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How a cell knows where to divide: Oscillation of MinD in vivo

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Project Description

Background

The development and spread of antibiotic resistance has become a major issue in the last few decades. This is quite frightening as these treatments have become central to our approach against disease and in performing surgeries. Over two-million people in the United States are infected by antibiotic resistant bacteria each year in the United States. Of this number 23,000 die from these infections and many more die from other complications made worse by these bacteria\(^1\). This makes it even more prudent to discover a new class of antibiotic. Many current antibiotics use target the cell wall, protein synthesis machinery or DNA replication. It is crucially necessary to find a novel target for new antibiotic therapies because of the widespread resistance to the known antibiotics.

Bacterial cells grow and reproduce using a series of proteins collectively known as the cell division machinery. This machinery promotes the division of one cell into two identical daughter cells. The cell division machinery is similar between bacterial taxa, making it an ideal target for new classes of antibiotics. Therefore, understanding the molecular mechanisms driving bacterial cell division will provide enormous potential.

In actively dividing cells, the cytoskeletal protein FtsZ drives the formation of a division septum at the cell center. FtsZ forms a large protein structure called the Z-Ring. FtsZ in the Z-Ring forms large linear polymers that assemble from the head to tail arrangement of individual FtsZ molecules. Protein interactions between FtsZ in the Z-Ring and additional cell division proteins facilitate the cleavage of the cell into two daughter cells\(^2\). Normal division and growth require the Z-Ring to form at the cell center. Mislocalization of the Z-Ring during cell division will result in abnormal growth, which often results in cell death. There are many different proteins that interact with FtsZ to ensure that it forms at the center.

In \textit{E. coli}, the position of the Z-Ring is regulated by the Min system, which includes the proteins MinC, MinD, and MinE. The Min system inhibits the formation of the Z-Ring at the cell poles by oscillating between each cell pole. MinD utilizes cellular energy in the form of ATP (adenosine triphosphate) to bind to the membrane. MinE binds to MinD, releasing it from the membrane\(^3\). MinE has the additional effect of stabilizing ATP hydrolysis upon binding to MinD.
as well. This allows for proper oscillation of the other Min proteins. Cycles of membrane binding and release by MinD form the basis of the oscillation\(^4\). MinC oscillates between the cell poles by binding to MinD and directly inhibits the formation of the Z-Ring\(^5\). As the functionality and survivability of a bacterial cell hinges on normal cell division, this makes the Min system particularly attractive as a target for novel antibiotics. The Min system regulates cell division through the interaction between different proteins. MinD and MinC are especially important as they form the gradient that directly inhibits the ability of the cell to divide normally.

**Introduction**

Those above facts are key factors in how I choose to go about choosing my honors project. I have always enjoyed both science and helping people and thus a path going into disease research or antibiotic research has always interested me greatly. It aligned with two defining facets of who I am. This interest led me to join Dr. Jodi Camberg’s lab almost two and a half years ago. She studies cellular division machinery and how they may be utilized as a novel target for antibiotics. Over the course of the time I have spent in her lab I have learned a great deal about how to conduct and design experiments in conjunction with a graduate student. When looking into what I wanted to do for my honors project I decided I wanted to test myself and see if I could design an experiment largely on my own. Looking back at what has been done in the lab I decided to look into something that no one has looked into yet, how the protein MinC affects the protein MinD’s movement within the cell. The main takeaways I want to get from this experience are how to design and conduct experiments on my own and to help propel this research into areas previously unexplored. An additional boon is that I will be working on something that has the potential to expand our knowledge of what may become a new target for antibiotics. This aligns very well with my interests as this research can help fight the emergence of new antibiotic resistant bacteria and the diseases they cause.

Looking at that specific interaction between MinD and MinC is the center piece of my proposed project. No one has looked at how MinC effects MinD’s oscillation in vivo. I want to change this. I aim to first observe normal oscillation of MinD via an observation method called fluorescence microscopy. This method involves tagging a protein with another that will emit fluorescence of a certain color at specific wavelengths of light. I will use a green-fluorescent
protein or gfp for short. I should see normal oscillation from pole to pole. I will then delete the minC gene from the cell and observe what happens to the oscillation of MinD in its absence. If this all goes well, then I can introduce mutagenized MinC into the cells that do not have MinC to observe how specific amino acid mutations effect the oscillation. Looking at these interactions will provide more insight into how the cellular division machinery works and therefore will also allow us to gain a better understanding of a possible novel target for antibiotics.

**Procedures and Methods**

The two major procedures that will be utilized in this project are fluorescence microscopy and bacterial transformations that will allow cells to take up engineered DNA. For fluorescence microscopy I will be using a fluorescence microscope to examine cell specimens and observe oscillation. The microscope will be turned on and set to a low power. After this it is required to wait approximately fifteen minutes for the microscope to provide full brightness. Next the prepared microscope slide with the sample will be affixed to the scope and analyzed. If a camera piece is fitted to the eye piece, then pictures can also be taken.

The next major procedure that I will utilize is called a bacterial transformation. To begin I would grow up a cell culture from a culture allowed to grow overnight to an optical density of about 0.3 AU. This is typically a culture of 3mL of LB, 30uL of overnight culture and 3uL of antibiotic. Afterwards this is spun down for 10 min at 10x gravity to pellet the cells. These cells are then resuspended 4 times in 1mL of 10% glycerol. They are spun down for about 2 min between each resuspension at 10x gravity. Afterwards the DNA that I will want the cells to take up will be added to the resuspended cells and electroporated at 2500V. The typical amount of DNA is around 400 nanograms of the engineered DNA. They are allowed to rest at 30 degrees Celsius for 1hr and then are plated on ampicillin plates at 30 degrees for 24hrs.
References


