People & Ideas

Martin Jonikas: Bringing high-throughput genetics to photosynthesis

Jonikas' career jumps have advanced *Chlamydomonas* systems biology.

Systems biologist Martin Jonikas was destined for a career in engineering until he was forced to take a molecular biology class. His aerospace engineering bachelor's degree from MIT required the course for graduation. Already an accomplished robotics designer, Jonikas discovered that the most fascinating machines are alive.

As a graduate student with Jonathan Weissman and Peter Walter at UC San Francisco, Jonikas was initiated into the world of high-throughput yeast genomics, discovering several genes required for protein folding (1). From there, Jonikas struck out on his own as a Young Investigator at the Carnegie Institution for Science, in Stanford, California. For the last six years, his group has been developing analogous high-throughput genetic tools for the single-celled photosynthetic eukaryote *Chlamydomonas reinhardtii* (2, 3). His group also discovered how a potassium antiporter helps plants deal with rapid light fluctuations (4). Most recently, they have begun to chart how cells build the pyrenoid, a photosynthetic organelle that concentrates carbon for efficient carbon fixing and how this organelle might be transferred to higher plants in an effort to enhance crop yields (5).

CHARTING NEW WATERS

How did an MIT graduation requirement turn into a career change?

I had delayed the biology classes until the end of college because, growing up, I thought biology was about memorizing long lists, like the bones in your body or of obscure organisms. I was extremely fortunate to have this wonderful professor, JoAnne Stubbe, who showed me that much of biology is actually about understanding cellular machines.

It opened my eyes to a new view of the world. Before that, I thought that airplanes and rockets were the most awesome machines one could ever hope to engineer. But that class showed me that organisms are actually far more impressive machines, capable of doing things like healing themselves, making more of themselves, and even thinking about themselves. I became very excited about the huge opportunities in engineering biological systems.

What inspired your graduate work to identify all of the genes involved in protein folding?

In Jonathan Weissman and Peter Walter's labs, we developed a tool that allowed us to systematically place poorly characterized yeast genes into genetic pathways, which often gives a good hint as to what the genes are doing.

I found these technologies exciting because they were rational efforts to deal with the incredible complexity of cellular systems. This is what systems biology is trying to do: perform simple experiments that give us a lot of insight into biology and make a complex system easier to understand.

> "[*Chlamydomonas*] is sometimes referred to as the 'green yeast.'"

And now you want to apply these approaches in* Chlamydomonas*?

Yes. At UCSF, we had no exposure whatsoever to photosynthetic organisms. When I finally understood how central these organisms are to life on Earth, I was struck by how little we know about them.

Photosynthetic organisms regulate the global carbon cycle, and produce the oxygen we breathe, the food we eat, the fuels we burn, as well as many of our materials and many of the drugs we use to treat disease. Our civilization faces major challenges in these areas in the coming decades, and the engineering of photosynthetic organisms holds major promise for addressing these challenges.

In the last year of my PhD, I started thinking about how I could help advance our understanding of these organisms by bringing yeast-style high-throughput genetic approaches to a photosynthetic model organism.

PHOTO COURTESY OF MARTIN JONIKAS

Martin Jonikas

GOING GREEN

Why* Chlamydomonas*?

Chlamy is the best system for doing this because it is the most advanced single-cell photosynthetic eukaryote in terms of tools and size of the research community. It is sometimes referred to as the "green yeast"—it is haploid, and all three of its genomes are sequenced and transformable.

Despite these benefits, Chlamy still presented challenges. To do high-throughput genetics, you need a genome-wide collection of mutants. However, there was no such collection, so we decided to make one.

We faced two major hurdles: first, it was hard to generate the desired mutants; and second, it was hard to maintain the mutants once we generated them.

The process of inserting foreign DNA into the Chlamy genome is very different from yeast. In yeast, where DNA goes in by homologous recombination, you can knock out whatever gene you want. But in Chlamy, DNA inserts into random sites in the genome. You have to generate lots of random insertion mutants, and determine which gene is disrupted in each mutant.

The insertions were much messier than we expected. Parts of the transforming DNA get chopped off by what appears to be an endonuclease. Another challenge was that fragments of genomic DNA get inserted between the cassette and the true flanking sequence. This all makes it hard to determine which gene is disrupted.

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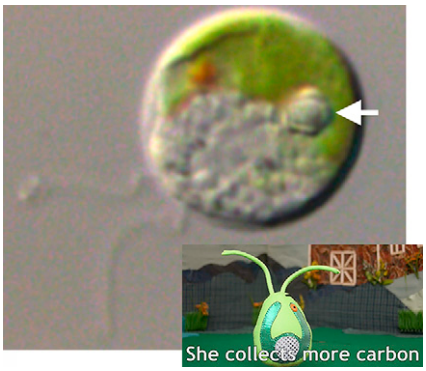

IMAGE COURTESY OF MORITZ MEYER/JONATHAN MANN, LIZ FREEMAN ROSENZWEIG, AND NINA IVANOVA

***Chlamydomonas*, as seen under the microscope and on YouTube (inset). The white arrow points to the real Chlamy's pyrenoid.**

How did you overcome these challenges?
The key was having a good team of people working on the problem. The hard part was figuring out what was happening during transformation to make the insertions so messy. Once we had a reasonable model, we were able to come up with simple solutions.

Finally, we found ways to maintain the mutants by adapting high-throughput robotics from yeast. Thanks to everyone's hard work and a lot of support from the community and the National Science Foundation, the dream became a reality. Over 60,000 mutants that cover most of the genome are now available from the Chlamy Resource Center at University of Minnesota.

Now that you've made a Chlamy mutant library, what's the goal?
The dream is to engineer photosynthetic organisms to do more good things for the world. For example, we'd like to help crops make more food, with the same resources, more sustainably.

To help meet those goals, we are going in two directions: advancing our systems-level understanding of photosynthetic organisms, and studying mechanisms that can be engineered to enhance photosynthesis.

A big challenge for engineering photosynthetic organisms is that the functions of most of their genes remain unknown. The mutant library will allow us to determine which genes are required for growth under which condition, and which genes work together to achieve a common goal.

The other direction in my lab is to understand evolutionary innovations in some organisms that enhance their photosynthetic efficiency. Specifically, we want to understand the eukaryotic algal carbon-concentrating mechanism (CCM).

CONCENTRATING ON CARBON

Why investigate the CCM?
The practical idea is to enhance crop yields. Many of our crops are struggling to assimilate carbon dioxide as a result of photosynthesis' own success. It has sucked all the carbon out of the atmosphere and starved itself.

Some organisms have invented CCMs, which allow them to suck carbon out of the atmosphere more efficiently. There's huge interest in understanding how these CCMs work and transferring them to the world's major crops that do not have them, like rice and wheat. If successful, we could potentially increase yields by up to 50%, and crops could grow with much less water and fertilizer.

> "The dream is to engineer photosynthetic organisms to do more good things for the world."

We are pursuing the algal CCM, which is built around an organelle called the pyrenoid that contains the algal cell's carbon-fixing enzyme Rubisco. About one-third of the planet's carbon fixation happens in the pyrenoid. Yet it is one of the most mysterious organelles.

What have you uncovered so far about the pyrenoid?
For the past 25 years, the pyrenoid was thought to be primarily composed of two proteins: Rubisco and its chaperone Rubisco activase. With our systematic approaches we have discovered many new components of the pyrenoid. These new components are giving us insights into how this organelle is assembled and how it functions. There's a ton of new stuff in there. We hope that many others will join us in figuring out how the pyrenoid works.

The aim of transferring the pyrenoid into higher plants is currently extremely risky and challenging. But the payoff would be huge, and a lot of solid science suggests that the approach should work. The major challenge is simply that we do not yet understand enough about the biology to enable engineering.

More broadly, the approach we are taking is just one of at least three different ways of engineering a CCM, and it's too early to tell which one is going to be practical in the end. But I'm very confident at least one of them is going to work, and it is exciting to be a part of the community working on this problem.

Your lab made a music video about Sammy the Chlamy (https://youtu.be/f1F4lxKF41g). Did you do vocals?
I'm not the one singing! That's Jonathan Mann, a songwriter and YouTube celebrity most famous for writing a song a day for more than 2,000 days now. He has this wonderful philosophy that by writing one song each day, overall he can produce more good stuff than if he wasn't forcing himself to be creative every day. I really admire him for that.

A few years ago, it occurred to me that it would be fun to explain our work to the broader public in the form of a song, working with Jonathan. Two lab members, Liz Freeman Rosenzweig and Nina Ivanova worked with Jonathan to come up with the lyrics and design the backgrounds and puppets. They worked with a lady on Etsy who makes plush toys. They did a fantastic job.

1. Jonikas, M.C., et al. 2009. *Science*. 323:1693–1697.
2. Zhang, R., et al. 2014. *Plant Cell*. 26:1398–1409.
3. Li, X., et al. 2016. *Plant Cell*. 28:367–387.
4. Armbruster, U., et al. 2014. *Nat. Commun.* 5:5439.
5. Atkinson, N., et al. *Plant Biotechnol. J.* http://dx.doi.org/10.1111/pbi.12497

PHOTO COURTESY OF MARTIN JONIKAS

The Jonikas lab hiking on Mt. Tamalpais