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Contact: Prof. Bassam Z. Shakhashiri

UW-Madison Department of Chemistry

bassam@chem.wisc.edu

www.scifun.org

Adaptive strategies employed by *Clostridioides difficile* in response to environmental and nutritional cues

by

Anna Gregory

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The dissertation is approved by the following members of the Final Oral Committee:

Kerri Coon, Associate Professor, Bacteriology

Johanna Elfenbein, Associate Professor, Pathobiological Sciences

Jo Handelsman, Professor, Plant Pathology

Andrew Hryckowian, Assistant Professor, Medical Microbiology and Immunology

Federico Rey, Professor, Bacteriology

A Metabolic Heist: Understanding how *Clostridioides difficile* sequesters host glutathione

This chapter was written at the invitation of the Wisconsin Initiative for Science Literacy (WISL) at UW-Madison. WISL encourages doctoral candidates in science and engineering to include a chapter in their Ph.D. thesis that describes their research to a non-science audience. In the following pages, I summarize what motivates my work, the scientific questions that drive my curiosity, and the core findings of my dissertation—written for a general, public audience. I would like to thank Professor Bassam Shakhashiri, Elizabeth Reynolds, and Cayce Osborne for their support and editorial guidance throughout the writing of this chapter.

Spilling my Guts: An Introduction

We reference “the gut” all the time! We follow our **gut** instincts, endure **gut-wrenching** news, and have the **guts** to do hard things. We often use the word without even thinking about the complex organ system it describes. I like to think that it’s because, even if we cannot describe the intricacies of the gut, on some level, we all know the gut matters.

Human history certainly reflects the centrality of the gut. Long before the function of the gut was understood, there seemed to be a recognition that the gut was important. The ancient Egyptians preserved the intestines of their dead in a separate jar during mummification, so the deceased would have them upon resurrection in the afterlife.[1] Centuries later, the Greek physician Hippocrates (460 – 370 BCE) famously claimed that “all disease begins in the gut.” There’s even a third-century patron saint of gastrointestinal and liver disease, Saint Erasmus of Formia, found in both the Catholic and Orthodox church traditions.[2] Even though they lacked a modern understanding of the gut, our ancestors knew intuitively that the gut played a prominent role in a person’s health.

My own fascination with the gut began when mine started to malfunction. It began with bright red blood in the toilet bowl. At the time I remember thinking “that’s weird,” but I was in college, so just tossed it up to stress. But the blood kept coming back. Sometimes, there would be months between episodes, sometimes years. The randomness of the bleeding combined with the mystery of its source led me to conclude that nothing serious was going on and life moved on.

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[1] *Egyptian Mummies*. Smithsonian Institution. https://www.si.edu/spotlight/ancient-egypt/mummies

[2] Walker-Smith, J. (2002). A god for guts. *Gut*, *50*(6), 886–887. https://doi.org/10.1136/gut.50.6.886

Then my aunt, who had Crohn's Disease, was diagnosed with colorectal cancer. She passed away not long after. It was shocking, fast, and devastating.

It also forced me to be more proactive about my own health. I could no longer ignore my gut. I made an appointment with a gastroenterologist (a doctor who specializes in the digestive system) and got a colonoscopy. During the colonoscopy, the doctor discovered some "active colitis with neutrophilic cryptitis, crypt abscesses, and crypt disarray," essentially there was some mild ulceration and inflammation at the end of my large intestines. The gastroenterologist informed me that these episodes of inflammation may be something I experience for the rest of my life, and to be more vigilant in monitoring my health.

My family's medical history and my own experience made me curious. How could a system of hollow tubes and organs cause so many people so much distress?

I have spent my Ph.D. pursuing a deeper understanding of the gut, the beneficial microbes that help it thrive, and the dangerous microbes (pathogens) that make our guts sick. I hoped that by studying the very organ system that sometimes revolts against me, I would gain a sense of empowerment rooted in knowledge.

I am writing this chapter to share what I have learned. I hope this information empowers you to understand your own gut a little better. Or to at least reclaim the power behind the word "gut" the next time you use a gut-focused idiom.

Public Service Announcement:
Colorectal cancer, colonoscopies and PEG

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second deadliest cancer worldwide.[3] It develops when normal, healthy cells in your large intestines begin to divide uncontrollably. The best way to prevent CRC is with routine colonoscopies (recommended every ten years starting at age 45). During this procedure, a doctor guides a thin, flexible camera through the sedated patient's large intestine (**Figure 5.1A**). The primary goal of this screening is to catch CRC in its early phases and remove pre-cancerous growths, called polyps, before they become something more serious. Colonoscopies can also be used to diagnose other GI diseases, like Inflammatory Bowel Disease (IBD). For a colonoscopy to be successful, the doctor needs a perfectly clear view of your large intestine, meaning your gut must be as clean as possible. Hence, the infamous prep! Preparing for a colonoscopy usually involves drinking a powerful laxative the day before your procedure. For my colonoscopy, I was prescribed the most common laxative version: polyethylene glycerol 3350 (PEG). I was given a giant plastic bottle, where I mixed the PEG powder with a gallon of water (**Figure 5.1B**). The night before, and the morning of your procedure, you drink this solution at regular intervals, which increases the frequency of bowel movements. By the time of your colonoscopy, you should have a clean large intestine, ready for inspection. The prep is uncomfortable and inconvenient but catching CRC early is worth the discomfort. I encourage everyone to be proactive regarding their gut health!

How does PEG work? Normally your intestines absorb the water you drink, which solidifies your stool. PEG, on the other hand, can't be absorbed. Furthermore, it binds to water, preventing your body from absorbing the fluid. This extra water in your gut softens stool and fills the bowel, triggering more trips to the bathroom.[4] (**Figure 5.1C**).

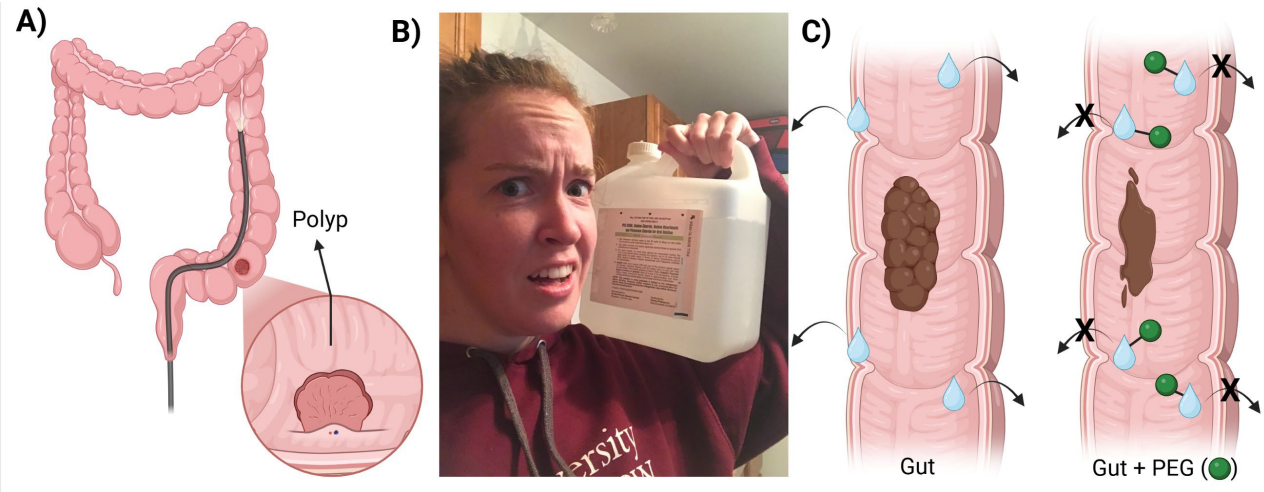

Figure 5.1. Preparing for a colonoscopy is key for success. (**A**) During a colonoscopy the doctor guides a thin, flexible camera to check your large intestine for polyps and cancer. (**B**) Beforehand, you drink a laxative solution (**C**) that prevents water absorption and increases bowel movements. This leaves your gut clean for the doctor to check for inflammation, polyps, and/or cancer.

[3] Keum, N., & Giovannucci, E. (2019). Global burden of colorectal cancer: Emerging trends, risk factors and prevention strategies. *Nature Reviews. Gastroenterology & Hepatology*, *16*(12), 713–732. https://doi.org/10.1038/s41575-019-0189-8

[4] Dabaja, A., Dabaja, A., & Abbas, M. (2025). Polyethylene Glycol. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK557652/

Gut Check: Your Gut Microbiota

Gut is a vague word that often gets thrown around to refer to the general gastrointestinal (GI) tract. The GI tract is the organ system that consists of the mouth, esophagus, stomach, small intestines, large intestines, and anus, with the primary functions of digesting food, absorbing nutrients, and expelling waste. While the GI tract may look like a series of hollow tubes, to think these structures are "empty" would be incorrect. The GI tract houses trillions of microorganisms. In fact, we are more microbe than human! The typical adult human body consists of 30 trillion human cells, and 38 trillion bacteria cells, and that's not even counting the fungi, viruses, archaea, and protists in and on us![5] The most complex microbial community is found in your large intestines (**Figure 5.2**). This specific community of organisms is called the **gut microbiota**.

I like to think of the gut microbiota like a symphony orchestra. In an orchestra, there's lots of different instruments, each with a unique sound, playing different notes. But those notes come together in harmony to form a single song. Similarly, in the gut, there are thousands of different species of microbes. Each species has its own preference for nutrients, generates its own unique byproducts, and interacts with other cells in a slightly different manner. But when you put these microbes together, they create a functioning large intestine ecosystem

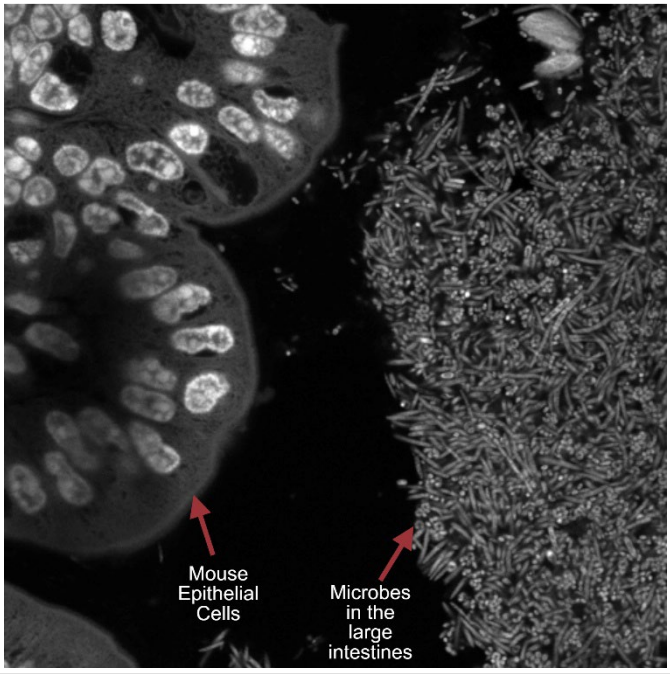

Figure 5.2. A close-up image of a mouse's large intestine, showing the community of gut bacteria (the gut microbiota) that lives inside it.

[5] Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

that is essential for our health. In fact, humans have co-lived with gut microbes for so long that we have developed a symbiotic relationship. We provide these microbes with a warm, nutrient-rich environment, and in return the microbes perform vital services for us. They help digest the food we eat, train our immune system to distinguish between “good” and “bad” microbes, impact our mental and physical health, and protect us from pathogens.

An orchestra requires the perfect balance of different instruments to achieve the correct sound, if some instruments drop out or others get too loud, the music goes awry. Similarly, your gut needs a balanced community of microbes to achieve ideal health. When this delicate balance is thrown into severe disarray, this disruption—often called dysbiosis—compromises the essential health benefits provided by the microbes, which contributes to various infections and chronic health issues.

A Punch in the Gut: **Dysbiosis Disrupts Gastrointestinal Health**

The word **dysbiosis** comes from the Greek: *dys*-, meaning “bad” or “difficult,” and *bios*, meaning “life.” Literally translated, it means “bad life.” This translation is fitting, because it refers to what happens when our normal, protective microbial community becomes imbalanced or severely disrupted. This imbalance can be triggered by many modern factors, such as antibiotic usage, poor diet, and lifestyle choices, such as a lack of exercise, poor sleep quality, smoking, and chronic stress.

A dysbiotic gut is characterized by a decrease in the overall diversity of microbes, meaning fewer species of beneficial microbes are present (**Figure 5.3**). The loss of these beneficial microbial impacts gut function in several ways. Initially, the loss of these microbes leads to an increase in inflammation and oxygen (O_2) into the large intestine. A healthy and diverse large intestine is considered anaerobic, meaning there’s no O_2. Most of the microbes living there have

adapted to survive without it, and for many of them, O_2 is actually toxic. Therefore, when inflammation and O_2 are introduced to this environment, good microbes struggle to recover, exacerbating dysbiosis. Simultaneously, these conditions also cause the protective mucus lining of the intestines to thin. This is problematic because this mucus acts like a shield, keeping microbes at a safe distance from our gut cells. When the mucus barrier breaks down, harmful microbes invade, increasing the risk of illness.

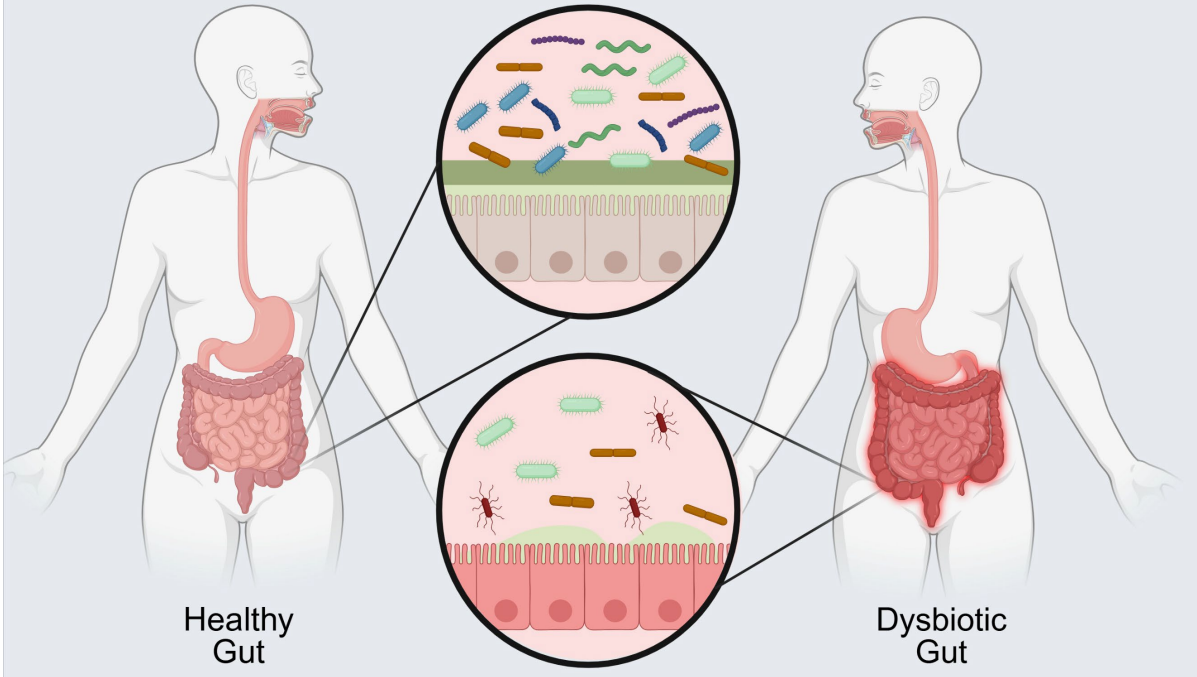

Figure 5.3. A healthy vs. dysbiotic gut. This diagram comparing a healthy gut (on the left) with a dysbiotic gut (on the right). The dysbiotic gut shows less variety in bacteria, has irritated (inflamed) gut cells, and has lost its protective mucus layer.

Researchers have found links between dysbiosis and health conditions ranging from inflammatory bowel disease (IBD) to gastrointestinal pathogens. IBD is an autoimmune disease where one's own immune system mistakenly attacks the cells of the gut, leading to chronic swelling and inflammation of the intestinal tissues. The disease is marked by recurrent "flare-ups," or periods of increased symptoms (e.g. abdominal pain, diarrhea, rectal bleeding, etc.). The inflammation can affect either the entire gastrointestinal tract (Crohn's disease) or be

confined to the rectum and colon (ulcerative colitis). While we don't fully understand what causes IBD, we know that dysbiosis is a key marker of the disease.[6,7]

A clearer connection exists between gut dysbiosis and opportunistic pathogens. Opportunistic pathogens are microbes that are harmless or neutral in a healthy person but start causing symptoms when an individual's health is compromised. These pathogens often proliferate in individuals who are immunocompromised, advanced in age, suffering a chronic disease, like IBD, or using antibiotics.

But antibiotics are supposed to help fight microbial infections! So how can antibiotic use lead to opportunistic pathogen infections? Let's say you go to the clinic because you are experiencing bad respiratory symptoms. At the clinic you are diagnosed with pneumonia and prescribed clindamycin. When you swallow the antibiotic, it travels through your digestive system. Most of the antibiotic will be absorbed to try to address your respiratory infection, but some of the antibiotic will inevitably reach your large intestines. There it will act like a bomb, killing off some of the good bacteria that are supposed to be protecting you. Without these key protective bacteria, you become more susceptible to GI infections caused by opportunistic pathogens, such as *Escherichia coli*, *Salmonella*, and *Clostridioides difficile*.

A Gut-wrenching Truth: My Research on how *Clostridioides difficile* Steals our Glutathione

When the gut falls out of balance (dysbiosis), opportunistic pathogens turn against us. And they don't just make us sick, they can also act like expert thieves, hijacking our cells' most valuable

[6] Winter, S. E., & Bäumler, A. J. (2023). Gut dysbiosis: Ecological causes and causative effects on human disease. PNAS 120(50), e2316579120. https://doi.org/10.1073/pnas.2316579120

[7] DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. *Inflammatory Bowel Diseases*, *22*(5), 1137–1150. https://doi.org/10.1097/MIB.0000000000000750

resources: nutrients. These opportunistic pathogens are remarkably resourceful at finding ways to tap into our stores of iron, sugars, and other essential molecules, to fuel their own growth.

One pathogen that is a master thief is *Clostridioides difficile* (*C. difficile*, or *C. diff*). This bacterium causes *Clostridioides difficile* infection (CDI), a condition infamous for its severe, recurring diarrhea. Think of the worst diarrhea you've ever had. Now imagine the pain and urgency doubled, lasting longer, and coming back again and again. That's what living with severe CDI can feel like. CDI symptoms can range from asymptomatic to life-threatening intestinal inflammation (colitis). Normally, a healthy gut microbiota keeps *C. difficile* in check. Even if you're exposed to the pathogen, your good microbes prevent it from taking hold. But when a person's gut is experiencing dysbiosis, *C. difficile* has the perfect opportunity to establish infection.

So, how does this master thief operate? My research focuses on understanding how *C. difficile* survives by stealing nutrients from our cells to maintain infection. When *C. difficile* establishes infection, it releases toxins that cause inflammation in the gut. This inflammation causes severe diarrhea, which is the hallmark symptom of CDI. In addition to causing diarrhea, the toxins make gut cells "leaky" and can even cause them to die. When this happens, cells accidentally spill valuable nutrients into the gut, including glutathione (GSH). GSH is a small molecule made up of three amino acids: glycine, cysteine, and glutamate (**Figure 5.4**). It is a molecule that is

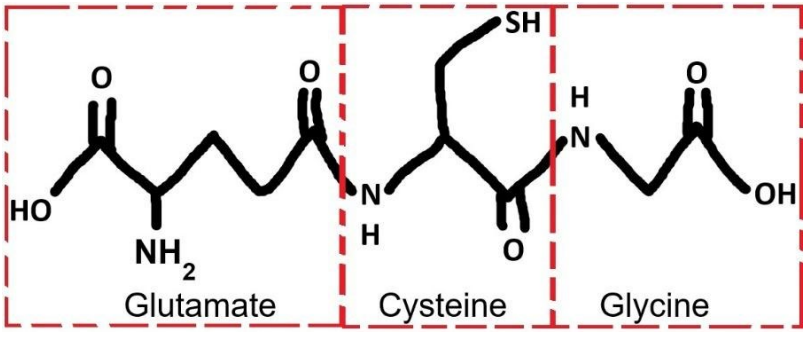

Figure 5.4. The structure of glutathione. It is made up of three building blocks (amino acids). Its most important part is the sulfur-containing group (-SH), which acts like a tiny shield, helping neutralize harmful stress and protect our cells from damage.

present at relatively high levels in our cells. GSH plays a critical role in protecting cells, especially when harmful molecules accumulate faster than the cell can neutralize. Our cells need GSH. In fact, GSH deficits have been associated with a wide range of health issues, including cancers, cardiovascular disease, liver disease, neurological disorders, and immune suppression.[8]

I spent the last five years studying a masterful heist, where GSH is the crown jewel. I found that *C. difficile* produces toxins to induce inflammation (**Figure 5.5**). This causes gut cells to leak GSH into the gut. *C. difficile* literally is forcing our gut cells to spill their valuable contents. *C. difficile* hijacks this leaked GSH and breaks it down using two specialized enzymes, named GecA and GecB (for **g**lutathione-degrading **e**nzymes in ***C****. difficile*). Why go through all this trouble? Because what *C. difficile* really wants is the cysteine molecule, found right in the middle of GSH. Cysteine is extremely important for *C. difficile* to live, but *C. difficile* cannot make cysteine itself. It must steal it from the external environment. Think of it like Vitamin C for humans. Our cells need it to function, but we can't produce it, so we have to eat it in oranges (otherwise, we'll get scurvy).

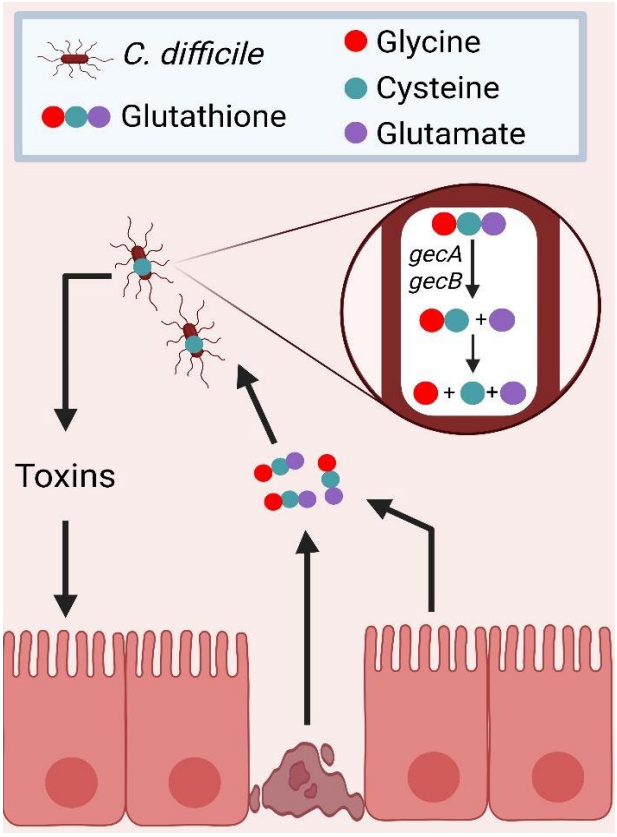

Figure 5.5. A diagram illustrating my research findings. I determined that *C. difficile* toxin force gut cells to leak GSH into the lumen where *C. difficile* metabolizes GSH via GecA and GecB to access the

[8] Alnasser, S. M. (2025). The role of glutathione S-transferases in human disease pathogenesis and their current inhibitors. *Genes & Diseases*, *12*(4), 101482. https://doi.org/10.1016/j.gendis.2024.101482

So how did I actually figure this out? What led me to discover that *C. difficile* acts like a thief? I performed a series of experiments both *in vitro* and *in vivo*. *In vitro* –Latin for "within the glass"—refers to experiments done in a controlled, artificial environment such as a test tube or petri dish. Because *C. difficile* lives in the gut, it is an anaerobic bacterium and O_2 is toxic to it. Therefore, all *in vitro* work with *C. difficile* must be done inside an anaerobic chamber (**Figure 5.6**). You can think of an anaerobic chamber like a sealed plastic bubble filled with nitrogen, carbon dioxide, and hydrogen, but no O_2. It's great for studying anaerobic bacteria, though the arm sleeves can get a little sweaty in the summer!

Figure 5.6. The anaerobic chamber in our lab.

For my *in vitro* experiments I measured how GSH affects *C. difficile* growth in a test tube (**Figure 5.7**). Turns out when you grow *C. difficile* with GSH, more of the bacterium grows at a faster rate. To confirm that the previously discussed GSH growth enhancement is due to GSH, I deleted the genes *gecA* and *gecB*, which I hypothesized *C. difficile* uses for eating GSH. Essentially, I used molecular scissors to cut out my genes of interest. Bacteria with deleted genes are called knockouts (KO) because a specific gene has been "knocked out" or removed. When I deleted *gecA* and *gecB*, the bacteria no longer showed a growth enhancement, even when GSH was added. This demonstrates the *C. difficile* depends on the enzymes GecA and GecB to metabolize GSH. To figure out which part of GSH the bacteria actually uses, I made a special growth liquid (called medium) that didn't include the three amino acids that make up

GSH: glycine, cysteine, and glutamate. Without these amino acids, *C. difficile* barely grew in the test tube. But when I added all three back—or just GSH—the bacteria grew normally again. Then I added each amino acid one by one to the test tube and found that only cysteine was able to restore normal growth. This experiment shows that cysteine is the key piece of GSH that *C. difficile* relies on.

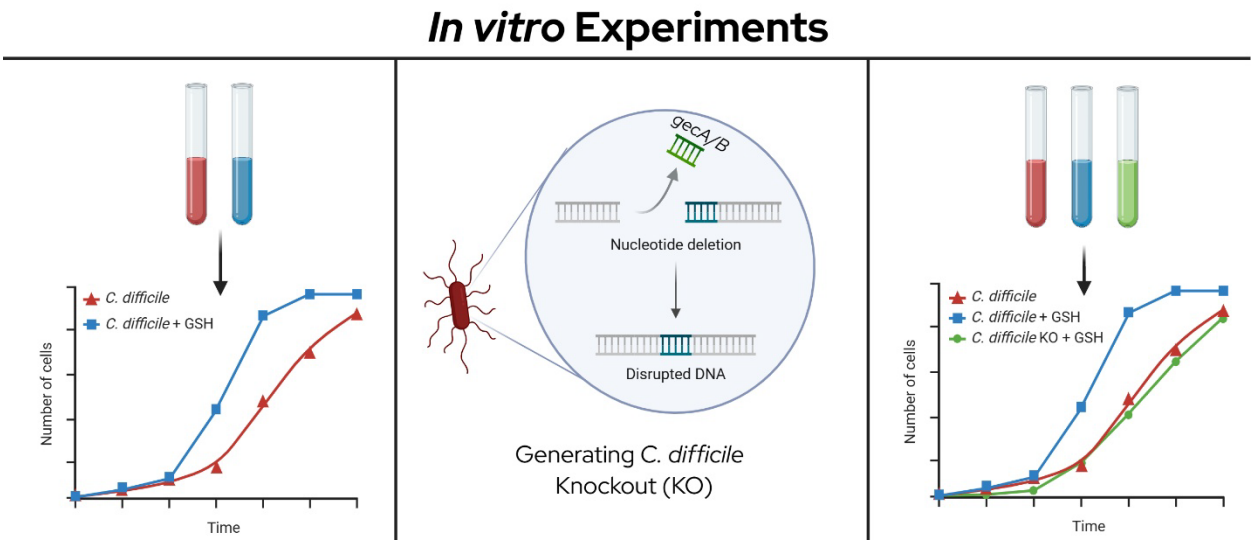

Figure 5.7. Examples of *in vitro* experiments I performed to study the relationship between *C. difficile* and GSH. I grew *C. difficile* in test tubes with and without GSH (left). The genes *gecA* and *gecB*, hypothesized to be involved in GSH metabolism, were deleted to create a *C. difficile* knockout (KO) (middle). These KO was grown with GSH to see if these deletions affected *C. difficile*'s ability to use GSH (right).

While *in vitro* studies are vital for microbiology, they're also simplified, focusing on a single type of bacterium. In reality, microbes live in complex communities. To test whether my *in vitro* findings held true in a living system, I also performed *in vivo* experiments (**Figure 5.8**). *In vivo*—Latin for "within the living,"—refers to experiments done with a living organism. For this I used a mouse model. First, I gave the mice antibiotics using a technique called oral gavage to induce dysbiosis. Once the gut community was disrupted, I introduced *C. difficile* or a *C. difficile* toxin KO, a version of *C. difficile* that does not produce toxins to induce host inflammation. Later I

would euthanize the mice, collect intestinal contents, and measure GSH. I found that mice infected with *C. difficile* had higher levels of GSH than mice infected with the *C. difficile* toxin KO. This indicates that *C. difficile* toxin is important in increasing GSH in the intestine where *C. difficile* can access it to metabolize. This approach allowed me to test whether *C. difficile*'s "thieving" behavior holds true inside a living host.

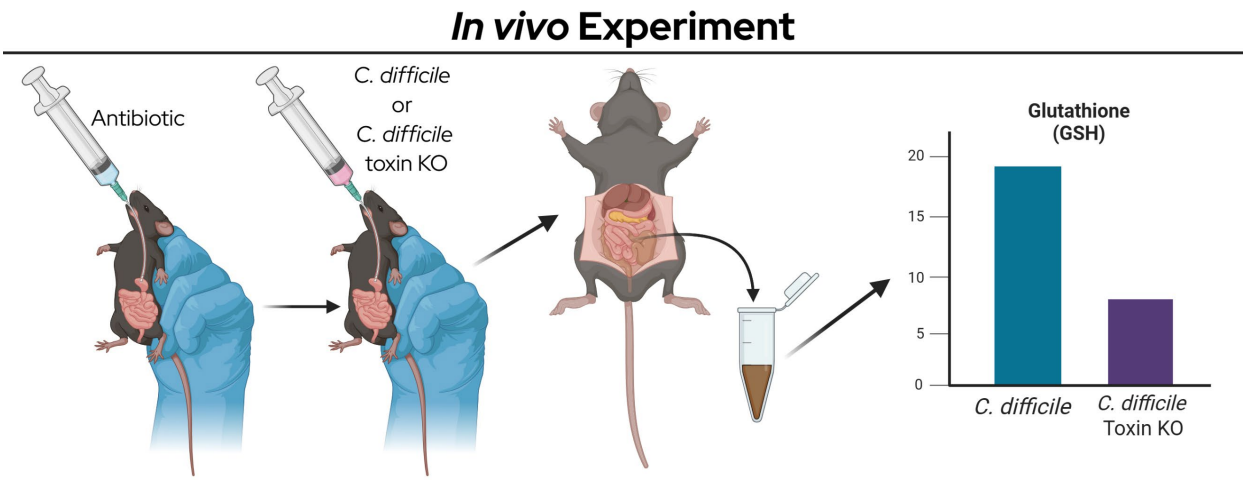

Figure 5.8. Example of an *in vivo* experiment I performed to study the relationship between *C. difficile* and GSH. Mice were treated with antibiotics to induce dysbiosis and then infected with *C. difficile* or a *C. difficile* toxin KO, a version of the bacterium with the toxin genes deleted, so it triggers less host inflammation. Intestinal contents were collected from the mice and GSH levels were measured.

I utilized a series of *in vitro* and *in vivo* experiments to determine that *C. difficile* employs a brilliant strategy to meet this essential need for cysteine. *C. difficile* produces toxins, which damage gut cells causing them to release GSH into the gut where *C. difficile* is waiting to access the essential cysteine. This bacteria survival strategy is somewhat gut-wrenching. We are unintentionally feeding the very microbe that is making us sick. But this is just one powerful example of how opportunistic pathogens can act like master thieves, exploiting us for the nutrients they need to thrive.

No Guts, No Glory: Why does "basic" science matter?

I spent over five years thinking about how *C. difficile* thrives in the dysbiotic gut and what *C. difficile* is eating during infection. But why? Why does this even matter? Why should you care that *C. difficile* steals GSH from gut cells to survive?

My research falls under what is called basic science, or basic research. This kind of science is exploratory, where we set out to expand our knowledge and deepen our understanding of how the world works. There's not always an immediate goal, product, or cure in sight with this type of research. It's often driven by simple questions and insatiable curiosity. Yet, basic science is the foundation for innovation. For example, in 1928 Alexander Fleming observed that *Penicillium* molds secrete a substance that can kill certain bacteria. On its own, this observation didn't have an obvious purpose except for adding to our understanding of the natural world. But later, scientists Howard Florey, Ernst Chain, and their colleagues built on Fleming's discovery, developing a way to purify this substance, called penicillin, turning it into a lifesaving drug. In 1941, a 43-year-old policeman, Albert Alexander, became the first recipient of purified penicillin.[9] It took over ten years and multiple scientists to take a basic research observation and turn it into a new treatment, the first antibiotic. Antibiotics have fundamentally changed medicine, and it all started *not* with a plan to cure infections, but with a basic science observation.

To be completely honest, I don't know yet if my research "matters" in the immediate, practical sense. It takes time—and imagination—for basic research to become applied. In fact, this sometimes makes basic research challenging because you don't always see where your question fits in the bigger pictures, or whether it will ever directly help people. But what I do

[9] *Alexander Fleming Discovery and development of penicillin - landmark.* American Chemical Society. https://www.acs.org/education/whatischemistry/landmarks/flemingpenicillin.html

know is that my research does add to our collective understanding of *C. difficile* and *C. difficile* infection. And the more we understand about *C. difficile*, the better equipped we'll be to develop target therapies in the future. Who knows—maybe in fifteen years patients with CDI will take a GecA and GecB inhibitor to block *C. difficile* from metabolizing GSH. Or maybe in fifty years, someone will design a molecule that binds to GSH in the gut, preventing *C. difficile* from using it altogether. My research shows that *C. difficile* can metabolize GSH stolen from the host. I may not yet know how that discovery will be applied, but I believe the only real limitations are our imagination, curiosity, and willingness to keep following the science.

Trust Your Gut: A Conclusion

I'm always fascinated by what drives scientists. There is so much to be curious about in the world, so why this topic? Why this question? I believe scientific discovery is rooted in a human story. Science is the practice of curiosity, and curiosity is driven by our experience.

For me, my own gut malfunctions and the loss of a loved one to colorectal cancer fueled my desire to understand the gut more deeply. I am not alone in these experiences. I am not alone in my curiosity. By sharing the knowledge gained during my Ph.D., I hope to recognize and honor this universality. This chapter is my effort to bridge the gap between scholarly research and the public, connecting the data to the human stories and experiences that inspire it.