability to wrap itself around ubiquitin, a protein found in all eukaryotic cells. This tight wrap leads to rapid bubbling of the membrane, resulting in cell death. ExoU is produced from the bacterium *P. aeruginosa*, which is found in the soil. In medical settings, equipment and surfaces are contaminated with this bacteria leading to infection. Infections often occur in the lungs and the death of these cells damages the airways. The bacterium is intrinsically resistant to antibiotics and adapts to different environments rapidly. Our mentor’s goal is to find a way to more effectively stop the spread of *P. aeruginosa* and lessen the toxic effects of its protein ExoU.

**What We Know About ExoU**

1. To be injected from *P. aeruginosa*, ExoU must associate with its chaperone, SpcU. SpcU is not injected but recycled.
2. The N-terminal domain of ExoU encodes a pataxin-like A2 phospholipase activity with two catalytic amino acids.
3. Dr. Frank’s group postulates that the C-terminal half of ExoU recognizes the activator ubiquitin.

**How ExoU Destroys Eukaryotic Cells:**

**Step 1:**

*Pseudomonas aeruginosa* enters the human body, and pierces the membrane of a eukaryotic cell with a needle-like apparatus.

**Step 2:**

The needle-like apparatus transfers ExoU, a toxic protein stored in *Pseudomonas aeruginosa*, into a eukaryotic cell containing ubiquitin. ExoU binds to ubiquitin by interacting with the activation domain.

**Step 3:**

The combined ExoU and ubiquitin mass, which is attached to the membrane of the eukaryotic cell, acts as a phospholipase and destroys both organelle and plasma membranes.

**Step 4:**

Because they are unable to fight the bacteria, the body’s cells that were injected with ExoU erupt and die shortly after.

**Effectiveness of ExoU vs. Length of Ubiquitin Chain**

This figure shows ExoU’s affinity to the molecule ubiquitin as the number of ubiquitin increases (chain length). As chain length increases, the affinity of the ExoU increases exponentially, as seen by the bar graph. This change in affinity is most apparent when the chain length of ubiquitin is increased from 2 to 4. In this case, there is a jump in ExoU affinity from 1 to almost 20.

**ExoU Active Sites**

Highlighted by the arrows, the active sites of ExoU, S142 and D344, are thought to be necessary in the ExoU’s role as a phospholipase A2.

**Conclusion**

As a phospholipase delivered into host cells by an apparatus in the membrane of *Pseudomonas aeruginosa*, ExoU becomes active after binding to ubiquitin and destroys cellular membranes. In patients with cystic fibrosis, *P. aeruginosa* aggravates the immune system by blocking the airway. In patients with acute pneumonia, ExoU destroys the airway. Once we understand the activation process of ExoU, we may discover new treatments for infections caused by *P. aeruginosa* and prevent future cases of acute infection.

**References and Citations:**


Background Image From: www.bioquell.com