Micro 201:
Bernhardt Lecture 1 - Protein Secretion and the Sec System
January 29, 2019

Overview:

This week, the course will start with a quick (re)introduction to the bacterial cell envelope. We will then begin a series of lectures exploring specialized topics in bacterial cell surfaces and cell biology starting with protein secretion and the Sec system.

Papers for discussion:

Context: Blobel and Dobberstein first formulated the signal hypothesis in 1975. In their pioneering work, they showed that proteins which were secreted into the endoplasmic reticulum were subjected to an N-terminal truncation of 15-30 predominantly hydrophobic aa residues. This N-terminal signal sequence distinguished the precursor and mature forms of secretory proteins. Since then, a major goal of biochemistry and molecular biology has been to work out what defines a signal sequence and to determine the molecular mechanisms involved in the export process. It was work by the Beckwith and Silhavy labs here at HMS that provided genetic proof for the signal hypothesis. In addition, their now classic genetic methods helped identify the important features of signal sequences and eventually helped identify all of the components of the secretion machinery. I can't think of a better set of papers to illustrate the awesome power of molecular genetics for generating new insights into complex biological phenomena.

1) Emr, Schwartz, and Silhavy, Mutations altering the cellular localization of the phage λ receptor, an *Escherichia coli* outer membrane protein. *PNAS* (1978) 75:5802-5806

Focus: Make sure you understand the logic of the genetics. What was the basis of their approach? How were uninteresting, unwanted mutations quickly eliminated? We'll discuss Table 2 and Figure 2. What is LamB? What class of proteins does it belong to? What is its function?

Skip the mapping and recombination sections on pg. 5804. These sections describe the use of lambda transducing phages to map the location of the mutations within lamB and move them from the lamB-lacZ fusion to native lamB. This lambda technology was necessary at the time, but has been superseded by modern cloning methods.

How would you map the mutations today? How would you test the importance of the signal sequence for protein transport if you were to do the experiment with modern day technology? How would you show the sequence is necessary for transport? How would you show it is sufficient?

2) van Stelten, Silva, Belin, and Silhavy. Effects of antibiotics and a proto-oncogene homolog on destruction of protein translocator SecY. *Science* (2009) 325:753-756

Thirty years after the original paper, LamB-LacZ fusions are still uncovering the secrets of protein translocation. This is a great example of how a good selection never loses its utility. In

this case, van Stelten and co-workers discover the mechanism of LamB-LacZ toxicity and it has interesting implications for understanding how cells deal with secretion stress. Please make sure you understand the logic of the study and why they chose to focus on YccA.

General Review:

Denks, Vogt, Sachelaru, Petriman, Kudva, and Koch. The Sec translocon mediated protein transport in prokaryotes and eukaryotes. *Mol Memb Biol* (2014) 31: 58-84.

This review will provide you with an in-depth overview of the process, what we know about it today, and what the questions for future work are. Tom Rapoport's lab in the Cell Biology Department here at HMS are leaders in the field and are currently performing beautiful structure/function studies aimed at understanding the mechanism of protein transport in atomic detail.

Further Reading:

Those interested in the art and design of genetic selections/screens should also see:

Oliver and Beckwith, *E. coli* mutant pleiotropically defective in the export of secreted proteins. *Cell* (1981) 25:765-772

Emr, Hanley-Way, and Silhavy, Suppressor mutations that restore export of a protein with a defective signal sequence. *Cell* (1981) 23:79-88