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Redox Control of Metabolic Remodeling in Inflammatory Cellular States

By

Nicholas Lawrence Arp

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Chapter 5: *Molecular traffic: how cells control their metabolic highways during inflammation*

By Nicholas L. Arp

Edited By Elizabeth Reynolds

supported by the Wisconsin Initiative for Scientific Literacy

Why I wrote this chapter

When I was growing up, science was an underappreciated topic. Not for any reason to science's credit, but rather it was not a major topic in the context of my upbringing. I grew up in small-town Iowa. If science was referenced, it was in the context of medicine, particularly careers in healthcare. Although some of my friends' parents were local healthcare workers (physical therapists, nurses, or medical doctors), I do not recall knowing anyone who was trained to be a scientist, or whose career involved scientific research. Being a scientist was a career that was depicted on TV and in movies. I remember watching *Dexter's Laboratory* on Cartoon Network and being entertained by this mad-scientist kid and his adventures with his sister, or *Bill Nye the Science Guy* on PBS and being mesmerized by the goofy approach to explain fascinating scientific concepts. In reality, science was simply a core curricular standard in the public school system and success in this subject was a ticket to enter the healthcare workforce.

After I graduated high school, I was selected for and attended the National Youth Science Camp in West Virginia as one of two Iowa Delegates ('15) for a month in the summer prior to starting college. Here, I began to understand what a scientist is, particularly university professors, and their role in advancing scientific knowledge and education. I recall a lecture at camp that described an "MD-PhD." At that time, the training length and career goals did not resonate with me. I was still trying to understand the concept of careers in science. Imposter syndrome was at its extreme during my time at the camp. Fellow campers were on track to start college at top universities I'd never heard of. Many discussed numerous Advanced Placement (AP) courses or competing in science fairs since middle school, an experience completely unknown to me. My enrollment in AP Biology and AP Calculus (the two main AP courses offered at my high school) seemed modest by

comparison. However, this experience taught me something crucial: different paths can lead to the same destination, and my path, although different, was equally valid.

In the Fall, I, a first-generation college student and Pell Grant recipient, attended Wartburg College, a liberal arts college in small-town Iowa, as a biochemistry major with future aspirations of applying to medical school and becoming a physician. Here I discovered what science could be.

I quickly became enthralled with my chemistry and biology coursework, which was recognized by professors in the departments. Dr. Shawn Ellerbroek, professor of biochemistry, invited me to join his small group of undergraduate students in his lab. Over the course of three years, I acquired a toolbox of scientific skills that helped me to excel in various scientific environments, including Massachusetts Institute of Technology (MIT) during their summer research experience. Dr. Ellerbroek was the first one who encouraged me to truly consider the MD-PhD career pathway, alongside other professors who I recognized as close mentors. I reflected on their advice and discerned that it was a perfect fit for my career goals.

I was the product of the small-town Iowa public school system, and navigated a path into science and medicine through embracing my own curiosity, capitalizing on opportunities, and accepting the support of mentors along the way. However, as I progressed through the academic system, the people in my life, family and friends, especially from my hometown, did not experience this alongside me. Therefore, each step of my academic journey has been a black box for them. Often, I would get the following questions: *What is an MD-PhD? What does a scientist really do? Will you still take care of patients? What is a manuscript? What is a NIH fellowship? How long do you have until you're a doctor?* These are questions that often come up after sharing my training and career plans. Over the past 7 years in the MD-PhD program, I had ample time to share and clarify the intricacies of my training path. The one aspect of my training that I have

admittedly been deficient in sharing adequately is my thesis research. This Wisconsin Initiative for Scientific Literacy (WISL) chapter is the perfect opportunity to carefully and thoughtfully relay my research to my family and friends, and the public (regardless of background), and share what I accomplished during my graduate school training. I appreciate Professor Bassam Shakhashiri and WISL staff members, including editor Elizabeth Reynolds, for this opportunity and guidance throughout the writing process.

Why did I select my PhD research mentor and lab?

I completed my PhD in the laboratory of Jing Fan, PhD, an expert in immunometabolism (a field that I will describe in detail later). I selected Jing's laboratory to complete my thesis research because she was well-known to be an excellent PhD mentor for graduate students. She supported me in my plans to pursue an MD-PhD, and pursue continued clinical training in residency and fellowship after my PhD. She studies cellular metabolism, a topic that is relevant to human health and disease. However, she studies the underlying foundation of biology, chemistry, and immunology at the molecular and cellular level. This is termed "basic science" research. This is different from "translational" research, which is specifically trying to take findings from a laboratory towards the development of new medicines, diagnostics, or treatments. Also, this is different from "clinical" research, which is medical research involving humans. Importantly, most translational and clinical research is based on discoveries established by basic science research. Therefore, I wanted to train under Jing to develop skills as a basic science researcher to establish new discoveries that will pave the way for future research to improve human health and disease. My training in medicine, where we become experts in human health and disease, including diagnosis, treatment, and clinical skills, will complement my research skills to bridge the two worlds.

Why does studying cellular metabolism matter for human health?

Consider the following: cancer cells require their metabolism to fuel rapid growth. Immune cells shift their metabolism when fighting infections. Heart cells change their fuel preferences during heart failure. Understanding these metabolic changes at the molecular level, the “basic science” research I do, reveals new targets for future therapies. My medical training taught me *what* goes wrong with disease, while my research training taught me *why* it goes wrong at the cellular level. To understand what I actually studied in Jing’s lab, I need to first explain the fundamental biology that underlies my work.

What is cellular metabolism?

A fundamental concept in biology is that our genes (made of DNA) contain instructions for building proteins. Proteins are the workhorses of the cell; they're the enzymes that facilitate the chemical reactions I'll describe next. Think of proteins as the construction crews, traffic controllers, and road maintenance teams that keep our metabolic highways running.

Now, I am going to focus more on the chemical reactions that proteins can support. A molecule in the cell can be broken down into new molecules, or multiple molecules can be combined to generate new molecules. The proteins described above help facilitate these changes to the molecules. Molecules can be used in many ways including to grow the cell, to provide energy to the cell, or to communicate with itself or other cells to perform a task. However, it can quickly become very complex as there are thousands of proteins, and each can support different chemical reactions. Think of it like a road map of a major city. The arrows are like roads, and the molecules are like cars traveling between destinations. When arrows merge, this indicates that the molecules are combining, whereas if arrows diverge, this indicates that the molecules are breaking into two.

Cellular metabolism can be defined as the collection of chemical reactions inside a cell. Therefore, the map described previously can be termed a "metabolic map" or a collection of "metabolic pathways" with pathways being a string of chemical reactions of similar purpose. We often think of metabolism in the context of the things we eat daily: sugar, protein, and fat. Here, the molecules that I have described so far are exactly those molecules or products of these molecules: sugar-derived molecules, protein-derived molecules, and fat-derived molecules. The nutrients we get from our diet fuel this metabolic map. However, rather than focusing on nutrients at the level of our entire body, I focus on what cells do with these nutrients, particularly how these molecules then proceed through this metabolic map to support the function of a cell (**Figure 1**).

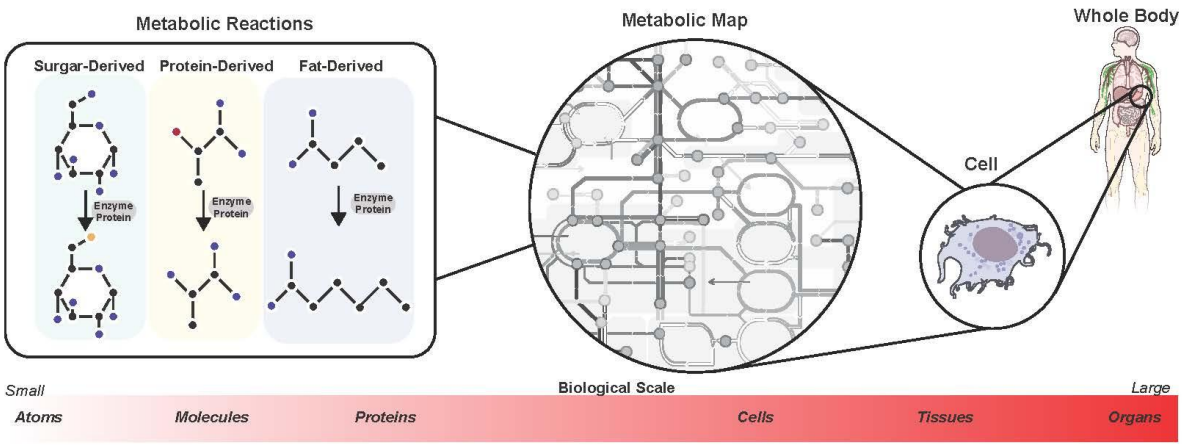

Figure 1. The "metabolic map" of cellular metabolism

The totality of chemical reactions that make up this metabolic map do not occur all the time or happen at the same speed. Just like how traffic patterns change based on the time of day, weather conditions, or road construction, cellular traffic patterns shift based on the type of cell (such as muscle cell, liver cell, or immune cell), the nutrient availability (such as more or less of one type of nutrient than others), any special functional state of the cell (such as skeletal muscle exercising),

or a disease state (such as a normal cell versus a cancer cell, or a cell in a high sugar environment as seen in diabetes).

You can imagine that just like a road map with changing traffic patterns, there are roads that are more or less traveled, rush hour congestion or empty highways, and road closures facing detours. *The proteins that facilitate these reactions?* They're like traffic signals, road signs, and even construction crews that control the flow, speeding up certain routes, blocking others entirely, or redirecting traffic when needed. You can begin to imagine how this metabolic map changes by context for all the factors described above.

If we continue the analogy, the molecules need to proceed to their destination within the cell. However, there are many routes a molecule can take to make it to their destination. I encourage you to consider the various ways in which we can make this process complex. For instance, when using a road map, we can change the type of vehicle we take (such as car or van, sports car or hybrid). We can change the destination we set off to (such as home, work, or vacation spot). We can change the preferences of the route (such as only taking county highways, avoiding tolls, least amount of expected traffic, fastest route by time, or shortest route by distance traveled). The choice of these (vehicle type, destination, or route preference) can be influenced by context. For example, if it is a random Tuesday, I may be taking my car to work on a city street. However, if it is a vacation in the Spring, I may be taking a van to accommodate all my friends and take a more scenic route towards the mountains.

This is similar for cells. The vehicle type is the type of molecule: sugar-derived molecules are like compact cars (quick and efficient) or fat-derived molecules are like semi-trucks (carrying lots of cargo for long-haul energy storage). The destination could be the part of the cell known for specific functions, such as the mitochondria (the cell's power plant) for energy production or to

make building blocks for growth. The route preference is determined by the type of cell and its environment. For example, a muscle cell during exercise is like a city during rush hour, pushing massive amounts of sugar-derived molecules down the fastest energy-producing routes and bypassing scenic detours. Meanwhile, a liver cell in a starving state is more like Sunday morning traffic, fewer sugar molecules coming in, so it's converting stored fat molecules and sending them out to other tissues that need fuel (**Figure 2**). As you can see, the specific context determines the traffic pattern of the map.

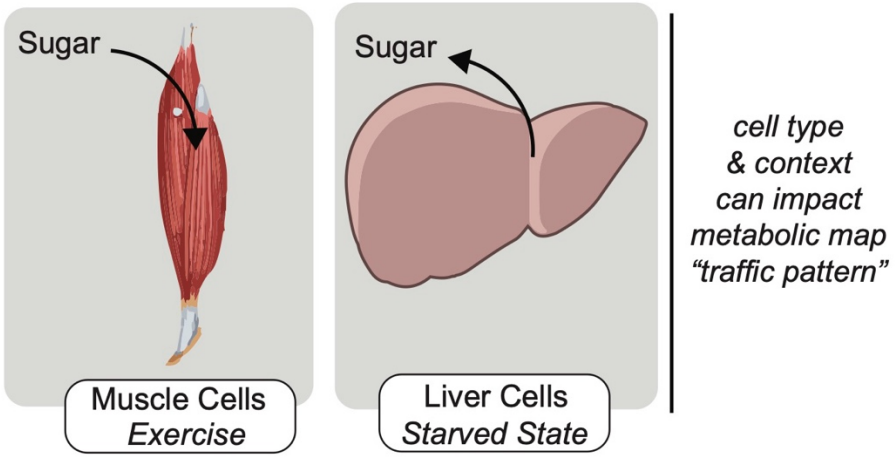

Figure 2. Cell type and context can impact metabolic map "traffic pattern"

What is immunometabolism and why does it matter?

Immunometabolism is the study of how immune cells use metabolism to perform their jobs. When your body encounters an infection, certain immune cells like macrophages act as first responders. They eat bacteria and produce antibacterial chemicals to kill these pathogens. To make these toxic chemicals and survive the battle, macrophages completely remodel, or shift, their metabolic highways. They change which roads they use, change their fuel preferences, and activate entirely new routes.

This metabolic remodeling isn't just interesting biology, it's critical for fighting infections, but it can also go wrong. For instance, in autoimmune diseases, macrophages get stuck in this "attack mode," continuously producing inflammatory signals that damage healthy tissue. Understanding how these metabolic shifts are controlled could reveal new ways to treat inflammatory diseases, help immune cells fight infections more effectively, or even reprogram tumor-associated macrophages that feed cancer growth.

Overview of my thesis research

During my time in my PhD, I focused on a type of immune cells called macrophages. Jing's lab has been one of the pioneering labs that identified the metabolic shifts that macrophages undergo when they interact with pro-inflammatory signals. These signals include the combination of molecules from the cell wall of a bacteria (lipopolysaccharide) and a secreted molecule from immune cells (interferon-gamma). The lab showed that these macrophages undergo a "two-stage" remodeling of their metabolic pathways that correspond to their immune cell function. Now, let me explain these two stages.

First, the macrophages ramp up their sugar breakdown pathway, called 'glycolysis'. At the end of this pathway, it feeds into their mitochondria. The main metabolic pathway of interest in mitochondria is called the 'tricarboxylic acid cycle' (TCA cycle). Unique to immune cells, there are detours of the TCA cycle. These detours make molecules that can act like signaling molecules themselves. To do this, the cell increases the amount of protein called IRG1. This protein functions to produce a molecule called 'itaconate' in the cell from the TCA cycle. Itaconate then acts like a city-wide emergency coordinator that travels to different departments (power grid, water treatment, emergency services) and issues specific activation orders to each. This ensures that all

parts of the cell's infrastructure work together to mount a coordinated immune defense with each system contributing its specialized function to the overall response (**Figure 3, Left**).

Second, the macrophages begin to shut down the point where glycolysis ends and its incorporation into the mitochondria's TCA cycle. Also, there is another break in the middle of the TCA cycle. You can imagine it like a traffic jam or a closed road. The result of this leads to a decrease in the amount of the molecule, itaconate, and its downstream effects. These two spots of a broken pathway were due to decreased function of two key proteins: pyruvate dehydrogenase complex (PDHC) and oxoglutarate dehydrogenase complex (OGDC) (**Figure 3, Right**).

The "two-stages" of metabolic remodeling that these immune cells undergo, which correlated with immune function, was a key finding to the scientific field of immunometabolism. The first stage being a pro-inflammatory state. The second stage being the transition back to a more suppressed state.

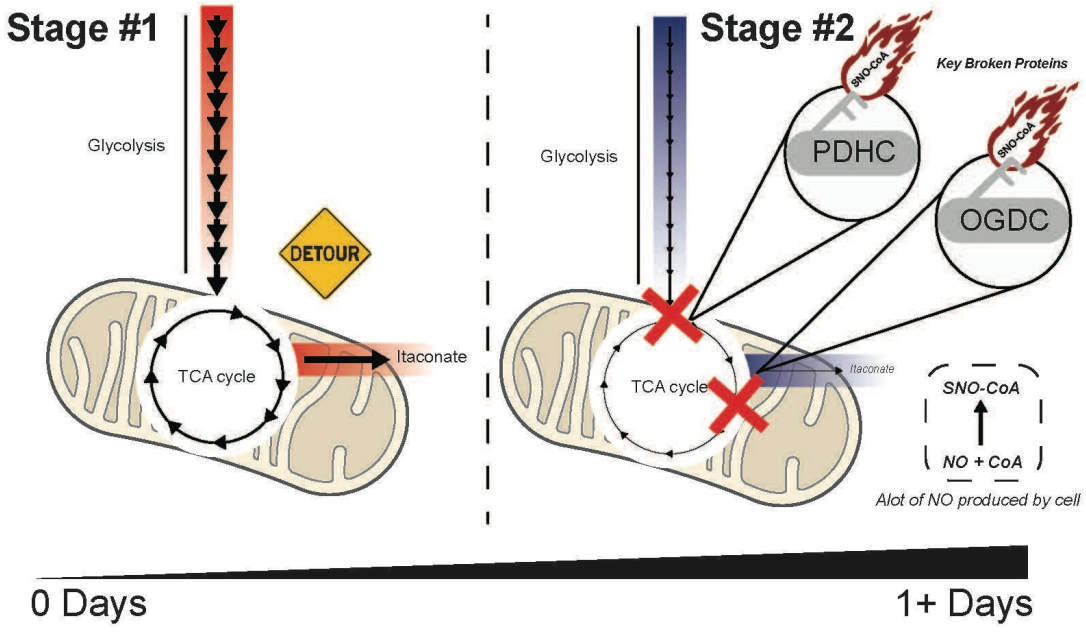

Figure 3. The "two-stage" remodeling of pro-inflammatory macrophages

In April 2022, I joined the lab after completing my first three years of medical school and my two medical licensing exams. At this time, Gretchen, a final year PhD student in the lab, was finalizing her second paper of her thesis research. Her work focused on understanding how these two stages were happening. The key questions remained: *What was causing the switch between the first to the second stage? What was happening with these two key proteins, PDHC and OGDC, that made them break down?* I arrived at the lab at the luckiest time. I was able to learn from Gretchen about the project, gain the relevant technical skills in the lab, and contribute to finishing the paper under her lead.

Gretchen's first author paper (my efforts for this work were recognized as third authorship on the paper, so it is included as Appendix I) identified the way the macrophage coordinated this specific breakdown of these two proteins to facilitate the transition from the first to the second stage. When macrophages are exposed to these pro-inflammatory signals, other researchers have shown that these cells produce a small molecule inside the cell called 'nitric oxide' by the protein called 'inducible nitric oxide synthase' (iNOS). Nitric oxide is a very small molecule that is very reactive due to its chemical properties, and there have been decades of research on this molecule not only in macrophages, but in other cell types like blood vessels, neurons, and muscle cells. However, we noticed that this two-stage remodeling matched the production of nitric oxide in the cell. When the first stage began to transition to the second stage, nitric oxide was in high amounts in the cell. So, we asked: *is nitric oxide causing the two-stage remodeling to occur and lead to the breakdown of these proteins?*

This paper went on to show that the answer to that question was '*yes*'. However, nitric oxide was not acting alone! It was working with a partner molecule. Think of nitric oxide as a key that needs to be attached to a special keychain before it can unlock certain doors in the cell. That

keychain is a molecule called ‘coenzyme A’ (CoA). When nitric oxide attaches to CoA, it creates a new molecule called ‘S-nitroso-CoA’ (SNO-CoA). This SNO-CoA is the actual molecular tool that interacts with PDHC and OGDC proteins to break them and causes the traffic jam in the TCA cycle (**Figure 2, Right**).

But this work went ever further! The research revealed exactly where on these proteins SNO-CoA was causing damage. Imagine these proteins as construction cranes with long mechanical arms that reach out to do work. Each protein has a special arm called a ‘lipoic arm’ that is essential for its function, like a hook on a crane that lifts materials. This work showed that SNO-CoA was specifically targeting and breaking these lipoic arms.

The discovery was important for two reasons. First, it revealed a completely new mechanism for how nitric oxide changes protein function in the cells: it doesn’t just block proteins, but strategically breaks functional pieces of those proteins. Second, because the lab had previously shown that this coordinated metabolic breaking was crucial for proper immune function, understanding how it happened at the molecular level opened doors for future research.

Now, this is where I took the lead on the next phase of research. Gretchen's discovery of the SNO-CoA mechanism raised three critical questions that would define my PhD work. Over the next four years, I pursued three interconnected projects that each tackled one of these questions.

First, *does this nitric oxide-mediated protein breaking extend beyond macrophages?* We wondered whether SNO-CoA could also break down a similar protein called branched-chain alpha-ketoacid dehydrogenase complex (BCKDC) in muscle cells, where nitric oxide is also produced during inflammation.

Second, macrophages clearly coordinate the production of nitric oxide and timing of the breaking of these critical metabolic proteins. *Are there fine-tuning mechanisms that control this shift?* Understanding this could reveal how cells maintain metabolic flexibility during immune responses.

Third, *what unique metabolic fingerprints emerge during inflammation?* We were curious about whether new molecules generated during this pro-inflammatory state might serve as biomarkers to identify pro-inflammatory macrophages or even act as signaling molecules themselves.

Nitric oxide-mediated metabolic regulation in muscle cells: the BCKDC story

In addition to the two key proteins important for the two-stage metabolic shift in macrophages, there is a third protein that looks very similar and it is called branched-chain alpha-ketoacid dehydrogenase complex (BCKDC). Importantly, this protein also has a 'lipoic' arm that is important for its function. This protein is known to reside in the mitochondria as well, where it is important for the breakdown of the building blocks of proteins called 'amino acids.' Specifically, this protein breaks down a certain type of amino acids called 'branched chain amino acids' (BCAAs). Think of these like specialized building materials that cells use for construction and energy.

This metabolic pathway is very important in muscle cells. *Why?* When we exercise, build muscle, or recover from injury, muscle cells need to carefully manage these BCAAs. Also, muscle cells, under inflammatory environments, such as during severe infection or injury, have the capacity to produce nitric oxide, just like macrophages. Therefore, I wondered: if the nitric oxide breaks similar proteins in macrophages to control their immune function, *could the same thing be happening in muscle cells during inflammation?*

This question led to a follow-up paper that described my findings (Chapter 2). I used C2C12 muscle-like cells, (a well-established cell type made from mouse tissue that scientists use to study muscle biology in the lab). I grew these cells in culture dishes, exposed them to similar pro-inflammatory signals that activate macrophages, and tracked what happened to their metabolism over time. Just as I hypothesized, when exposed to pro-inflammatory signals, these muscle cells produced nitric oxide from the protein iNOS (which I confirmed by measuring nitric oxide in the liquid that I grow the cells in called "growth media"). To investigate whether this nitric oxide and SNO-CoA was breaking BCKDC, I developed techniques to measure the activity of this protein to see if it was still functional. Sure enough, BCKDC activity dropped dramatically when nitric oxide production ramped up. Also, if I directly exposed BCKDC to SNO-CoA, I saw a drop in BCKDC function.

But I wanted to know exactly where the protein was breaking. Using immunoblotting, a technique that separates proteins from the cell and uses special probes to identify specific targets, like a functional lipoic arm, I analyzed the BCKDC protein and found the same loss of the functional lipoic arm that we observed in PDHC and OGDC in macrophages. This evidence was clear: SNO-CoA was breaking BCKDC in the same vulnerable spot!

But here is where it got even more interesting! These proteins are made up from three individual parts called "subunits" (**Figure 4**). It was known in scientific literature that the third subunit of these complexes can be inhibited by nitric oxide. However, I hypothesized that the broken lipoic arm is the intermediate step that leads to loss of activity, rather than nitric oxide doing the breaking on its own. So, I obtained a cell line that does not have the protein required to make the lipoic part of the lipoic arm of these complexes. So, these proteins exist in the cell *lipoic-*

less. When I measured the activity of the third subunit specifically with or without nitric oxide, I observed that the lipoic arm was required for the third subunit breakdown.

This pattern suggested something remarkable: the broken lipoic arm was not just sitting there inactive. Instead, it seems to be triggering damage to the neighboring protein subunit. Think of it like a domino effect or a chain reaction. It is like road debris from an accident-causing additional damage to other vehicles passing by, except in this case, the debris is chemically active and directly damages other molecular machinery.

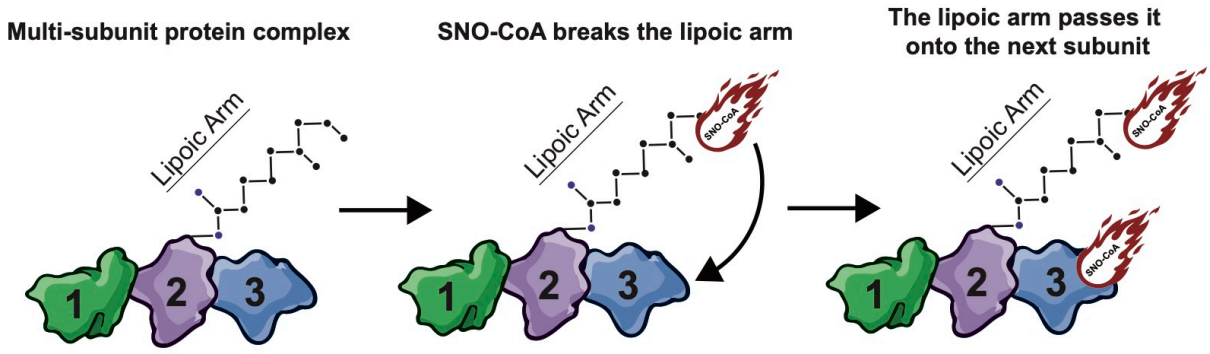

Figure 4. The three subunit proteins complexes with lipoic arm and how SNO-CoA breaks the lipoic arm and passes it to the third subunit.

Therefore, the main takeaways from this were twofold. First, this nitric oxide-mediated metabolic regulation was not exclusive to immune cells fighting infection, but it could be relevant to other cell types where we know nitric oxide is produced, like muscle cells during inflammation (**Figure 5**). This matters because it suggests a broader biological principle: cells across different tissues might use the same chemical strategy to coordinate their response to inflammation. Second, we demonstrated that there are additional steps in this mechanism where the broken lipoic arm propagates damage to additional proteins.

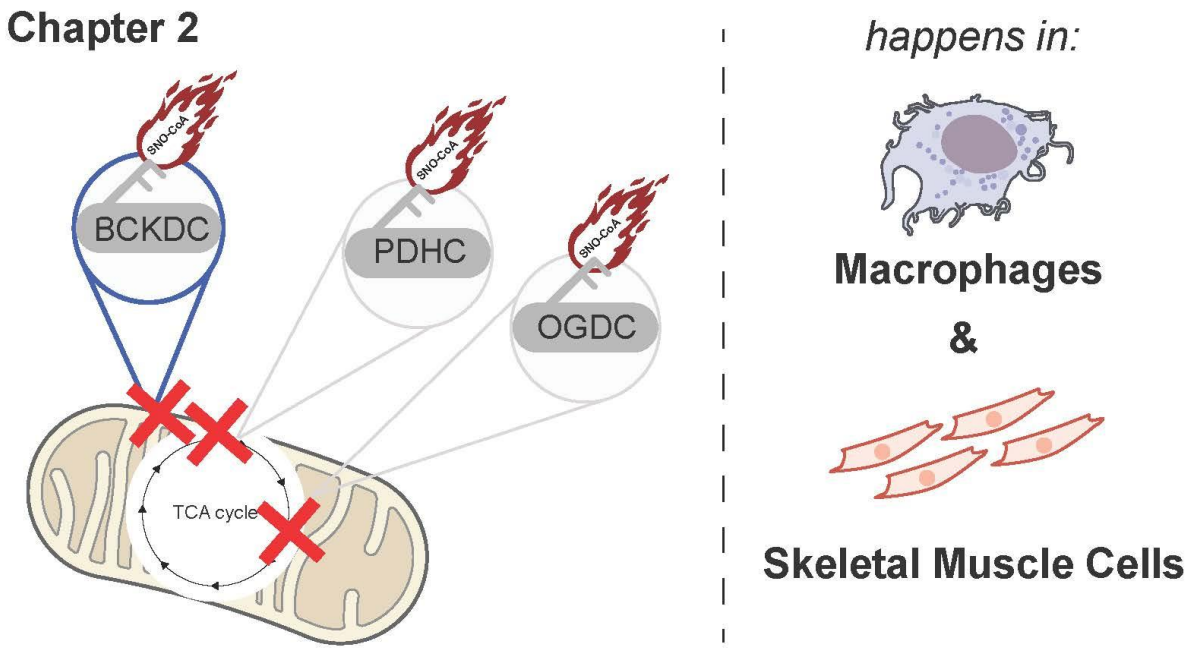

Figure 5. BCKDC's lipoic arm can be broken by SNO-CoA in macrophages and skeletal muscle cells

Metabolic fine-tuning in macrophages: the AKR1A1 mechanism

It was clear these cells were coordinating this two-stage shift in their metabolic pathways in a very carefully controlled way. However, I wanted to know if the cells were turning on new proteins that helped fine-tune the response in this coordinated shift. You can think of this as a car's cruise control. The car can go fast, or maintain speed, but you also need a system to regulate that speed and prevent overshooting or undershooting your target. This is where my second research paper was focused (Chapter 3).

One of the ways to identify fine-tuning proteins is to use a comparative approach. I measured thousands of proteins in two scenarios: macrophages exposed to pro-inflammatory signals (with nitric oxide production intact) versus macrophages exposed to the same signals but

genetically modified so they cannot produce nitric oxide. These genetically modified cells are called ‘knockout’ cells. Scientists use molecular biology techniques to permanently remove specific genes from the cell’s DNA. In this case, I collected cells from mice with the iNOS gene removed, meaning these cells physically cannot make the iNOS protein, and therefore cannot produce nitric oxide.

By comparing normal macrophages (which make nitric oxide) to these iNOS knockout macrophages (which don’t), I could identify proteins that specifically respond to nitric oxide’s presence. I used a technique called ‘proteomics’. This is essentially taking a snapshot of all the proteins present in the cell at once, like taking inventory of every vehicle on all the roads in our city at a given moment. Additionally, I measured which genes were being turned ‘on’ and ‘off’ using a technique called ‘transcriptomics’, which tells us what instructions the cell is reading from its DNA at the moment (**Figure 6**).

My reasoning was as follows: proteins that help fine-tune the nitric oxide response should increase with the pro-inflammatory signals, but only when nitric oxide is around. If they increase in both scenarios, they wouldn’t be specifically regulating nitric oxide’s effects.

From this approach, I identified a protein called AKR1A1. In the presence of both nitric oxide and pro-inflammatory signals, macrophages dramatically increased the amount of this protein they made. When I looked at what other researchers had discovered about AKR1A1, I found that it had been described as an enzyme capable of clearing away SNO-CoA. This is the exact molecule that Gretchen’s work had shown was breaking down PDHC and OGDC! And the molecule that my work showed could break down BCKDC! This was like finding the cleanup crew specifically designed to clear the road debris we have been studying.

I immediately wondered: could AKR1A1 be acting as the foot on a brake pedal, fine-tuning the extent of the nitric oxide-mediated metabolic regulation that we knew occurs in the cell?

To test this, I used genetic approaches to remove AKR1A1 from macrophages. Think of this as removing the cleanup crew. SNO-CoA accumulates without anything to clear it away. I then measured what happened to the metabolic pathways with a focus on PDHC.

The results confirmed my hypothesis. When AKR1A1 was absent, the metabolic breaking was more severe and happened more quickly. AKR1A1 was indeed a fine-tuning protein that helped the cell control exactly how much metabolic remodeling occurred and for how long.

Why does this matter? Immune responses need to be carefully calibrated. Too little, and you cannot fight off infections. Too much, and you damage your own tissues. By identifying AKR1A1 as a protective mechanism, we revealed how cells build in their own system to fine-tune their metabolic regulation, which is important for immune function.

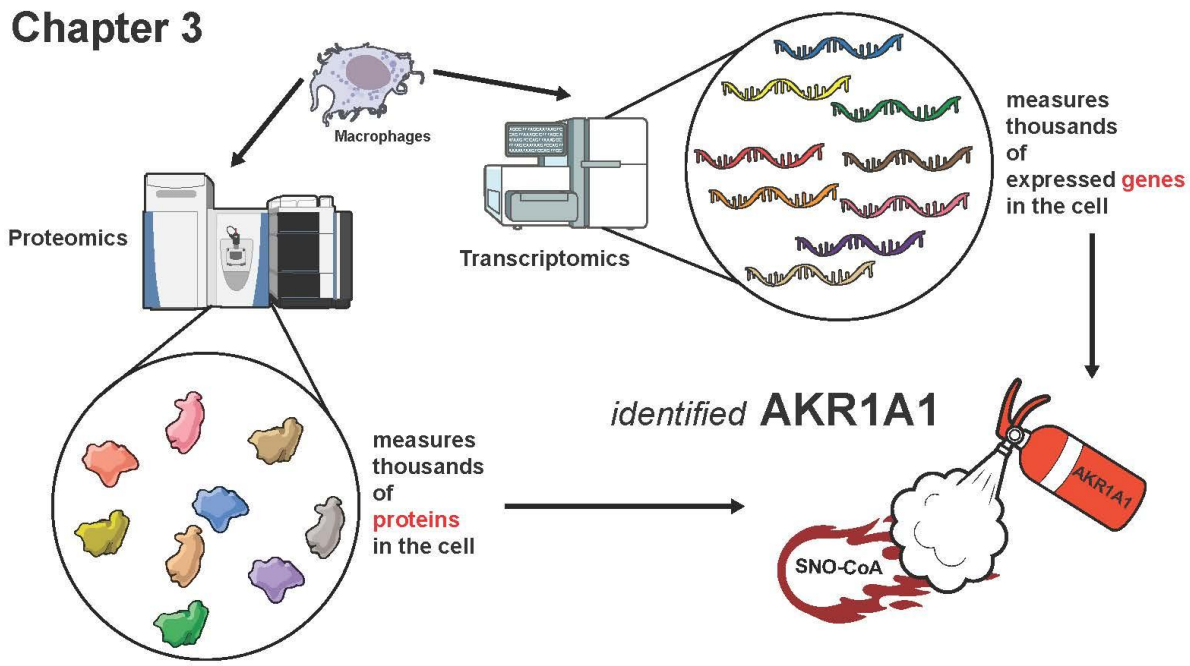

Figure 6. Integrating proteomics and transcriptomics uncovered AKR1A1, a protein that targets SNO-CoA

Metabolic fingerprints of inflammation: the cysteine conjugate discovery

In my work so far, I had been working with macrophages exposed to pro-inflammatory signals. What we call 'pro-inflammatory macrophages' or sometimes 'M1 macrophages.' We knew these cells were dramatically remodeling their metabolism and producing nitric oxide. But I began to wonder: *what if we could identify unique molecules inside these cells that would serve as an unmistakable marker of this pro-inflammatory state?* Think of it like identifying a specific license plate or bumper sticker that only appears on vehicles traveling certain routes. I set off to discover new molecules in the cell that might serve as these fingerprints. To continue the traffic analogy, I wanted to discover new pathways or highways in the cell that we hadn't mapped before. This was the focus of my third paper (Chapter 4).

I used a technique called ‘metabolomics’. Unlike proteomics, which measure proteins (the workers and machinery), metabolomics measures the much smaller molecules that are actually moving through the metabolic pathways, the cargo on our metabolic highways! Here is how it works: I extract all the small molecules from the cells, then use a sophisticated instrument called a mass spectrometer. Think of the mass spectrometer as a highly sensitive scale combined with a chemical fingerprint reader. It measures how heavy each molecule is and breaks it into characteristic fragments. This creates a unique signature for each molecule. By running thousands of these measurements, I can identify and quantify thousands of different small molecules in a single cell sample all at once. This gives me a comprehensive snapshot of the metabolic state of the cell. I compared pro-inflammatory macrophages to macrophages exposed to different signals (what we call ‘M2 macrophages’) and to resting macrophages that haven’t received any activation signals. I was specifically looking for molecules that appeared uniquely or at much higher levels in the pro-inflammatory state.

What I discovered was exciting! It was a new family of molecules formed by the combination of two smaller molecules stuck together. Cysteine, one of the twenty amino acids that serve as building blocks for proteins, was forming new conjugates (chemical pairs) with key molecules from the metabolic pathways I had been studying. For example, cysteine was forming conjugates with molecules from the sugar breakdown pathway (glycolysis) and from the TCA cycle. Imagine cars merging together to form a new type of vehicle that could only exist on certain roads under specific traffic conditions.

I identified multiple specific conjugates, including cysteine with alpha-ketoglutarate (a TCA cycle intermediate). Using mass spectrometry, a technique that identifies molecules based on their weight and chemical structure, I confirmed these were truly new molecules. I also used

chemical synthesis to create these conjugates in test tubes and showed they matched exactly what I was seeing in the cells.

The chemistry turned out to be so fascinating. These conjugates were forming through spontaneous chemical reactions. There were no proteins required! But they were forming only under the specific conditions present in pro-inflammatory macrophages (**Figure 7**).

This work leads to really interesting open questions about the role of these molecules. *Are they simply markers, like smoke indicating fire, that could help us identify pro-inflammatory macrophages in diseased tissues?* This could be valuable for diagnostics. For example, if we could measure these conjugates in samples from patients with inflammatory diseases, it might tell us how active the inflammation is or whether treatments are working. These are the fun, exciting ideas we get to generate based on this basic science research that can spark future research.

Or do these molecules have active roles in the cell as signaling molecules themselves? Could they communicate with other parts of the cell? Some similar conjugates in other systems have been shown to have biological activities. This is what makes discovery science so exciting! We have expanded the map with completely new roads and now we get to explore them!

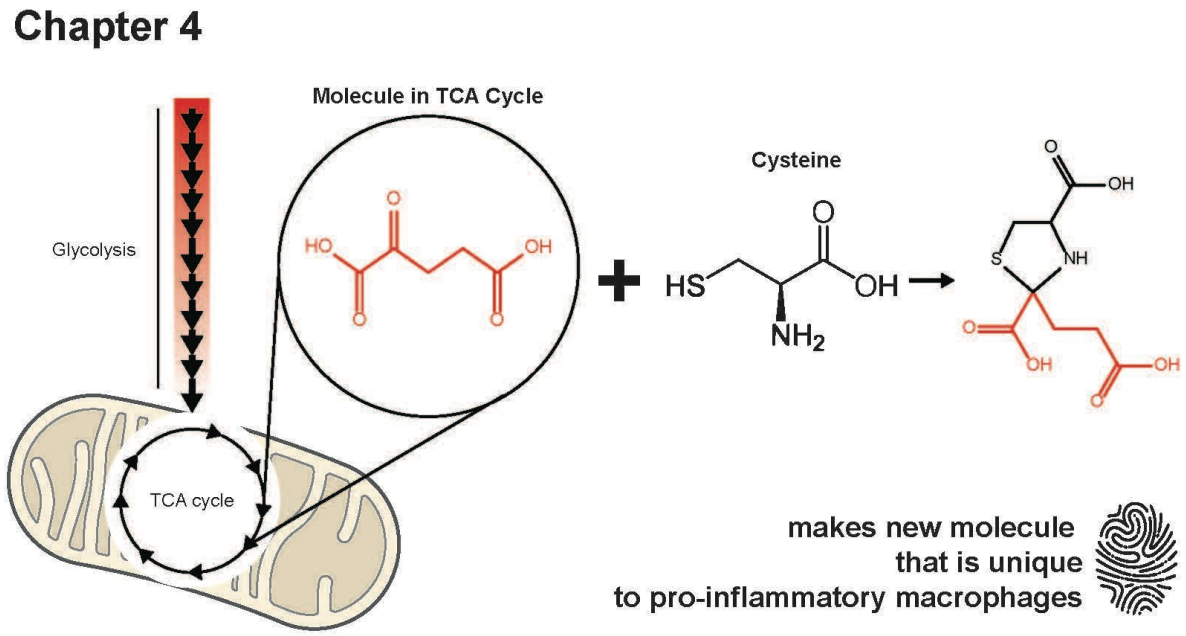

Figure 7. Molecules in metabolic pathways pair with cysteine to form unique molecules to pro-inflammatory macrophages

What this all means: connecting the dots

When I step back and look at these three projects together, a clear story emerges about how cells navigate inflammation.

Gretchen's work showed that macrophages use nitric oxide as a molecular switch to transition between their two metabolic stages, which is essential for immune function. My first project (Chapter 2) revealed this isn't just a macrophage trick. Rather, it is a broader biological strategy that muscle cells use too when facing inflammation. This suggests that nature has utilized the same chemical playbook across different tissues.

My second project (Chapter 3) showed that cells are not reckless. They have built-in safety mechanisms. AKR1A1 acts as a protective brake, ensuring that the metabolic remodeling doesn't go too far or too fast.

My third project (Chapter 4) took a different approach. Rather than studying the mechanism, I asked what unique chemical signatures emerge during inflammation. The cysteine conjugates I discovered are like chemical postcards that say "pro-inflammatory activity happened here," revealing previously unmapped metabolic chemistry.

Together, these projects paint a picture of cellular sophistication. Cells don't just flip switches randomly. They coordinate complex metabolic changes, build in protective mechanisms, and leave chemical fingerprints that we are just beginning to understand. This is the foundation that future research can build upon, whether for developing better diagnostics, understanding disease, or creating new therapies.

From small-town Iowa to the frontiers of discovery

As I write this, I think back to that teenager from small-town Iowa who barely knew what a scientist did. The person who felt imposter syndrome at the National Youth Science Camp, surrounded by students from elite prep schools. The first-generation college student who was wondering if he belonged in science at all.

Over the past four years, I have made discoveries that expanded our understanding of how cells work. I've identified new molecules that had never been characterized before. I've revealed protective mechanisms that cells use to regulate inflammation. I've extended a mechanism from immune cells to muscle cells, suggesting broader biological principles at play.

But perhaps what I am most proud of is that I did it all while staying true to my roots. The same curiosity that made me watch Bill Nye as a kid now drives me to explore cellular metabolism. The determination that got me from small-town Iowa to MIT's summer program carried me through late nights in the lab troubleshooting experiments. The mentorship I received taught me to pay it forward, as reflected in my role in mentoring undergraduates in the lab and serving as Co-President of the MSTP Student Executive Committee.

And if you are reading this from my hometown, or from any place where science feels distant or inaccessible, I hope my story shows you something important: scientists come from everywhere, and your path is valid.

In March 2026, I will defend this thesis. Then, I will return to medical school to complete my clinical training, with plans to become a physician-scientist in pulmonary and critical care medicine. The metabolic principles I've learned from studying macrophages will inform me how I think about lung inflammation and transplant rejection.

I am excited to see where this road leads next.

References

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