

Vamsi Mootha: Taking an inventory of mitochondria

Combining his medical training with his love of computational biology, Mootha is compiling an exhaustive list of mitochondrial genes and networks.

"I wanted to use knowledge of the human genome to understand mitochondrial diseases. I was in the right place at the right time."

Mitochondria, the energy reactors of the cell, are estimated to contain approximately 1,500 proteins, but the mitochondrial genome itself only encodes about 13 of them. The remaining proteins are immigrants that are encoded by the nuclear genome. Vamsi Mootha is

using an integrative genomics approach to identify all of these proteins and their functions and to understand how their mutated versions lead to disease.

Mootha, who built thermodynamic models of mitochondrial function as a medical student (1), honed his genetics skills during his years as a medical resident and a postdoc (2–5). In addition to expanding the database of mitochondrial proteins and networks, Mootha, now an assistant professor at the Broad Institute in Cambridge, MA, is also trying to figure out why mitochondria are often the source of drug toxicity (6).

His team has found that many commonly used drugs suppress mitochondrial gene expression or interrupt the cross-talk between the mitochondrial and nuclear genomes. Their discoveries help explain many physiological mysteries, such as why cholesterol-lowering statins cause muscle cramps in some users—because they increase the production of mitochondrial toxic byproducts and lower the cell's energy. Mootha, who was awarded the MacArthur Foundation's Genius grant in 2004, discussed his ventures into mitochondria in a recent interview.

MATH TO MEDICINE

How did you get interested in science?

I come from a family of physicians. My father is a general surgeon and my three siblings and their spouses are all physicians. So as a child, I wanted to be a physician as well. But, in my teens, I became enamored

by math and computer science. I was actually a math geek in high school who competed in various math contests.

You pursued your interest in math in college. Why did you pick Stanford?

I got into colleges on the East and West coasts, and in Texas, which is where I grew up. But, being an avid tennis player in high school, the weather in Northern California and the popularity of tennis there made Stanford really appealing to me. That, and the fact that math and computer science are so strong at Stanford, drew me there.

Did you end up playing tennis for Stanford too?

If I could play tennis for Stanford, I wouldn't be talking to you right now. I'd be on ESPN.

Fair enough. So how did you go from being a mathlete to becoming a med student?

I took a biology class during my junior year and absolutely fell in love with biochemistry and genetics. And although I was good at math, I realized that I didn't have that *je ne sais quoi* that's required to make a fundamentally deep impact in mathematics.

So my math advisor strongly urged me to think about mathematical biology or medicine as a career instead and told me about the M.D. program that is jointly offered by Harvard/MIT. He thought this program would be a fun way of entering the research world while, at the same time, giving me solid training in physiology and teaching me about human disease.

What was the program like?

It was small and spectacular. The first two years were heavy with courses that were highly quantitative. On the first day of our cardiovascular class, our professor said, "Let's model the heart as a thin, massless sphere." The final two years involved hospital rotations and thesis research.



Vamsi Mootha

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MEETING MITOCHONDRIA

When did you become fascinated with mitochondria?

During pathology class in medical school, we looked at EM images of a patient that had a mitochondrial disorder. I remember thinking that these organelles, which had their own DNA and evolutionary history, were amazing. And I also learned that mutations in mitochondrial DNA could give rise to maternally inherited disease also got me hooked.

At the end of our second year in medical school, some of my friends were going to the NIH to do their theses and I thought a change of scenery would be good for me as well—it was getting really cold in Boston. Also, there weren't any labs at Harvard that focused on mitochondrial research.

What was your project at the NIH?

I joined the lab of Bob Balaban who was working on mitochondrial physiology. My job was to build thermodynamic models to understand how ATP synthesis is regulated.

We made lots of measurements of membrane potential, oxygen consumption, ATP production rates, and other features that make up the mitochondrial circuit and then asked how these processes were linked. I guess that our approach would be called "systems biology" today, but back then it was simply biochemical or quantitative physiology.

MERGING CLINIC WITH LAB

What happened after you got back to Boston?

My internship advisor, Jeff Flier, talked me into setting up a physiology rig like the one I used at the NIH to study how mitochondrial respiration is influenced by factors called uncoupling proteins. These are proton channels that disrupt the proton gradient and prevent it from transmitting the energy needed for ATP synthesis.

And just after I graduated from medical school, Stan Korsmeyer, who had discovered the apoptosis gene BCL2 and its localization in the mitochondria, moved his lab to Boston from St. Louis. He agreed to let me explore the effect of Bcl2 family members on mitochondrial physiology, and so I started moonlighting in his lab while doing my residency at the Brigham and Women's Hospital.

What did you work on in his lab?

Stan's lab had previously discovered a proapoptotic molecule called Bid that somehow triggered cell death when it translocated to the outer membrane of the mitochondria. My work showed that Bid was puncturing mitochondrial membranes and causing cytochrome c, a member of the electron transport chain, to leak out. This resulted in a respiratory block. But adding back cytochrome c at early time points could rescue the respiration.

Did you also get to learn about mitochondrial disorders during your residency?

Because mitochondrial mutations are so rare, I only occasionally got to see patients with these disorders, which were mostly muscle- or cardiomyopathies. At the time, we knew of about 50 mitochondrial DNA mutations that had been linked to these diseases. But epidemiological studies showed that a majority of patients with mitochondrial disease did not have mitochondrial DNA mutations. I thought that in order to understand how mutations in nuclear DNA were

causing these diseases, we needed to figure out the functions of all the proteins in the mitochondria.

INTEGRATING DATA

How did you go about your mission?

I was a clinician with a college background in math and computer science, who also had some DNA sequence analysis experience. It was 2000, I was in Boston, and I wanted to use knowledge of the human genome to understand mitochondrial diseases. As it turned out, I was in the right place at the right time.

You mean because of Eric Lander and the Human Genome Project?

Exactly. Eric Lander was leading some of the genome sequencing efforts right here in Cambridge. I joined his lab at the Broad Institute as a postdoc right after I finished my residency. While brainstorming on how to tackle my problem, I heard Matthias Mann give a talk about tandem mass spectrometry-based proteomics.

For tandem mass spec, you need two

things: a sample to analyze and a genome sequence so that you can figure out what your spectra correspond to. So I spent six months collecting mitochondrial mass spec data in Matthias's lab in Denmark. The genome sequencing was completed

just after I got back to Boston and so I could start interpreting my mass spec data. We found 150 or so new mitochondrial proteins this way, which was a big advance at the time.

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Extracts of isolated mitochondria are the starting point for Mootha's protein hunt.

Did your expanded database help you find disease-associated genes?

Yes, it did. A genome-wide association study had mapped a fatal mitochondrial neurodegenerative disease called Leigh syndrome to a large chromosome segment but hadn't pinpointed the gene. I combined the human genetics data with our proteomic and genome data to identify the disease gene, which induces a chronic energy shortfall by causing a deficiency in cytochrome c oxidase—an enzyme required for electron transport. Our discovery had a big impact: there is now a prenatal screening process to prevent parents that are both carriers from losing children to the disease.

FUTURE PLANS

What are you working on now?

My lab is using a combination of microscopy, mass spectrometry, and computation to identify all the component machinery of the mitochondria. We've clocked about 1,100 proteins so far. We've now mapped the evolutionary history of each of these proteins and are using this information to figure out protein function and identify disease genes. We're also using chemical genomics to find disease biomarkers and identify therapeutic strategies.

What's chemical genomics?

We model these disorders in cell culture by using cells from diseased patients or by engineering the mutation into normal cells. We then screen large collections of small molecules or drugs to see if any of them suppress or aggravate the disease phenotype. This approach is helping us understand disease pathogenesis and is giving us clues for new therapies. My hope is that over the next few years, it will help us transform mitochondrial medicine.

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