Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included 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—as well as their excitement for and journey through their area of study—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.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

Contact: Prof. Bassam Z. Shakhashiri

UW-Madison Department of Chemistry

bassam@chem.wisc.edu

www.scifun.org

Decoding the Epigenetic Language of Gut Microbiome:

How diet, gut microbiome, and metabolism converge to remodel epigenetics and health

By

Jessica Haye-Joe Han

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Cellular and Molecular Biology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2025

Date of final oral examination: 7/8/2025

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

John M. Denu, Professor, Department of Biomolecular Chemistry
Federico E. Rey, Associate Professor, Department of Bacteriology
Luigi Puglielli, Professor, Department of Medicine
Reid Alisch, Associate Professor, Department of Neurological Surgery

CHAPTER 5

WISL

5.1 Prelude

Writing this chapter gave me a chance to reflect on why my research matters, not just to fellow scientists, but to anyone curious about how the human body works and how we might care for it better. At its heart, science is a shared endeavor, supported by public trust and funding, and there is a collective responsibility to make its findings accessible to those it ultimately benefits. I'm grateful to be part of University of Wisconsin-Madison that values public engagement through initiatives like the Wisconsin Initiative for Science Literacy. I am especially thankful to Elizabeth Reynolds, Dr. Bassam Shakhashiri, and Cayce Osborne for their generous support in helping me refining this draft. Figures were created and edited in Biorender to enhance visual communications and engagement.

5.2 Germs Are Not All Bad

When most people hear the word "germs," they think of illness, something to avoid, sanitize, or eliminate. But the truth is far more nuanced. Not all germs are bad. In fact, many are absolutely essential to keeping us alive. Trillions of microorganisms including bacteria, fungi, viruses, and more, live in and on our bodies, especially in the gut[1,2]. Collectively known as the microbiome, these tiny tenants help us digest food, train our immune systems, and even produce vitamins we can't make ourselves[2].

Imagine your gut as a bustling city (Figure 1). Microbes are the workers, engineers, street cleaners, and messengers, keeping the system humming. While some microbes can cause disease, most coexist with us peacefully, or even beneficially! The more diverse and well-balanced this microbial metropolis, the healthier we tend to be.

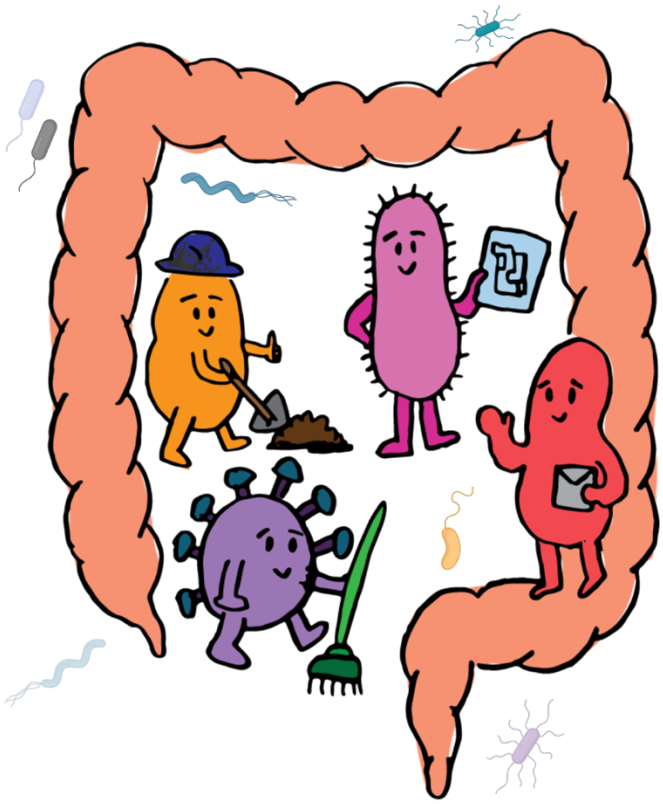

Figure 1. The microbial metropolis inside us. A cartoon representation of the gut microbiome as a vibrant community of microbes playing distinct roles: the orange microbe as a construction worker building the gut lining, the pink microbe as an engineer overseeing system plans, the purple spiky microbe as a street cleaner maintaining balance, and the red microbe as a messenger carrying signals. Together, they illustrate the diverse and cooperative functions of gut microbes in digestion, immunity, and metabolic health.

5.3 Microbial Byproducts from Our Diet

What do microbes live on? Primarily, what we eat. When dietary fiber reaches the colon, it's fermented by gut microbes to produce short-chain fatty acids (SCFAs), tiny chemical messengers like butyrate[3], which I will explain in more detail later. These aren't just waste products. SCFAs regulate inflammation, appetite, and even brain function[4–6]. Microbes also metabolize nutrients like amino acids (building blocks that form proteins) and polyphenols (which act as antioxidants in our bodies) into molecules that our bodies absorb and respond to[7,8]. This is called host-microbe co-metabolism, which is a true, inherent partnership. Some byproducts help heal the gut lining or regulate insulin[9,10]. Others, however, may do more harm than good.

Think of your microbes as chefs preparing meals with the ingredients you provide. In figure 2, on the left, a diet rich in vegetables, fiber, and polyphenol-containing foods supports a diverse, balanced (eubiotic) microbiota. These beneficial microbes act like chefs in a well-stocked kitchen, turning healthy ingredients into bioactive compounds such as SCFAs, vitamins, and phenolic acids. These "recipes" promote gut integrity, immune balance, and even brain health via the gut-brain axis. On the right, a diet high in processed meats, saturated fats, and refined sugars fosters a less diverse, unbalanced microbiota (dysbiosis). These microbes generate a different set of byproducts, which may compromise gut health and increase the risk of chronic diseases, including cardiovascular and neurological disorders.

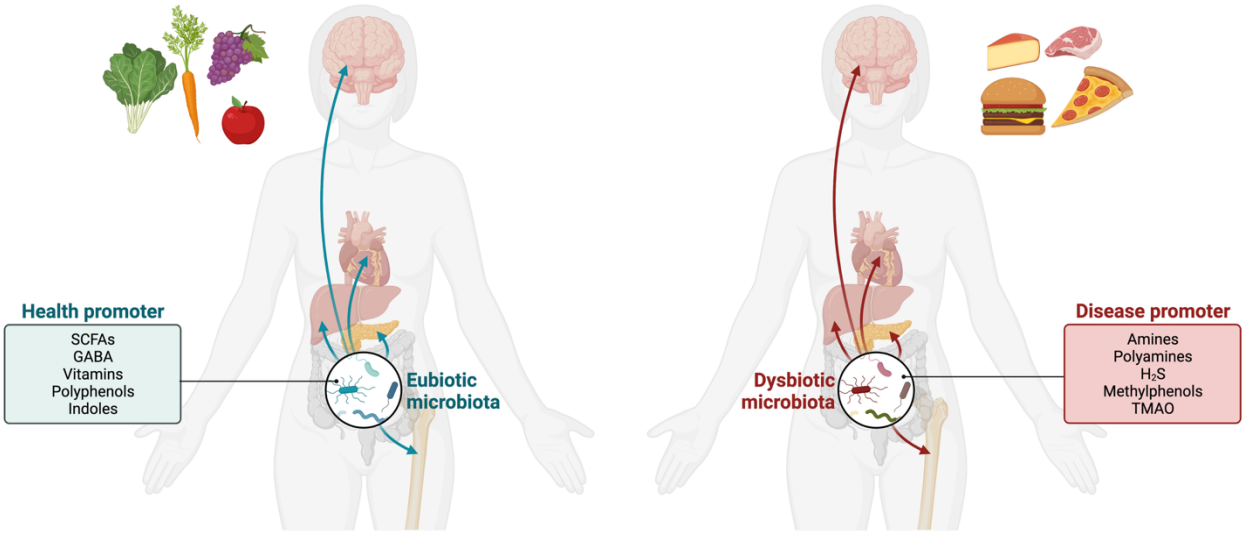

Figure 2. How diet shapes microbial metabolites and their impact on the brain and the body. This illustration highlights the powerful role of the diet in determining the composition and activity of the gut microbiome and how these microbial communities influence health.

5.4 How DNA Folding Impacts Our Body Function

Now let's shift gears. Inside every cell is a full copy of your DNA, a vast library of instruction manuals for running the body. But just like a cookbook, you don't need to use every recipe every day. So how does your body decide which genes to read and which to ignore?

The answer lies in how DNA is packaged. Rather than floating freely, DNA is wrapped around spool-like proteins called histones[11]. Picture winding a very long thread around tiny reels, that's how over six feet of DNA fits into the nucleus of a single cell! This structure, called chromatin, plays a major role in gene control[12]. Tightly wound chromatin keeps genes inaccessible, effectively silenced. Loosely packed chromatin exposes genes, making them easier to activate.

Histones aren't just passive spools, though. They have tails, tiny extensions that can be chemically modified. These chemical tags are like switches or dimmers, fine-tuning how tightly DNA is wound. Scientists call this system epigenetics: changes "on top of" the DNA that affect gene expression without altering the genetic code itself.

The two most well-known types of histone modifications are acetylation and methylation[13,14]. Acetylation usually opens chromatin. Think of it like loosening your belt after a big meal. When histones are acetylated, the DNA wraps more loosely around them, making it easier for cellular machinery to access and read the genes. This tends to increase gene activity. Methylation is a bit more nuanced. Adding methyl groups to histone can either silence or activate genes, depending on exactly where the tag is placed. Some methyl marks tighten the chromatin, others do the opposite. It's like adding bookmarks or highlighter ink – sometimes you're marking something important to come back to, other times you're indicating a section that should be skipped.

Now let's zoom out. Beyond the histones, DNA itself can be modified too. One of the most common changes is DNA methylation, where a methyl group is added directly to the DNA strand. When lots of these methyl tags are present in a gene's promoter regions (the "on switch" of a gene), that gene tends to be turned off. It's like taping a note over a light switch that says, "Do Not Touch."

Together, as figure 3 illustrates, histone modifications and DNA methylation form a flexible, responsive system that helps the body adapt to its environment. These epigenetic changes are essential to development, allowing the *same* genetic code to produce *different* cell types. But they're not fixed. Your diet, stress levels, exercise habits, sleep, and exposure to toxins can all influence your epigenome.

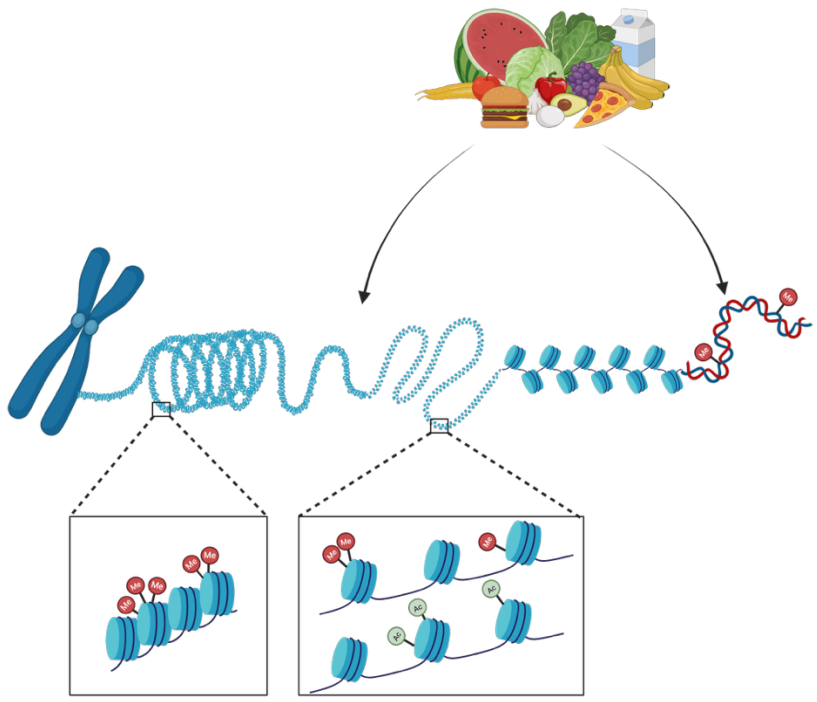

Figure 3. DNA folding regulates gene activity through epigenetic marks. This illustration shows how gene activity is controlled by the packaging of DNA around histones and the addition of chemical tags. Tightly wound chromatin with dense methylation and minimal acetylation silences gene expression. Acetylation relaxes the chromatin, exposing genes and allowing them to be read and activated. These reversible, dynamic modifications help the cell respond to its environment and are influenced by factors such as diet, stress, and sleep.

Let's take a real-world example in disease. If your body is constantly exposed to inflammatory signals due to stress, processed foods, or chronic infection, it may start turning on genes that promote inflammation and turning off those that regulate it. Epigenetic changes play a key role in this shift. Over time, these changes can raise your risk for conditions like heart disease, diabetes, or autoimmune disorders[15–18].

On the flip side, positive changes like a high-fiber diet, regular exercise, or good sleep can introduce beneficial histone marks and promote the expression of genes involved in repair, detoxification, and metabolic balance. What's especially fascinating is that some of these changes may be heritable. That means the epigenetic marks added to your DNA today might affect your children or grandchildren.

In summary, chromatin acts like a molecular filing system, helping the cell decide which genes to use and when. Histone modifications and DNA methylation are the color-coded tabs, sticky notes, and bookmarks guiding this process. And through your lifestyle – what you eat, how much you move, how well you sleep – you are constantly editing these notes. Understanding this gives us a powerful tool: the ability to influence our biology, not by rewriting our genes, but by guiding how they're used.

5.5 Microbes Influence Gene Expression Through DNA Folding

Here's where the story comes full circle. We've seen how the structure of DNA – how it's wrapped, tagged, and folded – controls which genes are turned on or off. And we've seen how the microbiome, the ecosystem of microbes in your gut, generates a host of chemical byproducts from what you eat. Now we get to the big question: how do those tiny microbial metabolites affect *your* gene expression?

Let's start with one of the microbiome's most famous products: butyrate, a short-chain fatty acid (SCFA). Butyrate is made when fiber is fermented by gut bacteria in your colon. It's a humble molecule, but it's a molecular multitool. Not only does it feed the cells that line your gut, but it also modifies gene expression by interacting with enzymes known as histone deacetylases (HDACs)[5]. HDACs normally tighten up the chromatin, packing DNA away and turning genes off. But butyrate inhibits HDACs, loosening the chromatin and making it easier for genes to be turned on. Think of it like unlocking a file cabinet and pulling the drawer open, you've just made that section of the DNA accessible, allowing helpful proteins to read the DNA and use the information stored there!

Another example is B vitamins, including folate, B6, and B12, that are either synthesized or activated by gut microbes. These vitamins play essential roles in producing the universal donor of methyl groups (known as *S*-adenosylmethionine)[20,21]. In other words, they help tag DNA with methyl groups, a key part of epigenetic regulation. Without enough methyl donors, your body may struggle to maintain proper DNA methylation patterns, which can affect everything from development to cancer risk.

In this sense, your microbes act like remote controls for your genetic expression. They don't change the channels (your genes), but they adjust the volume, brightness, and contrast. They turn some genes up, quiet others down, and do so in real time based on what you eat, how you feel, and how healthy your gut ecosystem is.

And these influences are not confined to the gut. These microbial metabolites can travel through the bloodstream and affect distant organs[22]. Studies suggest they may influence the brain, liver, and immune system by adjusting gene expression in those tissues[23]. In fact, some researchers

believe that part of the mental health benefit of a fiber-rich diet comes from these microbial metabolites acting on genes in the brain.

One emerging area of interest is how this microbial influence plays out over time. For example, could a prolonged Western diet gradually lead to epigenetic changes that predispose someone to metabolic disease? Conversely, could changing the microbiome through diet, probiotics, or lifestyle reset some of these epigenetic marks?

Scientists are just beginning to scratch the surface. But what we know so far is compelling: your microbiome doesn't just digest your food. It *talks* to your genome. And the language it uses? Molecules that tweak the way your DNA is folded, tagged, and read – the *epigenetics*. It's as if you had a team of microscopic editors living in your gut, constantly annotating your body's instruction manual. Depending on what you feed them, they can highlight healthy recipes or scribble in some risky footnotes. By understanding how these interactions work, we open the door to new strategies for preventing and treating disease.

5.6 What Do We Know About TMAO?

While many microbial byproducts are helpful, some might be harmful, especially when produced in excess or in certain contexts. One of the most intriguing and controversial of these is trimethylamine N-oxide (TMAO). When we eat foods like red meat, eggs, or dairy, our gut microbes go to work digesting nutrients such as choline, lecithin, and L-carnitine[15,24]. In the process, they produce a gas called trimethylamine (TMA). This gas is then absorbed into the bloodstream and sent to the liver, where enzymes convert it into TMAO, a more stable molecule that circulates throughout the body.

At first glance, TMAO may seem harmless. But research over the past decade has painted a more complicated picture. Elevated levels of TMAO in the blood have been linked to a higher risk of cardiovascular disease, including heart attack, stroke, and atherosclerosis (the buildup of plaque in arteries). Some studies have also found associations between TMAO and kidney disease, type II diabetes, and neurodegenerative conditions like Alzheimer's disease[25]. So, what might TMAO be doing to cause trouble?

5.7 How a Gut Microbially-Derived TMAO May Be Rewiring Our Epigenetic Story

One hypothesis gaining attention is TMAO's potential role in epigenetic regulation. Could this molecule produced by gut microbes actually influence which genes get turned on or off in human cells? Preliminary evidence suggests that it might. Some cell and animal studies indicate that TMAO can alter DNA methylation and histone modifications, the same mechanisms we've discussed earlier that control how tightly DNA is packaged and how genes are expressed. If TMAO affects these epigenetic marks, it could shift gene expression patterns in ways that promote inflammation, impair metabolism, or accelerate aging. Yet despite exciting discoveries, the story is far from complete. Some of the most important questions are only beginning to be asked, and answering them could reshape how we understand and possibly treat the long-term effects of microbial metabolism on human health. Here are some of the big questions scientists are now exploring:

- Does the way we deliver or structure diets, such as fasting, timing, or composition, affect TMAO levels in circulation and in distant organs like the brain or liver?
- Can TMAO influence how DNA is folded and packaged in specific tissues?

- What molecular machinery enables TMAO to place or erase epigenetic marks like DNA methylation or histone modifications?
- Could we monitor these epigenetic changes over time using reliable biomarkers to assess how TMAO impacts health?
- If TMAO induces harmful epigenetic changes, can they be reversed with targeted drugs, nutrients, or microbiome interventions, and can such reversal lead to better health outcomes?

To understand how TMAO might be affecting our biology, we designed a study using mice and a controlled diet. One group of mice received extra choline in their food, a nutrient their gut bacteria could use to make TMAO naturally. Another group was given water laced with TMAO directly. After eight weeks, we measured TMAO levels in the blood and tissue. Both the choline and TMAO-water groups showed elevated TMAO in their bodies, but the TMAO-water group had especially high levels in the brain. This suggested that TMAO can travel to distant organs, including the brain, where it may influence important functions.

Next, we asked how does TMAO change the molecular machinery of different organs? To find out, we analyzed proteins in the cortex (part of the brain involved in thinking), the hippocampus (critical for memory), and the liver (a key metabolic hub). In the brain, we found that TMAO reshapes protein landscapes, particularly those involved in neural communication, memory, and energy production. Proteins that help nerve cells send signals and maintain connections were altered, hinting that TMAO could affect how the brain processes information. The liver, meanwhile, showed changes in proteins tied to fat metabolism and detoxification, reinforcing TMAO's wide-ranging impact.

To dig deeper, we looked at whether TMAO influences epigenetic marks in these tissues, specifically, chemical modifications on the histone proteins that help package and organize DNA. Remember, these marks determine how tightly DNA is wrapped, which in turn controls gene accessibility. We found that high TMAO levels led to broad shifts in these histone modifications, especially in the brain. This pattern of changes varied between tissues, but the brain's cortex and hippocampus showed the most dramatic differences.

5.8 The Methionine Cycle: Where TMAO Interferes

We then turned our attention to the methionine cycle, a critical metabolic pathway that produces the raw materials for adding epigenetic marks. One key compound in this cycle is SAM, the cell's main source of methyl groups used to tag DNA and histones. When methylation happens correctly, our genes stay balanced. But when this cycle is disrupted, it's like running out of ink for your highlighters. Interestingly, we discovered that TMAO disrupts this balance by blocking an enzyme called SAH hydrolase (AHCY). This enzyme's job is to break down SAH, a byproduct of methylation that, if allowed to build up, inhibits further methylation. As illustrated in figure 4, with AHCY blocked by TMAO, SAH levels climb, and the overall methylation machinery gets jammed.

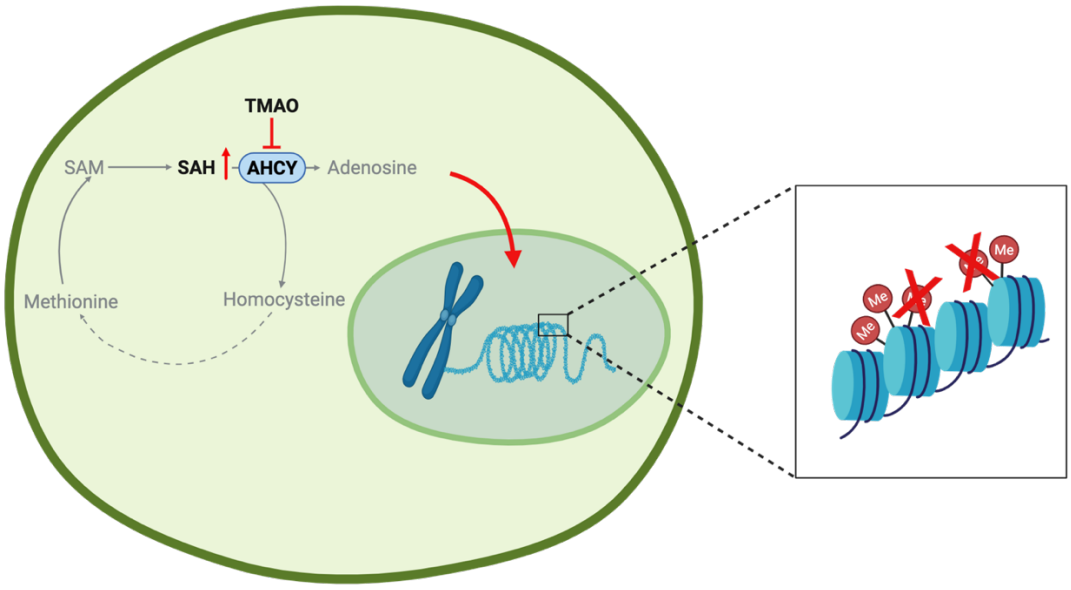

Figure 4. TMAO disrupts the methionine cycle. This diagram shows how TMAO interferes with a key metabolic enzyme called SAH hydrolase (AHCY), causing a buildup in the cycle and jamming the system that adds chemical tags to DNA and proteins.

To validate this mechanism, we treated human cells in the lab with increasing concentrations of TMAO. The results mirrored what we saw in mice: SAH went up, and the histone methylation decreased. Importantly, TMAO did not harm cell survival. But it clearly reprogrammed the cell's epigenetic landscape. That led us to an important question: Can we push back? Could increasing the supply of SAM help cells maintain their epigenetic balance even when TMAO is around? To test this, we overexpressed a gene called MAT2A, which boosts SAM production. The results were encouraging. While TMAO still disrupted some methylation marks, overall methylation capacity was partially restored. Cells with more MAT2A retained higher SAM/SAH ratios, preserving their ability to methylate DNA and histones. Interestingly, we also found that restoring methylation indirectly reduced abnormal histone acetylation. This suggests that fixing one part of the system, methylation, can ripple out to other epigenetic processes, helping to restore balance across the chromatin landscape.

5.9 Why This Matters

Our findings reveal a previously unrecognized way in which a diet-derived microbial molecule can interfere with gene regulation. TMAO not only changes protein expression in key tissues, but also rewires the epigenetic control panel by disrupting methylation balance at the biochemical level. These shifts may be silent in the short term, but over time, they could contribute to disease by gradually nudging gene expression away from health and toward harm. The good news? These changes appear reversible. By bettering understanding the molecular dialogue between diet, microbes, and metabolism, and the genome, we can begin to design interventions that protect or even restore healthy epigenetic states. Whether it's through dietary choices, targeted supplements, or future therapeutics, we may one day able to tune our gene expression by simply adjusting the microbial signals we feed. In the story of TMAO, we're not just reading the genome, but we're helping write its footnotes. I hope these findings inspire you to choose nutrient-rich foods over simple convenience, nurturing not just your own body but also the microbes that help keep it healthy. By paying closer attention to what we eat and how it affects our gut ecosystem, we can steer our gene expression toward wellness and away from disease.

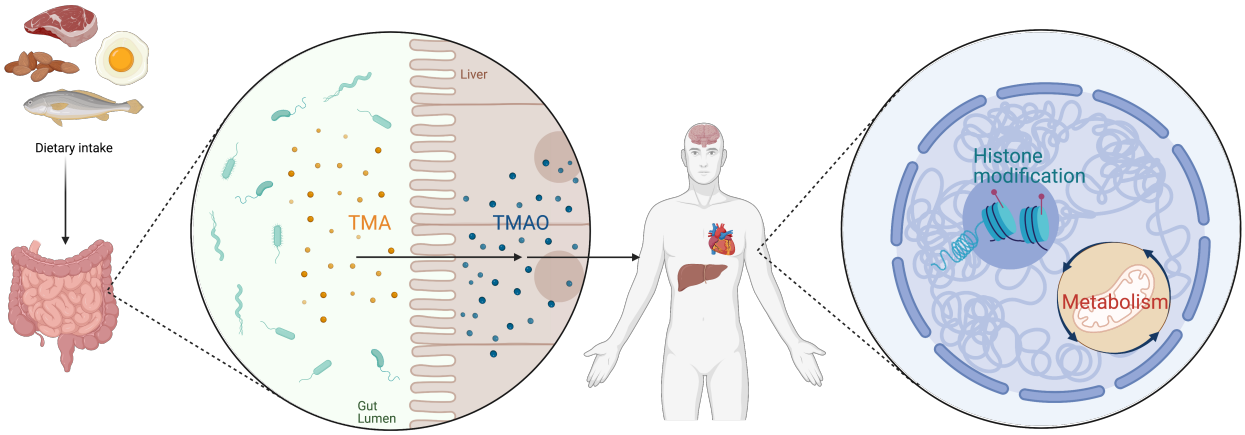

Figure 5. From dinner plate to DNA: TMAO travels and talks to our cells. This figure follows the journey of dietary nutrients like choline and carnitine, abundant in red meat, eggs, and fish. As they reach the gut, where microbes convert them to TMA (Trimethylamine), the liver then transforms TMA into TMAO (trimethylamine N-oxide), which enters the bloodstream and travels to tissues like the brain and heart. There, TMAO can influence key cellular processes like metabolism and gene expression by affecting histone modifications and other epigenetic marks. It's a powerful example of how what we eat and how our microbes process it can send molecular messages that reshape our biology from the inside out.

5.10 References

1. Heintz-Buschart, A. & Wilmes, P. Human Gut Microbiome: Function Matters. *Trends Microbiol* 26, 563–574 (2018).
2. Bäckhed, F. *et al.* Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host Microbe* 12, 611–622 (2012).
3. Brouns, F., Kettlitz, B. & Arrigoni, E. Resistant starch and "the butyrate revolution". *Trends Food Sci Technol* 13, 251–261 (2002).
4. Chang, P. V., Hao, L., Offermanns, S. & Medzhitov, R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci U S A* 111, 2247–2252 (2014).
5. Donohoe, D. R. *et al.* The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 13, 517–526 (2011).
6. Carabotti, M., Scirocco, A., Maselli, M. A. & Severi, C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology : Quarterly Publication of the Hellenic Society of Gastroenterology* 28, 203 (2015).
7. Guzior, D. V. & Quinn, R. A. Review: microbial transformations of human bile acids. *Microbiome* 9, 1–13 (2021).
8. Corrêa, T. A. F., Rogero, M. M., Hassimotto, N. M. A. & Lajolo, F. M. The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases. *Front Nutr* 6, 485842 (2019).
9. Kimura, I. *et al.* The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nature Communications 2013 4:1* 4, 1–12 (2013).

10. Cani, P. D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56, 1761–1772 (2007).

11. Richmond, T. J. & Davey, C. A. The structure of DNA in the nucleosome core. *Nature* 423, 145–150 (2003).

12. Kouzarides, T. Chromatin Modifications and Their Function. *Cell* 128, 693–705 (2007).

13. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science (1979)* 293, 1074–1080 (2001).

14. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* 403, 41–45 (2000).

15. Tang, W. H. W. & Hazen, S. L. Microbiome, trimethylamine N-oxide, and cardiometabolic disease. *Translational Research* 179, 108–115 (2017).

16. Musso, G., Gambino, R. & Cassader, M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? *Diabetes Care* 33, 2277–84 (2010).

17. Maslowski, K. M. & MacKay, C. R. Diet, gut microbiota and immune responses. *Nat Immunol* 12, 5–9 (2011).

18. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature 2011 474:7351* 474, 327–336 (2011).

19. Roager, H. M. & Licht, T. R. Microbial tryptophan catabolites in health and disease. *Nature Communications 2018 9:1* 9, 1–10 (2018).

20. LeBlanc, J. G. *et al.* Bacteria as vitamin suppliers to their host: A gut microbiota perspective. *Curr Opin Biotechnol* 24, 160–168 (2013).

21. Guetterman, H. M. *et al.* Vitamin B-12 and the Gastrointestinal Microbiome: A Systematic Review. *Advances in Nutrition* 13, 530–558 (2022).

22. Thomas, S. P. & Denu, J. M. Short-chain fatty acids activate acetyltransferase p300. *Elife* 10, (2021).

23. Krautkramer, K. A. *et al.* Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues. *Mol Cell* 64, 982–992 (2016).

24. Koeth, R. A. *et al.* Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 19, 576–585 (2013).

25. Zarbock, K. R. *et al.* Trimethylamine N-Oxide Reduces Neurite Density and Plaque Intensity in a Murine Model of Alzheimer's Disease. *Journal of Alzheimer's Disease* 90, 585–597 (2022).