

Feel the Burn, then Feel the Death. ExoU as a Phospholipase

The Brown Deer SMART Team: Evan Bord, Zack Farrell, Wongsai Heur, William Keslin, Robert Laughlin, Hannah Leedom, Andrew LeMense, Seriah Lucre, Chad Marable, Suzie Mielke, Sara Olk, Carlos Orozco, Charlie Rosio, Jordan Schubert, Sarah Smith, Gina Wade and Mike Weeden
Teacher: David Sampe

Mentor: Dara W. Frank, Ph.D., Medical College of Wisconsin, Department of Microbiology and Molecular Genetics

Abstract

A major cause of infection-related deaths in immunocompromised patients is the protein toxin ExoU, encoded by the bacterium *Pseudomonas aeruginosa*. In cooperation with MSOE, the Brown Deer SMART Team (Students Modeling A Research Topic) has modeled ExoU using 3D printing technology to have a better grasp on how the toxin interacts with eukaryotic cells. This soil bacterium infects those with a weakened immune system. *P. aeruginosa* uses a type 3 secretion system (T3SS) to inject toxins including ExoU into the cell to disrupt its functionality. The T3SS is a needle-like structure comprised of proteins that allow the bacterium to transfer effector proteins into innate immune cells. ExoU travels through the T3SS using a chaperone protein (SpcU). Once inside the eukaryotic cell, ExoU interacts with ubiquitin, where it refolds into an active potent phospholipase that breaks down cellular membranes using Ser142 and Asp344 as the catalytic amino acids. The exact mechanism is unknown but the C-terminus (residues 580-683) helps in targeting the membrane, allowing ExoU to break it down. *P. aeruginosa* is able to freely reproduce inside the environment of the host organism as the immune system is not able to compensate for the infected cells and bacterium. Left unchecked, this infection will prove fatal. Research is being conducted to create an ExoU inhibitor to reduce the deaths it causes in patients with compromised immune systems.

Feel the Burn: Infection in the Hospitals

Hospitalized patients have far more to worry about than swelling and fever-typical infection symptoms. *Pseudomonas aeruginosa* (see figure 1) is a bacterium that thrives by colonizing damaged tissue, often resulting in the death of the host. The problem isn't the bacterium alone; *P. aeruginosa* is everywhere—the ground you walk on, the air you breathe, on almost every surface you touch, even within the human intestines. *P. aeruginosa* is an opportunistic pathogen because it's unable to successfully infect a healthy body, but can be fatal to those in weakened states. Patients with such diseases like AIDS or Cystic Fibrosis, or those that have suffered from severe burns (see figure 2), have little to no chance of fighting the bacterium off.



Figure 1: *P. aeruginosa* bacterium.



Figure 2: Burn wounds on a 17 year old infected with *P. aeruginosa*

Injection into the Host

If *P. aeruginosa* is in a host organism, the host's defensive cells, called macrophages and neutrophils, attack the foreign bacterium. These cells specifically "hunt" for invaders to destroy them. In order to survive the immune system's attack, the type 3 secretion system (T3SS) injects toxins into the host cells. This system is a needle shaped protein, and is employed by *P. aeruginosa* (see Figure 3). These toxins then destroy the host cells, allowing *P. aeruginosa* to feed off the cells and utilize mucus to protect itself from the body's attempt to counter their invasion.

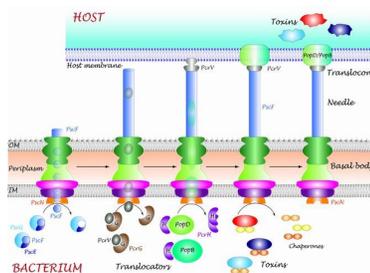


Figure 3: Type 3 Secretion System

Feel the Death

All eukaryotic cells are specifically vulnerable because of their use of a protein called ubiquitin, which helps to direct other proteins in their duties throughout the cell. While ExoU is in the bacterium, it is inactive and will not destroy the bacterial cell membrane. Once it enters a eukaryotic cell and comes into contact with ubiquitin, the shape of ExoU changes into a new confirmation in which the phospholipase amino acids are exposed. The enzyme phospholipase breaks down the main component of cell membranes, phospholipids (Figure 4). Without a cell membrane, the cell dies.

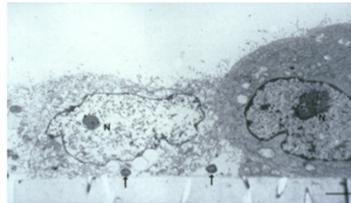


Figure 4: An electron micrograph. N = the nucleus. Arrows show the bacteria beneath the cells. The cell on the left is dead and the membranes are deteriorating. The cell on the right has a dense cytoplasm and an intact nucleus.

What part of ExoU binds to Ubiquitin?

ExoU binds with ubiquitin in the C-terminal region of the four-helix bundle. It is inferred that the points of binding on ubiquitin are at the hydrophobic patch (green) and the E24 region on the flipside (orange).

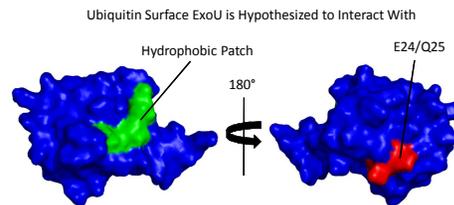


Figure 5: Theorized ExoU-Ubiquitin binding site

Mapping the Ubiquitin Binding Domain

The Frank lab constructed and tested protein expression constructs in a solid phase binding assay (Figure 6). Each construct was fused with the protein glutathione S-transferase, abbreviated GST to provide a way to detect the ExoU fragment as there are commercial antibodies for GST. The X axis indicates the amount of GST-tagged protein fragment added to the assay. The Y axis shows the amount of signal (relative fluorescent units) is measured. If the protein fragment binds to ubiquitin, which is coated on a surface in this assay, then the fluorescent signal is higher. PcrV is the needle protein and is a negative control that does not bind ubiquitin. These data indicate that the major ubiquitin binding domain of ExoU is localized to amino acids 480-683. Minor binding is also detected in fragments 480-599 and 480-659.

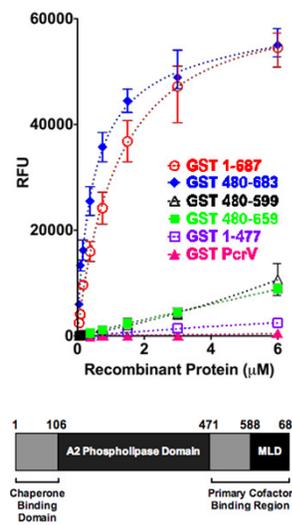


Figure 6: ExoU-ubiquitin binding assay (Dave Anderson)

What does ExoU look Like?

The model is a representation of ExoU with attached chaperone SpcU (chartreuse) as it would be found in *P. aeruginosa*. The catalytic amino acid Ser142 (medium blue) is required for phospholipase activity in eukaryotic cells. Other residues, 619, and 679-683 (cyan) help in ubiquitin binding. They are located on the 4-helix C-terminus bundle (red) where ubiquitin binds.

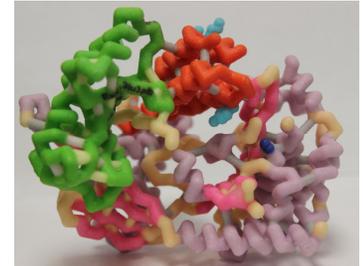


Figure 7: ExoU model based on 3tu3.pdb

ExoU-Ubiquitin Interaction Diagram

With knowledge on where ubiquitin binds to ExoU and where ExoU touches ubiquitin, models can be proposed to understand how ExoU would become activated.

Once ExoU reacts with di-ubiquitin, the catalytic reaction site is exposed; it breaks down eukaryotic cell walls by functioning as a phospholipase. The speculated confirmation change is as shown in figure 8.

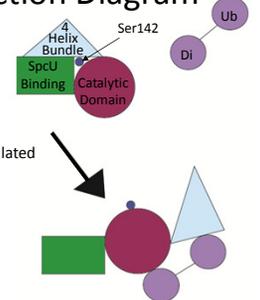


Figure 8: ExoU represented with the SpcU binding region in green, the catalytic domain in maroon, and with Ser142 in dark blue. The 4-helix bundle is represented in light blue. Di-ubiquitin is light purple.

Future Directions for Study

The binding points between ExoU and ubiquitin need to be known in order to accurately model ExoU in a eukaryotic cell and to find a way to prevent the detrimental effects of *P. aeruginosa*. The range where they bind has been determined (480-683) but more experimentation is needed to find the exact location. With this information, an ExoU inhibitor can be developed to prevent it from interacting with ubiquitin. Hypothetically, this inhibitor could interact with ExoU at the same site as ubiquitin to obstruct binding. The Frank lab has also developed an inhibitor that blocks the action of the injection protein and prevents ExoU delivery. Both of these proposals could stop *P. aeruginosa* from killing defensive cells and possibly save lives.

References

1. Medical Discovery. (n.d.). *Pseudomonas aeruginosa* bacteria. *GMD*. Retrieved February 5, 2013, from <http://globalmedicaldiscovery.com/>
2. Lakhota, S. (n.d.). *Pseudomonas aeruginosa* infection - DermAtlas - Dermatology image atlas. *Search - DermAtlas - Dermatology image atlas*. Retrieved February 5, 2013, from <http://dermatlas.med.jhmi.edu/>
3. Dessen, A. Current research projects - LNBio - Brazilian biosciences national laboratory. Retrieved February 13, 2013, from <http://lnbio.cnpem.br/dessen/>
4. Apodaca, G., M Bomsel, R. Lindstedt, J. Engel, D. Frank, K. E. Mostov and J. Wiener-Kronish. 1995. Characterization of *Pseudomonas aeruginosa*-induced MDCK Cell injury: Glycosylation-defective host cells are resistant to bacterial killing. *Infect. Immun.* 63: 1541-1551.
- 5.6. Anderson, D., & Frank, D. (2013). [ExoU assay]. Unpublished raw data.