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**OBY:** From the campus of Harvard Medical School, this is *ThinkResearch*-- a podcast devoted to the stories behind clinical research. I'm Oby.

**BRENDAN:** And I'm Brendan. And we are your hosts. *ThinkResearch* is brought to you by Harvard Catalyst-- Harvard University's Clinical and Translational Science Center.

**OBY:** And by NCATS, the National Center for Advancing Translational Sciences.

**BRENDAN:** The microbiome is home to some of the best chemists in nature. Microbes themselves can do chemistry that scientists don't yet understand. This phenomenon takes place at the intersection of biology and chemistry that is not often studied. Combining her experience as a chemist and her passion for biology, Dr. Emily Balskus is conducting new research to understand how the chemistry that microbes perform affects human health.

Dr. Emily Balskus is a chemist and microbiologist in the chemistry and chemical biology department at Harvard University. Dr. Balskus, thank you for joining us.

**EMILY BALSKUS:** Thank you. It's really exciting to be here.

**BRENDAN:** To start off, can you just describe the focus of your research?

**EMILY BALSKUS:** Sure. So I am a chemist. I am in the Department of Chemistry and Chemical Biology here at Harvard. And I really work at the interface of chemistry and biology. My research focuses on the chemistry of microorganisms. We attempt to understand how they make molecules, how they consume molecules in their environment, and how they interact using chemistry with other surrounding organisms, including humans.

**BRENDAN:** So part of your research focuses on the microbiome, which is the collection of bacteria that live inside and on our bodies. How did you become interested in the microbiome?

**EMILY BALSKUS:** That's a great question. My path to the microbiome was kind of a very non-intuitive one. I started my scientific training, actually, far from biology. I was a synthetic organic chemist. So I was trained to make molecules in a lab setting. And in the context of that research, I became really interested in learning about how nature made molecules.

And so I gradually transitioned the focus of my research and training to investigate how organisms like microbes actually did chemistry. Microbes are amazing chemists, and actually many drugs that we use clinically come from microorganisms, mostly organisms that live in habitats like the soil or the ocean.

And right when I was finishing my postdoctoral training and starting my own independent faculty career, there was sort of a resurgence of interest in microbes that live in close association with humans, the human microbiota. And there had been a lot of sequencing of this community. So there'd been great advances in DNA sequencing, technology. We were kind of just starting to really appreciate the genetic potential of these organisms, the diversity of the genes present in the microbiome, the proteins they encoded.

And I think there was a realization-- certainly I had had a realization right at this critical time-- that we really didn't understand that much about the type of chemistry the microbiota could do, or really how these organisms were accomplishing chemistry in the body. And from my perspective, really understanding the impact of the microbiota on human biology requires knowing what these organisms are capable of doing in this habitat. So it seemed like there was a great opportunity for someone with chemical training to jump in and start investigating the chemistry of these organisms.

One of the very first microbio projects in my lab focused on how gut microbes metabolize a nutrient that's very important for human health. And this nutrient is choline. So choline plays lots of important roles in the human body. It's a really important component of cell membrane lipids. It can be converted to a neurotransmitter, acetylcholine. And it's a donor of one carbon unit, a donor of methyl groups. And that plays a lot of important roles in cellular metabolism.

And so there was an understanding that choline was really important in the context of human biology, but very little was known about how the microbiota consumed this nutrient. So right as I was kind of starting my lab, there was a very interesting study that had been performed on human patients, so a lab-- Stan Hazan's lab at the Cleveland Clinic-- had done a metabolomics study in patients. They had looked at the concentrations of many, many, many small molecules present in the blood of patients and looked to see whether there were molecules whose abundance correlated with risk for cardiovascular disease.

And so they found that there was a strong correlation between a molecule called trimethylamine and oxide, or TMAO, and risk for adverse cardiac outcomes. And they did some animal work that really suggested there could be a causal role. They fed this compound to mice and they saw more severe atherosclerotic plaque formation. And what was intriguing about TMAO was that it's a molecule whose production depends on the microbiome.

So the precursor to TMAO is a molecule called trimethylamine. I think many of us are actually familiar with trimethylamine. It's a compound that's responsible for the odor of rotting fish. And the microbiome is the sole producer trimethylamine in the human body. Choline was known to be a major source of trimethylamine, and it was known that the microbiota could convert choline to trimethylamine, but no one had ever figured out which organisms were responsible, and more specifically, which genes and enzymes were actually performing this chemistry.

So this really provided my lab with a great opportunity to kind of use our detective skills to try to discover how this was happening. And this is kind of a theme, I think, in a lot of our work is that we try to identify interesting chemical transformations that are happening in the microbiome and then find the genes and enzymes that are responsible for performing them.

And so in the case of choline, we thought a lot about the chemistry of how choline was being converted to trimethylamine. We were able to sort of recognize some chemical parallels between that metabolic activity and bacterial metabolic activities that had been better characterized. And so we generated a hypothesis for the types of enzymes that might be involved, searched a bunch of genomes for them, and managed to uncover a set of genes that encoded a pathway for choline metabolism.

So this set of genes we named the choline utilization gene cluster or the cut gene cluster. And they included an enzyme that was able to take choline and metabolize it to trimethylamine. This discovery allowed us for the first time to start searching microbiome sequencing data and really identifying this gene in patient data sets so we could start to ask the question of whether there was a correlation between the abundance of this microbial pathway and TMAO levels and disease.

And a big interest of ours is actually trying to manipulate choline metabolism-- stop it from happening so that we can test whether or not this pathway plays a causal role in disease.

**BRENDAN:** Is your work the kind of evolution of microbiome research? Is it going more towards understanding the chemistry? Like, I know there's a lot of-- you know, DNA sequencing is really important in discovering which microbes live where. But at some point do we need to understand the chemistry in order to really advance the field?

**EMILY BALSKUS:** Yeah. So I think microbiome research is definitely evolving. So to give some context, we have known that we have a microbiome pretty much since the birth of the field of microbiology. I mean, one of the first things that van Leeuwenhoek looked at with his microscope was dental plaque from humans. And there's actually a lot of research spanning many, many, many decades that had investigated gut microbes and their roles in human health.

But it turns out that microbes are often very hard to cultivate in the lab. And so for many years we really lacked a complete understanding of which microbes were present in the human body and the diversity of different individual's microbiomes. And so it was really DNA sequencing technology that allowed us to get our first answers to those questions.

And so sort of back in the late '90s, early 2000s, as DNA sequencing started to become more and more powerful and people started isolating DNA directly from the human microbiome, the gut microbiome, and in particular was a-- was a very important community of interest. And you know, what researchers started to do was actually apply methods that had first been developed by environmental microbiologists who were interested in studying microbes out there in soils and marine habitats.

And these DNA sequencing methods looked for marker genes that are diagnostic of bacterial phylogeny or relatedness. So you basically pick a universal gene that all living organisms share. The one that's typically used for bacteria is a gene that encodes an RNA subunit of the bacterial ribosome.

And you can tell because that gene evolves-- is essential. It evolves very slowly. You can look at the sequence of the gene and how it's evolved and you can kind of work out how bacteria must be related to one another. And so these early attempts to kind of look at DNA sequencing data and get information about microbes involved looking for that specific kind of gene-- that marker of a phylogeny.

And so this really revealed for the first time the incredible diversity of the human microbiome, that there are hundreds of different species and that each one of us actually has a different collection of species in our microbiome. And I think that really was a surprise to the scientific and medical community.

And so these types of sequencing endeavors got scaled up a lot. So you've probably heard of the Human Microbiome Project. And that was a big success in trying to characterize the organisms present in the healthy human microbiome. There've been follow up projects that have focused on comparative analyses, so looking at what's different between the healthy microbiome and microbiomes of patients with disease.

And really there's been sort of an explosion of efforts to use this sort of sequencing-based approach to look at genes that are present in the microbiome and try to gain information about the microbiome's role in human health. But that goal has actually been really, really challenging to achieve just through sequencing.

And so I think this gets at the crux of why I think chemistry is so important in microbiome studies. Another reason why we're focused on microbial chemistry is that the gut microbiome just is an environment where there's a lot of chemistry happening. So if you think about microbiomes' interactions with molecules, you know, we consume a tremendous diversity of compounds in our diet. Many of those compounds actually can be digested by human enzymes. So they make their way down to the large intestine where they're metabolized by bacteria.

So plant-derived compounds, fiber, complex polysaccharides, are a great example of compounds that are metabolized by the microbiome. Drugs we take also interact with the microbiome. We often take drugs thinking that they are intended to manipulate things that are happening in our cells. But they also can interact with microbes in both positive and negative ways. So we're learning the microbiome can affect drug efficacy.

Microbes also produce their own small molecules. So they can also make complex molecules that enter the human body and bloodstream and can interact with targets in human cells. And we know very little, actually, about the diversity of molecules that gut microbes are making and how they're actually interacting with and influencing cells in the body.

**BRENDAN:** Great. So you mentioned drug metabolism. And I wanted to touch on the work you've done looking at Parkinson's treatment. So could you talk a little bit about that specific work and how it ties back into this drug metabolism idea?

**EMILY BALSKUS:** Sure. So it's been known for a long time that gut microbes can actually transform the chemical structures of drugs. So they will actually perform chemistry on orally administered drugs, changing their properties like toxicity. In some cases, these types of microbially derived transformations are beneficial. They can be even critical for drugs to be efficacious.

But they can also be very harmful. So in some cases, there have even been patient deaths that have been linked to gut microbial drug metabolism. But even though we've known about a lot of examples of this phenomenon, just like other types of gut microbial chemistry, the actual organisms and genes and enzymes that are responsible are often unknown.

And so we got into this problem because of an interest in the gut microbial metabolism of the Parkinson's disease medication levidopa or L-DOPA. So L-DOPA is one of the oldest and still most effective treatments for Parkinson's. It is administered orally, and it has to cross the blood brain barrier where it actually gets metabolized by an enzyme in the brain to dopamine.

And so this type of transformation is known as a decarboxylation. And this reaction is absolutely critical for drug efficacy. So it replenishes dopamine in the brain, where it's needed. But one of the things that can really limit the effectiveness of L-DOPA is if metabolism happens in the wrong place.

So it turns out that the brain is not the only place in the body where L-DOPA can be decarboxylated. There is expression of the host decarboxylase enzyme in peripheral tissues. And so this-- the problem with this peripheral metabolism-- metabolism in other parts of the body-- is that if dopamine is generated outside of the brain, it can no longer cross the blood brain barrier.

So one major advance in Parkinson's therapy many years ago was the development of drugs that block the host enzyme outside of the brain. So these inhibitors are co-administered with L-DOPA and they help to increase the concentrations of L-DOPA that actually make it into the brain.

But one intriguing observation that's been made is that even when patients are taking these extra enzyme inhibitors, they can still have a lot of metabolism happening in the periphery. So up to 60% of L-DOPA can still be metabolized outside of the brain. And there really wasn't a great explanation for this observation.

However, it had also been noted many years ago when L-DOPA was first kind of being developed that the gut microbiota also had the potential to metabolize L-DOPA. So it turns out they can perform exactly the same reaction that the human enzyme can. They can do decarboxylation.

But the contribution of the gut microbiota to this metabolism outside of the brain was really unknown. And so that's what we kind of decided to try to investigate. Well, we wanted to learn how the microbiota was performing this transformation and whether or not it might be having a negative effect on patients who were taking this drug.

So we started with a hypothesis that gut bacteria might be performing this decarboxylation reaction in a very similar way to the human enzyme. And so we basically searched gut bacterial genomes for enzymes that resembled the human decarboxylase and found a really promising looking candidate enzyme that was present universally in one specific species of human gut bacteria called Enterococcus faecalis or E. faecalis.

So we did genetics in that organism to show that this enzyme, which is called tyrosine decarboxylase, was actually responsible for L-DOPA metabolism. And then we actually purified and studied the enzyme to learn more about how it worked and its activity. From those studies, we learned that the bacterial enzyme actually prefers a different substrate, the amino acid tyrosine, but displays a very high activity towards L-DOPA.

So even in the presence of its preferred substrate, tyrosine, it still metabolizes L-DOPA. We were then able to sort of show that this particular organism and enzyme really were potentially relevant in the human gut by actually looking at activity of gut microbiome samples ex vivo. So we were able to sort of take a set of samples from human donors, both neurologically healthy individuals and patients with Parkinson's disease, and show that these communities do have the potential to metabolize L-DOPA and that there's actually a large degree of variability between different individuals.

So some samples we saw absolutely no metabolism of the drug. Other samples we saw very extensive metabolism. And I think that variability is quite interesting from the perspective of Parkinson's patients because it's known that patient response to L-DOPA and L-DOPA efficacy can differ quite substantially between different patients. And the basis for that is not at all clear.

And so we also were able to show that in the samples that could metabolize a drug, there was an increased abundance of the tyrosine decarboxylase gene and an increased abundance of E. faecalis. So it really does appear that the specific organism and enzyme we've discovered really might be responsible for this chemistry in the human microbiome, which is very exciting.

**BRENDAN:** And could be responsible for this variability in the efficacy of the drug across patients.

**EMILY BALSKUS:** Potentially. Yeah. We're during follow-up studies to really try to investigate that hypothesis more thoroughly in patient populations. But that is certainly a hypothesis that we have. One of the other things that we were able to show was that the drugs patients are currently taking to block the human decarboxylase enzyme don't work against the bacterial enzyme.

So even if patients are taking L-DOPA and a decarboxylase inhibitor, they are likely not affecting decarboxylation by the microbiome. And so then that raises the question of, could one develop an inhibitor that could target the bacterial activity? And we were able to do this.

So by thinking about the substrate preference of the bacterial enzyme, we were actually able to find a inhibitor that better mimicked tyrosine, the enzyme's preferred substrate. And that small molecule is effective at preventing decarboxylation by the gut microbiota. And so you could imagine potentially someday Parkinson's patients who happen to have this activity present in their microbiome could take L-DOPA, a human decarboxylase inhibitor, and potentially an additional medication that could target metabolism by the microbiome.

**BRENDAN:** As a closing thought, where do you see research in your lab going, and where do you hope that understanding the chemistry of the microbiome leads medicine?

**EMILY BALSKUS:** My lab's research is going to continue to focus on trying to understand how gut microbial chemical transformations are affecting human health and disease. And I think a few major goals for us in the future, that also maybe kind of extend to the field of microbiome research, are to really start to do more of our work in humar patients, so to find ways of doing mechanistic microbiota research in humans.

And I think drugs are actually kind of a great platform for doing that, because a drug is a very defined perturbation of the microbiome, a very defined interaction with the microbiome, that you can actually deliver to subjects. And so I think that rather than just describing microbiomes in patients, we can maybe transition to the idea of perturbing them and seeing how they respond.

And I think that that will give us a lot of fundamental information about how the microbiome works. We'd like to continue working towards this goal of trying to develop small molecules that interfere with specific microbial chemistry. I mean, I think it's very possible that these types of compounds could be interesting from a therapeutic perspective, but also purely from a research perspective. And I hope that they will be really transformational tools for allowing us to perturb and then understand the roles of different chemistry in this complex habitat.

And I think the field more broadly really should be trying to address this huge gap in our knowledge of what genes in the microbiome are actually doing. And I think this is a problem that applies to more fields than just the microbiome research. If you think about the whole genomic era of biology, we're accumulating vast amounts of sequencing data. That's no longer a bottleneck.

You know, what really limits progress now is really interpreting that information and really gaining an understanding of what genetic change means from a functional perspective. And so I think that a big goal that I would like to pursue in my lab in the future, and I hope others will embrace, as well, is the challenge of accelerating our ability to connect genetic information to functions.

And so we're thinking about how we can start to link enzymes and genes to activities in a higher throughput. So up until now, we've mostly focused on one reaction at a time, or one set of enzymes at a time. And so how can we find ways to really apply this type of kind of chemical thinking, you know, more massively, and apply it in parallel to accelerate this process of functional characterisation? I think that's a major goal for the future.

**BRENDAN:** Great. Well, Dr. Balskus, thank you very much. It's a pleasure to have this conversation with you.

**EMILY BALSKUS:** Oh, thank you for the invitation.

**BRENDAN:** Next time on *ThinkResearch*--

**OSAMA RAHMA:** Well, it's been called the breakthrough of cancer treatment in the past decade is cancer immunotherapy where we actually try to mobilize or activate the immune cells to go after cancer.

**BRENDAN:** Dr. Osama Rahma of Dana Farber Cancer Institute answers our questions about his research in understanding cancers and their various treatments. Thank you for listening. If you've enjoyed this podcast, please rate us on iTunes and help us spread the word about the amazing research taking place across the Harvard community.

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