Miklós Müller: The deep history of eukaryotic metabolism

Müller initiated the comparative analysis of metabolic pathways in anaerobic eukaryotes.

Back in the deep, dark reaches of time, there existed a cell that became the ancestor of all eukaryotes, from unicellular microorganisms to dinosaurs to humans. What was that first eukaryote like? What were its metabolic capabilities, and how were they organized?

In the early 1970s, as a research associate at The Rockefeller University in New York, Miklós Müller started investigating these questions by examining unicellular anaerobic eukaryotes (1). These organisms do not have mitochondria, and Müller discovered that some of them instead possess a novel organelle that he dubbed the hydrogenosome (2). The Müller lab's studies of this organelle, and comparative analyses of the organization of metabolic pathways in different unicellular anaerobic organisms (3), have strongly influenced current ideas on eukaryotic evolution and the origins of mitochondria (3, 4). Now Professor Emeritus at Rockefeller, Müller continues to keep tabs on his colleagues' investigations into biological history, but these days his own inquiries focus on the history of biology.

FROM THE RUBBLE

What was your childhood like?

I come from the pre–World War II Hungarian middle class. I had a sheltered life as a child. My father was an architect and my mother studied art, so I grew up in a very intellectual environment. Our apartment was full of books in many languages. But my life drastically changed during the 1944–1945 Russian siege of Budapest. I saw battle from the windows, and my father passed away due to an illness just a few months later. My school was half ruined, so in the first school year after the war we spent part of our time removing the rubble.

Hungarian politics was quickly shifting toward communism, but I was not interested in that. What interested me was that I wanted to become a scientist, so in 1949 I went to university. I had been admitted to both the biology and medical faculties, but in Hungary the biological sciences were in complete disarray. So I studied medicine.

What were the challenges facing the biological sciences in Hungary?

Russia during Stalin's time wanted to develop a completely Soviet scientific system that was not dependent on Western "imperialist-capitalist" science. Trofim Lysenko was a Soviet agronomist who rejected Mendelian genetics and suggested a different inheritance model. This was not valid, of course. He was a charlatan. But he was a very powerful person in Russia, and in the 1940s his ideas strongly distorted Hungarian research in the biological sciences.

While I was in medical school I translated the work of another prominent Russian scientist, Olga Lepeshinskaya, into Hungarian for the Hungarian Academy of Sciences. She believed that cells could arise de novo from nonliving material. My professor in Budapest actually claimed to have results that confirmed this false notion, and he was awarded a big state prize for it.

I witnessed this, but by then I had completed my medical training and become interested in the physiology of unicellular organisms. I started studying the mechanisms of intracellular digestion in these cells, and I realized a year or two after I started that the food vacuoles where digestion occurs are really lysosomes.

> "[De Duve] taught me how to do science."

On the basis of that work I was invited to the first international conference on lysosomes in London in 1963. This changed my life. I met several prominent colleagues there, including Christian de Duve, who a year later invited me to join him in the department he was organizing at The Rockefeller University.

A BIG YEAR

You worked closely with de Duve…

Yes. He was my first real scientific mentor. He taught me how to do science: how to evaluate and interpret results. He received the Nobel Prize in 1974 for describing lysosomes and peroxisomes. Our work led to the discovery of peroxisomes in a unicellular eukaryote, or protist, *Tetrahymena*. These organelles were described earlier in mammals, but it turned out that peroxisomes in *Tetrahymena* are very unusual. In addition to the typical peroxisomal enzymes you find in mammals, they had some metabolic enzymes, including those involved in the so-called glyoxylate bypass.

PHOTO COURTESY OF JUDIT MÜLLER

Miklós Müller

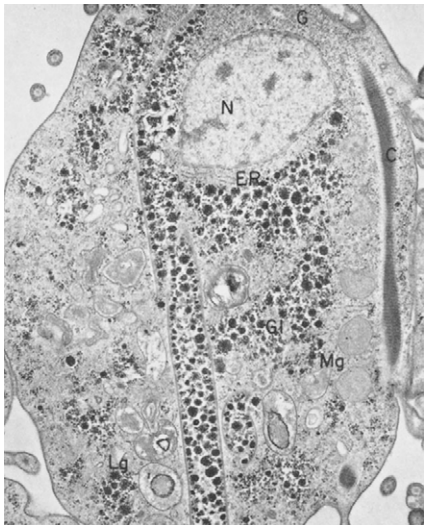
De Duve wanted to reconstruct the ancestral peroxisome. His idea at that time was that there could be eukaryotic organisms that do not contain mitochondria but have only peroxisomes as an oxidative organelle. This was really what pushed me to start working on anaerobic protists. I started working on trichomonad flagellates, which do not use the Krebs cycle or the electron transport chain for ATP synthesis.

Trichomonads do not have mitochondria but do have organelles that look like peroxisomes. It turned out that these organelles are biochemically similar to neither mitochondria nor peroxisomes and have an unusual metabolic end product: they produce hydrogen. When we isolated our new organelles, we demonstrated that they contained enzymes for hydrogen production. That's why they got the name "hydrogenosomes."

It must have been very exciting to discover a new organelle…

It was. That year—1973—was a major year for me. I became an American citizen,

Text and Interview by Caitlin Sedwick
csedwick@gmail.com



Reproduction of an electron micrograph of a *T. foetus* cell containing hydrogenosomes (labeled Lg)

I got tenure at Rockefeller, I discovered hydrogenosomes, and I got married! My wife, who just retired as the chief parasitologist for New York State, was a postdoc at Rockefeller when we met.

What's the role of hydrogenosomes?

Trichomonad hydrogenosomes produce ATP. In contrast to mitochondria, where you get 36–38 ATP molecules for each pyruvate oxidized, here we get only two. But hydrogenosomes serve some of the same functions as mitochondria. They contain enzymes—pyruvate ferredoxin, oxidoreductase, and hydrogenase—that reoxidize the reduced components of glycolysis.

Our data raised the question: what are these organelles? Are they really a completely different organelle from mitochondria, or could they be some kind of transformation of mitochondrial structure? There was no molecular genetics technology available then, so we started looking at potential biochemical similarities between mitochondria and hydrogenosomes. In doing this we opened up a window on a comparative aspect of eukaryotic cell biology that was completely novel at that time.

As an aside, the basic metabolic map of the *Trichomonas* hydrogenosome that we made in the '70s has not changed since, except that several additional enzymes have been found there. I am very pleased with that work. It has withstood the test of time.

HISTORY OF BIOLOGY

While investigating hydrogenosomes, you looked at many anaerobic organisms…

Several organisms were known not to require oxygen in their metabolism. Don Lindmark in my laboratory looked at whether the anaerobic protist *Giardia* has hydrogenosomes or not. We found the enzymes characteristic of this metabolic pathway but no organelles. The enzymes were in the cytoplasm.

We then spent many years comparing the biology of different protists. It turned out this is a vast group of unicellular organisms that organize their metabolic pathways in many different ways. I retired in 2005, and in 2012 we published a wide overview of the metabolic pathways and organellar compartmentalization in anaerobic eukaryotic organisms. I regard that paper as my swan song.

How did hydrogenosomes arise?

When investigating the enzymes present in mitochondria and hydrogenosomes, we did not detect any functional overlap. It seemed possible that the two organelles could have appeared in eukaryotes through separate endosymbiotic events, in which bacteria with different metabolic capabilities became incorporated into nucleated cells. In terms of their metabolic functions, hydrogenosomes and mitochondria are different. But others have since shown that they are very similar in their biogenesis. Most likely, hydrogenosomes are modified from mitochondria, and there was just one endosymbiotic event.

"Our data raised the question: what are these organelles?"

I think that the ancestral mitochondrion likely had both aerobic and anaerobic capabilities. In some eukaryotic lineages, there remain mitochondria that perform only aerobic metabolism, whereas in others the organelle has evolved into the hydrogenosome. And as we showed, some eukaryotes don't even have the organelles anymore but have these metabolic pathways in their cytoplasm. No one has yet found any bacteria today that resemble the ancestral mitochondrion. Perhaps we should not expect to. After all, bacteria have continued evolving for the same billion and a half years as eukaryotes.

Are you still involved in the field?

I think often about all my past mentors, collaborators, and postdoctoral students—I wish I could list and thank them all! But yes, I am still in contact with my colleagues. I just had a visitor from Vienna with whom we collaborated 15 years ago. Some seem to regard me as a grandfather within the field. [*Laughs*]

I am interested in the ongoing work on the origin of mitochondria and the ancestral eukaryotic cell. There are several hundred thousand species of anaerobic protists, and we only know the metabolism of about six of them. So there is still a tremendous amount of work to be done there.

But I will no longer publish in protist research. I have become a science historian, and I am now working on the history of biology during Lysenko's period of influence in Eastern Europe. This is partly a return to my youth, when I decided to study medicine because Lysenko had ruined Hungarian biology. I want to understand what happened in those times. I am doing archival research on that topic in Budapest, Prague, Berlin, St. Petersburg, and other cities, and I attend international meetings on Lysenkoism. That's what I'm doing today.

1. Müller, M., P. Baudhuin, and C. de Duve. 1966. *J. Cell. Physiol.* 68:165–175.
2. Lindmark, D.G., and M. Müller. 1973. *J. Biol. Chem.* 248:7724–7728.
3. Müller, M., et al. 2012. *Microbiol. Mol. Biol. Rev.* 76:444–495.
4. Müller, M. 2012. Discovering the Hydrogenosome. *In* Entering an Unseen World. 305–309.

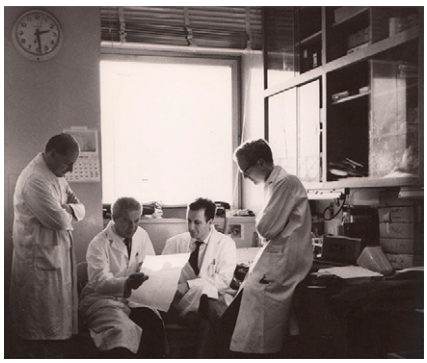


PHOTO COURTESY OF MIKLÓS MÜLLER

Müller (standing, left) and de Duve (seated, left) discuss data with colleagues.