FALSE DICHOTOMIES: BRIDGING SEPARATE WORLDS IN RESEARCH

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ABSTRACT This article follows the author's research experiences with cotton biology and biosynthetic enzymes, using them to explore insights into the nature of research. Key topics include basic vs applied research, natural vs synthetic systems, and tool development.

When I came to North Carolina State University as a freshman, I had already decided to pursue an engineering degree, combining my interest in the natural sciences with my desire to create. After hearing a guest lecture about the biology of cotton fiber, I decided to write a term paper examining the possibility of using cotton as a feedstock for cellulosic ethanol since part of the fiber is almost pure cellulose. When I was looking into undergraduate research opportunities for the summer, I reconnected with the guest lecturer, Dr. Haigler, and set up a summer research internship in her laboratory.

A main focus of the work in Dr. Haigler's lab is to elucidate cotton fiber growth mechanisms, especially those related to length and other characteristics important to the quality of the fiber for textile applications. Although the production of cellulosic ethanol from cotton fibers is not economically feasible, the nearly pure cellulosic structure of the cotton fiber's secondary cell wall makes it an interesting model for studies on this naturally abundant material. Through a better understanding of cellulose synthesis in cotton, the cellulose pathways in more economical bio-energy crops could be manipulated more successfully. By looking beyond the native function of cellulose synthesis in cotton fiber (and plants in general) and understanding the machinery involved in this process, it is feasible that we could one day engineer plants to produce functionalized fibers.

When I began working in Dr. Haigler's lab, I had very little knowledge of the day to day workings of a research laboratory. From my research paper, I gained an understanding of the well-established knowledge regarding the cotton fiber, but I knew very little about current controversies and questions in the literature and even less about how we would go about answering these questions. At the beginning of my work, I shadowed a postdoctoral assistant and learned how to set up standard cotton ovule cultures, as well as how to view these cultures with different microscopy techniques. I remember my excitement working against me during these early stages of research since I had very naïve ideas about the pace of research and was ready to do many experiments and produce tons of interesting data and conclusions. In reality, the process of taking a scientific idea through experimental

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testing can take months or years, and I must confess occasional frustration at the slow pace. My excitement did not fade though, and soon I was working on a set of culture modifications, more or less independently. My interest and curiosity kept me thinking and digging deeper into the methods that I was using and the papers I was reading, prompting me to ask fellow researchers and Dr. Haigler questions that clarified the research and gave me new ideas.

At the end of the summer, I decided to work part time through my sophomore year and began a new project to figure out whether cotton fibers elongate through tip growth, a specialized form of cell growth. In order to do this, I worked on identifying proteins in cotton that were similar to proteins involved in tip growth from other plant species. As I was gathering information about tip growth proteins, I also worked to collect cotton plant tissue and flash freeze it in liquid nitrogen to preserve the ribonucleic acid (RNA). While deoxyribonucleic acid (DNA) stores all of the information cells might need at any time, RNA is a messenger molecule that cells use to direct their protein building machinery. Cells in different developmental stages will have different types and quantities of RNA while their DNA remains relatively constant. After collecting tissue from different time points during the plant's development, we extracted the RNA and used a variant of the polymerase chain reaction (PCR) to amplify RNA corresponding to individual tip growth proteins. PCR uses short 'barcodes' that are specific to different DNA and RNA molecules, allowing researchers to selectively amplify them from very small amounts of starting material. Using these methods, we were able to measure how much RNA was being made for different tip growth proteins at different timepoints in cotton's life cycle. Although being able to amplify specific DNA or RNA sequences may not seem impressive at first glance, PCR and the myriad of techniques built around it allow researchers to study and engineer biology at the molecular level. Thinking about how PCR has truly revolutionized research in the biological sciences, I began to consider developing new research methods as a path to making a large impact in the sciences and engineering.

In all scientific and engineering disciplines, we are limited by the tools we use. Investments made in improving research tools and methods now can provide us with exponentially greater dividends from the resources we apply in the future. Based on this, it might be tempting to pursue tool development exclusively and expect greater yields in the future; however, a great number of revolutionary tools have come directly from basic research into observing and characterizing some phenomenon, which at the time had no application whatsoever. Paradoxically, these discoveries, and therefore the tools that they now support, would not have been made without substantial resources dedicated to basic research and discovery. Basic research and tool development complement each other, and are often inseparable, although there is fierce debate about how to prioritize and fund efforts focused on one or the other.

Through my undergraduate research experiences it became increasingly clear that working in the Haigler lab was exactly the type of research I wanted to be involved in: studying nature's fascinating abilities to synthesize chemicals and materials with the goal of eventually engineering those systems to improve people's lives. Although there are many ways to go about making useful chemicals and materials, a large part of my chemical engineering education had focused on separating side products and impurities after carrying out a reaction, which gave me concrete examples of how many resources could be saved if these purification steps were unnecessary; in the Haigler lab I learned about systems in nature that displayed amazing specificity for both their substrates and products and was drawn to the

idea of 'perfect processes' that did not lead to side products requiring separation. Studying and mimicking natural processes that possessed high specificity was an appealing route to pursue these perfect processes.

My personal fascination with the natural world has led me to focus my studies and research on characterizing and engineering preexisting biological systems. I currently work on polyketide synthases: giant proteins that synthesize complex natural products that have been used as antibiotics, insecticides, and cancer treatments. Although examples of nature's specificity originally attracted me to characterizing and engineering natural systems, developing synthetic routes is a viable alternative, though both have advantages and disadvantages. Synthetic routes may be simpler if they are developed rationally for a single purpose and haven't gone through evolutionary iterations that are dependent on many other factors. Thus, you can design, test, and redesign your synthetic system with a specific goal in mind, without other complicating variables imposed by biological systems.

For example, an enzyme may catalyze a useful reaction that you wish to use on a chemical; because the enzyme evolved in the cellular context it may only function in a specific environment or if certain regulatory signals are present. Additionally, the enzyme may be highly specific for a particular substrate and function poorly with your desired chemical (although as previously mentioned, this specificity can be highly advantageous in the right situation). On the other hand, biosynthetic machinery is somewhat modular already, suggesting that nature has evolved a great deal of functionality that we can take advantage of if we understand the systems' limits and interactions. Some of the domains in different polyketide synthases have simply been swapped into other systems and have functioned well enough to yield novel and useful molecules.

Penultimately, a large advantage of studying and engineering preexisting biological systems is that they are already biased to produce chemicals that interact with biological systems. Chemical space is more or less infinite, with the estimate for possible small organic molecules at around 1060, so trying to produce every possible chemical is not a realistic option. Given this context, biasing the chemicals you are producing towards a specific purpose is not only wise, but a necessity to get the most 'bang for your buck.' That being said, even if you are trying to make chemicals or materials that work in biological systems, you may be looking for things that specifically don't interact with your host organism such as materials for long-term medical implants.

The final advantage of studying pre-existing biosynthetic machinery is that, in addition to characterizing it for engineering purposes, it is also furthering our understanding of how these systems work and function in biology. Some of the molecules made by pathogens are required for disease to develop, so even if we're not trying to modify a system for engineering goals, understanding how it works and is regulated could lead to better treatments for disease. For example, certain strains of E. coli produce a compound called colibactin, which can cause DNA damage and is strongly implicated in the development of colon cancer.

Although there are many differences between the various avenues of research, in studying the natural sciences it quickly becomes clear that seemingly distinct fields are truly inseparable and complement each other in unexpected ways. Many small pieces of the puzzle may be required before a broader model emerges; sometimes different components were characterized decades ago and were sitting idly until someone was able to connect the fragments. Other pieces can push the boundaries of our understanding, making us

aware of puzzles and fields that we didn't even know existed. Engaging in research is one of the most unique aspects of humankind, leading to our ever increasing understanding and mastery of the natural world. Societies face many current and impending problems, and though no panacea, research is a strong tool to aid us in confronting the challenges that our world faces.