Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 20 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

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Chemical-biological studies of cell division and intracellular organization in bacteria

By

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A place for everything and everything in its place

A tale of bacteria, old sayings, and new findings

Bacteria have a fundamental impact on our lives. For many years, scientists thought that these microscopic organisms were simple, featureless, and disorganized cells. Recent studies have shown that bacteria are far more complex than we ever imagined. In this article, we discuss new findings about the fascinating bacterial world and how these discoveries affect us.

The good (bacteria) pay for the bad. What is the first thing that comes to your mind when you think of the word "bacteria"? For many people, the word "bacteria" sounds scary and is often associated with diseases, hospital-acquired infections, food poisoning, bioterrorism, you name it. Bacteria acquired their bad reputation partially because most people learn about bacteria in the context of a disease. Thus, it is natural to think about the harm they do. However, there are a lot of myths and misconceptions about bacteria. In fact, the vast majority of bacteria we know of are harmless to humans , and a lot of them have enormous beneficial effects on our lives.

The following are a few examples of the benefits that bacteria provide to humans: (1) bacteria that live inside our gut help us digest our food, protect us from getting infections, and produce some of the vitamins we need to stay healthy; (2) soil bacteria provide nutrients to plants and are important for agriculture; (3) bacteria in the soil and in the ocean are major players in the decomposition of organic matter; and (4) many bacteria are used to produce fermented food (yogurt and some types of cheese and sausage, for example), and pharmaceutical products (antibiotics and anticancer drugs, for example). The bottom line is: We would not be able to live without bacteria! Because we are surrounded by these microorganisms[1,2] (organisms that are invisible to the naked eye) and they are so essential in our lives, it is important to study their lifestyles, their behaviors, and how they affect the world around us.

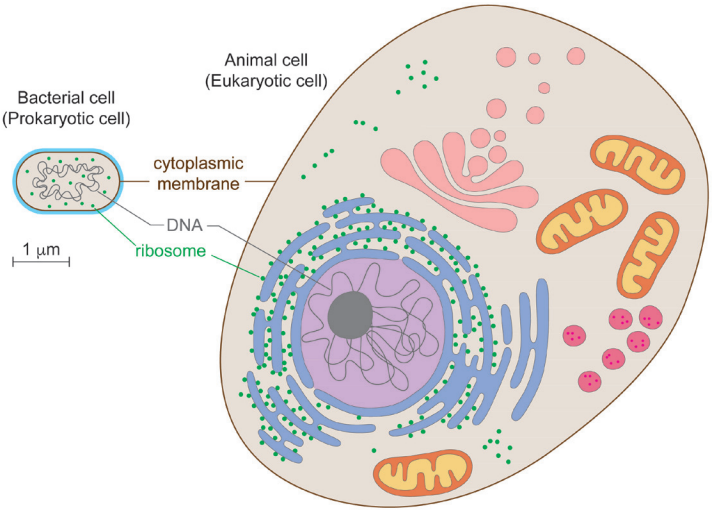

Figure 1. Cartoon depicting some of the differences between prokaryotic (bacterial) and eukaryotic cells.

First impressions are the most lasting. Microbiology is the branch of science that investigates microorganisms such as bacteria, viruses, molds, and yeast. This field of study started around the second half of the 17th century with the first documented observations of microorganisms by the English scientist Robert Hooke and the Dutch businessman and scientist Antonie van Leeuwenhoek. The latter was the first to report the existence of microorganisms that were later confirmed to be bacteria. Using a simple and small microscope of his own invention, van Leeuwenhoek observed different types of bacteria[3] in various samples, including dental plaques scraped from his teeth. However, the study of microbiology did not develop rapidly after these initial observations because microscopes were rare and underdeveloped, and the interest in microorganisms was not high.

As more sophisticated and powerful microscopes became available in the 19th century, scientists interested in studying cell biology observed remarkable differences between bacteria and cells from other organisms. The two most striking differences that could be seen through a microscope were the size and the internal complexity of their features. Microscopy images revealed that bacteria (1) typically are much smaller than cells from other organisms[4]; and (2) lack almost all internal structural features that are common in plant or animal cells. Based primarily on these two differences, scientists separated all organisms into two types: the "prokaryotes" to represent bacteria[5]

[1] The estimated number of bacteria on the planet Earth is around 1×10^{30}. That is almost 100 million trillion bacteria for every human on the planet.
[2] The number of bacteria on and in the human body—mainly the gut, skin, mouth, and some regions of the eye—is estimated to be 3.8×10^{8}. About 0.45 pounds of a person who weights, approximately, 145 pounds is made up of bacteria.
[3] Antonie van Leeuwenhoek named the group of microorganisms he discovered, including bacteria, "animalcules."
[4] Bacteria are about 1-10 micrometers (µm) long and 1 µm wide (1 µm is 1 meter divided by 1 million). Cells from animal or plants, for example, are about 10-100 times bigger than bacterial cells. There are many exceptions though.

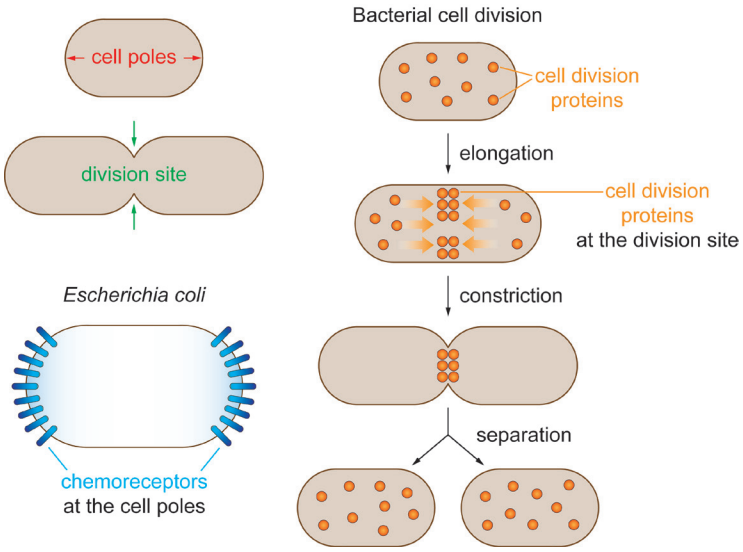

Figure 2. Cartoon depicting regions in a bacterial cell where proteins localize. This example highlights chemoreceptors and cell division proteins in *Escherichia coli*.

and the "eukaryotes" to represent animals (including humans), plants, fungi (mold, yeast, and mushrooms, for example), algae, and protozoa (amoeba, for example).

Bacteria have a membrane (called the cytoplasmic membrane)[6] surrounding a gel-like substance called cytoplasm, which contains the DNA (the genetic material), ribosomes (a machine necessary for synthesis of proteins), and all the other components that are important for cell survival (Figure 1). Eukaryotic cells possess many internal structures called organelles in addition to the cytoplasmic membrane and ribosomes. Some of the most prominent organelles of eukaryotic cells are: endoplasmic reticulum, mitochondrion, plastid[7], Golgi apparatus, lysosome, vacuole, and nucleus (where the DNA is stored) (Figure 1). These organelles can be thought of as the organs of the cell. In fact, the word "organelle" means small organ. Each organelle forms a separate space inside the cell and holds a characteristic set of proteins and other biomolecules[8] that perform a specific task (or biochemical reaction). Thus, eukaryotic cells have an organized internal space.

Eukaryotic cells and their organelles are often compared to a factory. Factories have an external wall with security cabins that protect them and control what crosses the border of the factory. They also have interior walls to separate offices and different departments. There is an executive department where major decisions are made, including what product the factory will produce. The receiving department receives and brings in all the components the factory needs to make the product, and the communication department allows it to contact suppliers. There is a production line where the product is assembled and a finishing/packaging department to process and prepare the product for shipping. The power plant provides all the energy and the cleaning department mantains cleanliness.

Using this same analogy to describe bacteria, it is tempting to imagine that they would be the equivalent of a small shed lacking any internal walls to separate the departments. Can you imagine how messy and, perhaps, inefficient this factory would be? For many years, scientists believed that bacteria were simple, disorganized "sacks," devoid of intracellular features such as organelles and, therefore, unable to physically separate all the biochemical reactions. This simplistic description of bacteria implied that all the processes happening inside the cell were not as controlled as in eukaryotes, and all the biomolecules being produced were randomly distributed.

Don't judge a book by its cover. After further developments in microscopy and other methods of studying bacteria in the 20th century advanced our understanding of cell biology, scientists renewed their focus on the study of bacteria as they realized that these microorganisms are far more complex than many researchers previously thought. Studies from the early 1990s revealed that some bacteria are able to place specific proteins at specific locations within the cell. Some of these proteins are enriched at the extreme ends (the cell poles) of rod-shaped cells; others, at the center of the cell. These studies showed that bacteria are able to maintain internal organization of the cell even in the absence of organelles (Figure 2). By keeping certain proteins in specific regions of the cell, bacteria guarantee that some cellular processes will only happen at these defined regions. This is similar to what eukaryotic organelles do; they confine each cellular process to a specific region of the cell.

These seminal studies on bacterial internal organization changed the way scientists thought about bacterial cells and laid the groundwork for identifying other cellular components that localize to specific regions of the cell. We now know that, in addition to proteins, some types of RNAs and

[5] Prokaryotes are unicellular microorganisms. In addition to bacteria, there is another type of prokaryotic cell called archaea. They resemble bacteria, but differ in their evolutionary history.
[6] Bacteria contain an additional layer called the cell envelope (or cell wall) surrounding the cytoplasmic membrane. There are 2 major types of cell wall. The differences in composition of these types are used to separate bacteria into 2 groups: the Gram-negative and the Gram-positive bacteria.
[7] Plastids are organelles found in the cells of plants and algae.
[8] Biomolecules are molecules present in living organisms. Some important large biomolecules are: nucleic acids (DNA and RNA), proteins, carbohydrates, and lipids. They all perform a vast array of functions within the cell.

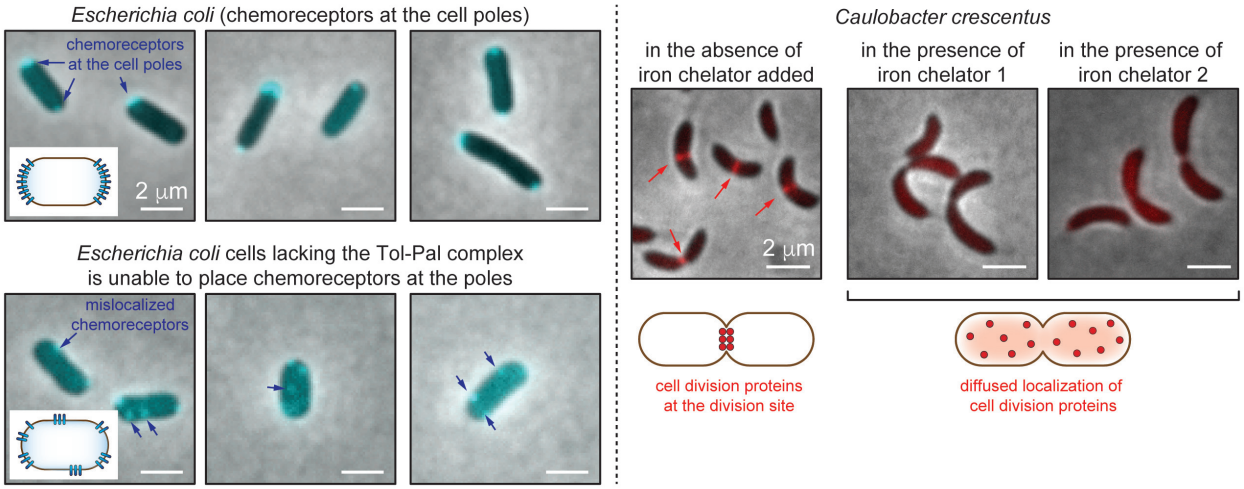

Figure 3. Microscopy images of fluorescently-labeled chemoreceptors in *Escherichia coli* (right panel) and cell division proteins in *Caulobacter crescentus* (left panel).

lipids also prefer certain regions of the cell. This finding that biomolecules are not moving aimlessly inside bacteria was a great discovery in microbiology and it changed the way we look at these microorganisms. It is now accepted as fact that bacteria once thought of as "simple" are able to maintain internal organization. Going back to our factory analogy: Even though the small shed lacks internal walls to separate departments[9], some of the workers (proteins and other biomolecules) concentrate at specific places to maintain the level of organization the factory needs to be efficient.

Seeing is believing. At this point, you might be asking yourself: How does one know where proteins are located inside a bacterial cell? Most studies rely on fluorescence to visualize where the protein goes. Using genetic manipulations, scientists can add an artificial fluorescent tag to their protein of interest and use microscopy to watch the protein in real time inside the cell (Figure 3). Generally, this technique is very powerful. We can basically learn the intracellular "address" of every protein for any bacteria of interest. Many studies have made great contributions to this field; however, they also show that we are only beginning to learn where proteins live inside bacterial cells.

All these findings also raise another important question: How do these proteins that prefer certain regions of the cell find their way around? In eukaryotes, this question has been investigated for many years and researchers have solved several parts of the puzzle. With few exceptions, eukaryotic proteins carry a small tag that contains their "address." The eukaryotic cell uses a sophisticated mechanism to read the information in the tag and direct each protein to their correct location. Using our factory analogy, it would be as if the employees of the factory carried a tag in their uniform with the directions to the department where they work. The factory has a transport system with a GPS that makes sure employees finds their department. How about bacterial proteins?

Unlike eukaryotic proteins, bacterial proteins do not seem to carry a tag with their addresses. At least, scientists have not yet been able to identify any such tags. Instead, proteins that have a preference for certain regions of the bacterial cell use other mechanisms to find their correct location. Some proteins find their way because of differences in geometry of the cell surface or by recognizing specific features inside the cell. In the factory analogy, it would be as if employees found their department in the factory by searching for specific regions like a corner, or a chandelier hanging in the center of the shed.

Why is it important to understand how proteins find their correct location inside the cell? There are many reasons: (1) some proteins only function properly if they are in the correct place; (2) in some cases, "being in the wrong place at the wrong time" can result in negative outcomes to the cell or even death. This is particularly true in human cells, where mislocalization of proteins can lead to human diseases, such as cardiovascular and neurodegenerative diseases, as well as cancer. Finally, (3) if scientists understand the mechanisms by which proteins find their destination in the cell, they can manipulate these systems to treat diseases.

There's a time and place for everything. In my doctoral work at the University of Wisconsin–Madison, my colleagues and I investigated how a specific family of bacterial proteins finds

[9] Recent studies have shown that some types of bacteria contain internal structures that resemble eukaryotic organelles.
[10] In science, a model organism is a simplified system that is accessible and easily manipulated.

their destination inside the cell. Specifically, we were interested in studying the localization mechanism of a family of bacterial proteins called chemoreceptors. These proteins are important sensorial components of bacteria. Chemoreceptors allow the cell to sense the amount of nutrients in the environment and move towards regions that are more favorable for growth. It basically helps bacteria to find nutrients and to run from toxic substances. As you can imagine, these proteins are very important; if a cell is not able to find nutrients or escape poisonous environments, they will not be able to survive. Another interesting fact about chemoreceptors is that they are concentrated at the extreme ends of rod-shaped cells (the cell poles) (Figure 2). We decided to study how these proteins find the cell poles in the model[10] bacterium *Escherichia coli* (*E. coli*) and what problems mislocalization of chemoreceptors can cause.

To investigate this problem, we first labeled *E. coli* chemoreceptors with a fluorescent tag so we could easily visualize where they are located (Figure 3). Next, we created variants of *E. coli* (mutants) that do not make some proteins that we predicted to contribute to polar localization of chemoreceptors. Finally, we used microscopy to image these mutants and see which ones were no longer able to localize the chemoreceptors at the cell poles. We found some mutants that were unable to send their chemoreceptors to the cell poles (Figure 3). In these mutants, chemoreceptors were dispersed all around the cell. The mutants that are unable to localize chemoreceptors at the cell poles do not have the genetic information necessary to make five proteins that form a large protein system called Tol-Pal complex. In other words, if the Tol-Pal complex is not present in the cell, chemoreceptors are not able to stay at the poles. These results suggest that chemoreceptors need the Tol-Pal complex to find the cell poles and stay there. We performed additional experiments and found that chemoreceptors associate with the Tol-Pal complex and this association is important to keep them at the cell poles.

Next, we investigated what happens to the cell when chemoreceptors fail to localize at the cell poles. We found that bacteria that do not have Tol-Pal complexes and, therefore, are unable to place their chemoreceptors at the poles develop sensorial defects and fail to move towards nutrients efficiently. These findings confirm that when proteins fail to find their correct location inside the cell, the cell does not function properly. The ability to sense and move towards nutrients is very important for some pathogenic bacteria to infect humans. If these pathogens lose the ability to sense nutrients around them, they can no longer cause disease. We can explore this system to fight diseases caused by bacteria. For example, we could develop drugs that affect the pathways used to direct chemoreceptors to the bacterial poles.

Another part of my research was designed to understand how bacteria reproduce and what signals are important for this process to happen. During this process (also called cell division), a rod-shaped bacterium such as *E. coli* elongates, constricts at the central region, and separates into 2 cells (Figure 3). Many proteins are required for replication and they must all be recruited to the central region of the cell when the cell is ready to divide. If these proteins fail to localize at the division site, the cell will not be able to replicate. We decided to investigate strategies to block bacterial replication by preventing these proteins from finding the division site. Our first approach was to explore if nutritional starvation could prevent proper localization of proteins involved in cell division. We focused our studies on iron deprivation, since iron is an essential nutrient for most cells, including bacteria. In addition to *E. coli*, we used another model bacterium called *Caulobacter crescentus*. To easily visualize the proteins that are important for cell division in bacteria, we separately labeled them with a fluorescent tag and treated the labeled bacteria with chemical compounds called iron chelators. These iron chelators hold iron and make it unavailable to the bacteria. In other words, iron chelators prevent bacteria from using iron. Therefore, bacteria behave as if there was no iron in the environment. Following the treatment with the iron chelators, we examined the cells to see where the proteins involved in division were located. We found that bacteria are unable to properly localize their proteins at the division site in the absence of iron and, as a consequence, are unable to divide (Figure 3). This discovery has important clinical applications. We are interested in using these iron chelators to develop new therapies against pathogenic bacteria that cause human diseases.

A drop in the ocean. For many years, bacteria were considered to be simple, featureless microorganisms devoid of internal organization. Many recent studies have demonstrated that localization of proteins and other biomolecules is common in bacteria and is necessary for a variety of processes. Scientists have made significant progress towards understanding how bacteria control the localization of their proteins, but there is still a lot to be learned. There are many types of mechanisms of localization awaiting to be discovered. The research my colleagues and I presented here adds a little piece to this complex puzzle. Understanding how bacteria establish and maintain the internal organization of their biomolecules will help us understand similar processes in other organisms and to develop new strategies to treat disease.