GOING GREEN

What we can learn from a little alga

By Yasemin Saplakoglu

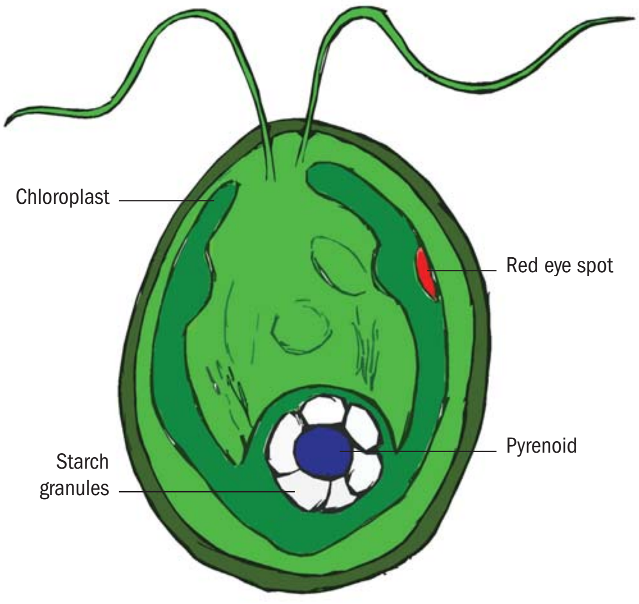
IMAGE COURTESY OF URSULA GOODENOUGH, UNIVERSITY OF WASHINGTON IN ST. LOUIS

WE ARE CONCERNED, rightly so, about the amount of carbon dioxide accumulating in the Earth's atmosphere. But to most plants, which use carbon for photosynthesis, the amount we have is not enough.

Far back in Earth's history, the atmosphere is thought to have contained a thousand times as much carbon dioxide as it does today. Over the years, photosynthetic bacteria, and later, algae and plants, gradually consumed the carbon dioxide to a point where it now makes up just a tiny fraction of air. In today's atmosphere, many plants, including most crops, are literally starving for carbon dioxide.

But some of these enterprising organisms have come up with ingenious ways to overcome this limitation by siphoning carbon dioxide from the air and concentrating it for use in photosynthesis. These carbon-concentrating mechanisms make algae and a handful of land plants able to grow faster than they otherwise would. Could scientists learn the secrets of carbon-concentrating mechanisms so that they can engineer crops to grow more quickly?

ILLUSTRATION BY YASEMIN SAPLAKOGLU



Researchers are exploring how single-celled algae like the one pictured are able to grow so quickly. The secret of this growth stems from a structure called the pyrenoid, which concentrates carbon dioxide from the air to make photosynthesis run faster. By learning the secrets of algae's success, the researchers hope eventually to engineer the faster growth of crops such as wheat and rice. Opposite page: A cryo-electron micrograph of the pyrenoid.

One scientist who thinks this may eventually be possible is Martin Jonikas, an assistant professor of molecular biology at Princeton. He and his team are trying to reverse engineer the carbon-concentrating machinery in algae to find out how it works, with the idea that researchers could apply some of these tricks to crops.

Jonikas' companion in this quest is a single-celled, freshwater alga named *Chlamydomonas reinhardtii*, which grows naturally in ponds and lakes throughout eastern North America and is known to the plant biology community by the nickname "Chlamy." In Jonikas' lab, Chlamy is the main character. Deep green soups of Chlamy fill the flasks that sit in the laboratory incubator. Plastic plates dotted with Chlamy colonies are stacked on the laboratory benches. This unassuming alga plays the starring role in the researchers' mission to understand the molecular machinery that enables carbon concentration.

"Chlamy is evolutionarily related to higher plants, so most of what we learn from it still applies to them," Jonikas said. "But because Chlamy is a single-celled organism, we can work with it much more easily and much more rapidly than we can with other organisms."

From the sky to the lakes

That Jonikas studies molecular machines for a living is no surprise. Since childhood he has been fascinated with machines, especially those that fly. As a child, he would build remote-controlled airplanes and spend his afternoons chasing after them. He followed this fascination by studying aerospace engineering at the Massachusetts Institute of Technology.

His career took an unexpected turn late during his undergraduate years. "An inspiring biology professor opened my eyes to the concept that living organisms are actually complex machines that can be engineered," he said. He soon transitioned from aerospace engineering to pursuing a Ph.D. in biochemistry and molecular biology at the University of California-San Francisco. One day, a colleague gave a talk on biofuels — fuels produced from plants. "It got me interested in biofuels, but more generally it opened my eyes to the importance of photosynthetic organisms to life on Earth," Jonikas said.

By 2010, Jonikas had earned his Ph.D. and had started his own laboratory at the Carnegie Institution for Science's Department of Plant Biology, located on the campus of Stanford University. He began studying the molecular machinery that makes photosynthesis possible using Chlamy as a model, and moved his laboratory to Princeton in the fall of 2016. Soon after his arrival, Jonikas was named a Howard Hughes Medical Institute-Simons Foundation Faculty Scholar.

Alga as muse

In his office at Princeton, Jonikas picks up an oval stuffed animal from the windowsill. It's a green, plush version of Chlamy. It has a red dot representing the part that the alga uses like an eye, two antenna-like protrusions that the alga uses for movement, and a silver spot on its abdomen where carbon is concentrated. Jonikas' lab members had the toy made by a craftsperson they found online.

Pointing to the circle of silver cloth sewn on its front, Jonikas says it represents a structure inside the cell called the pyrenoid.

He explains that all photosynthetic plants, including algae, use an enzyme called Rubisco to "fix" carbon dioxide as the first step in converting carbon into sugars that the plant can use for growth. But in most plants, Rubisco does not work at maximum capacity because there isn't enough carbon dioxide to keep it running at full steam. "Humans have been working for decades to make Rubisco run faster, and nature has been working on it for much longer than we have, but so far, neither has been successful," Jonikas said.

Many plants — including rice and potatoes — have tried to deal with this problem by making huge amounts of the enzyme. As a result, Rubisco makes up nearly half of all the protein in the leaves of many plants, making it the most abundant protein on Earth. But this strategy only goes so far, because making Rubisco uses up resources that plants could otherwise use for growth.

Algae and some other fast-growing plants like corn have found other solutions. They use carbon-concentrating mechanisms to suck carbon dioxide from the environment and force-feed it to Rubisco. This solution allows Rubisco to run at maximum speed, leading to faster growth.

Chamy's solution is to crowd its Rubisco into the pyrenoid, ensuring that high amounts of carbon dioxide come in contact with Rubisco enzymes. The pyrenoid remains something of a black box, which is what makes it so interesting to study, Jonikas said. "Researchers have been very limited by the few tools that are available for studying algae, so we know almost nothing about the protein composition of the pyrenoid, or even how it functions," he said. "Even the simplest of experiments reveals a lot of interesting new biology."

Learning how nature builds a pyrenoid, so we can too

One of the key questions has to do with understanding the structure of the pyrenoid. Without a basic understanding of these organelles, building one from scratch in a higher plant seems like an unachievable dream.

Years ago, scientists who took some of the first electron microscopy images of the pyrenoid concluded that the structure was a crystalline solid. But something wasn't adding up for Jonikas and his colleagues. They observed that when algae reproduce — which they do by dividing in half — the pyrenoids also usually divide, with a portion going to each daughter cell. But how could they do this if the structure is so rigid?

To explore this question, Elizabeth Freeman Rosenzweig, then a graduate student, and Luke Mackinder, then a postdoctoral researcher in the lab, attached a fluorescent protein called Venus to Rubisco enzymes in some Chlamy cells. In each cell, this fluorescently labeled Rubisco made the pyrenoid light up. The researchers used a high-powered laser to turn off the fluorescence in one half of the pyrenoid and observed what happened to the fluorescent Rubisco remaining in the other half. Within minutes, some of the fluorescent Rubisco had moved to the dark side, indicating that the contents of the pyrenoid were mixing like a liquid.

The finding that the inside of the pyrenoid is liquid-like, published in a September 2017 issue of the journal *Cell*, is a step in the direction of transferring the carbon-concentrating mechanism to higher plants, said Freeman Rosenzweig, who recently earned her Ph.D. in Jonikas' lab. "If we can figure out how nature does it, maybe we can engineer it."

In another set of experiments, Mackinder and colleagues identified a protein that they think serves as a molecular glue that holds Rubiscos in the pyrenoid. This protein, which they named Essential Pyrenoid Component, or EPYC-1, appears to be necessary for packaging Rubisco into the pyrenoid. Another study, published in the same issue of *Cell,* revealed 89 new pyrenoid proteins and gave the most detailed look yet at how the pyrenoid is structured.

To learn more about what is going on in the pyrenoid, Jonikas and his team are looking at the genes involved, starting with those involved in concentrating carbon. To do that, the researchers created thousands of Chlamy strains, identical except for the fact that each one has a different single gene disrupted in its genetic code. Each one of these cells is a mutant.

"To study the function of a gene, you can make its mutant — a strain that lacks that gene — so that you can watch how the mutant performs differently than the original organism," said Xiaobo Li, an associate research scholar in Jonikas' lab. "But in algae, for many years, we only had a few mutants so we had no idea what most genes were doing."

Li led the development of a "mutant library" that helped the team study the pyrenoid, revealing dozens of candidate pyrenoid proteins. The library is also helping researchers around the world, who frequently request mutants that they can use to do their own research. "This is the first genome-wide collection of mutants in any single-celled photosynthetic organism," Jonikas said. In the past year, through a collaboration with the Chlamydomonas Resource Center at the University of Minnesota and funding from the National Science Foundation, Jonikas and his team have made available over 2,300 mutants to nearly 200 labs around the world.

Martin Jonikas, assistant professor of molecular biology, studies how algae are able to grow so quickly, with the goal of eventually engineering faster growth of crops such as wheat and rice.

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Xiaobo Li, an associate research scholar, extracts frozen trays of algae from a storage vessel filled with liquid nitrogen. The trays contain individual strains of algae that have been mutated so that each strain lacks a single gene, allowing researchers to study how the loss of that gene affects growth and other functions.

When Jonikas first moved his lab to Princeton, he sat down with Ned Wingreen, the Howard A. Prior Professor in the Life Sciences and professor of molecular biology and the Lewis-Sigler Institute for Integrative Genomics. Wingreen, whose team uses computational models to study biological systems, remembered that within 10 minutes both professors had an “aha!” moment.

Wingreen was working on how different polymers could undergo phase separations, such as when steam condenses into water droplets. He became interested in pyrenoids after speaking with Jonikas. His team created a simulation of Rubisco and the linker protein that glues Rubiscos together. The researchers found that the number of binding sites on both the Rubiscos and the linker proteins precisely dictated whether they were condensed into a liquid-like structure, like the pyrenoid, or dissolved into the surrounding chloroplast, as happens during cell division. The work provided a possible explanation for how the pyrenoid can rapidly switch between the two forms.

“Martin has been a tremendous force in the field by taking the modern tools of genomics and adapting them to this understudied organism,” Wingreen said. “He has this wonderfully powerful and motivating vision that what we learn from Chlamy may eventually be immensely beneficial for society.”

The dream

Jonikas and his team have a vision of meeting the world’s rising food demand by engineering pyrenoids into crops such as rice and wheat, so the crops can feed on carbon as efficiently as Chlamy does.

In parallel to studying the genes of the pyrenoid to explore how it photosynthesizes so efficiently, the team has already begun early efforts to inject Rubisco and other algal proteins into higher plants. In collaboration with Alistair McCormick, a molecular plant scientist, and his team at the University of Edinburgh, the researchers found that most of the carbon-concentrating proteins taken from Chlamy traveled to the correct location in tobacco when injected into the plant, as published in the journal *Plant Biotechnology* in 2015.

"These two organisms are a billion years evolutionarily diverged and yet the little ZIP codes on the algal proteins that point them to their destinations still work in higher plants," Jonikas said. "They know where to go in a new land."

Jonikas and his team hope in the coming years to successfully identify and transfer enough components of Chlamy's carbon-concentrating machinery to produce a functional pyrenoid in higher plants and increase crop yields.

On Jonikas' windowsill, propped up next to the Chlamy, are other plush toys in the shape of crops — a pea pod, a cabbage, a carrot and stalk of wheat. These characters starred in a video that Jonikas' team created and posted online to explain their research to a broader audience. In the video, the other plants make fun of Chlamy for being different, but when they realize that Chlamy is so good at photosynthesis, they come to respect the lime-colored alga. From Chlamy, they learn the secrets of carbon concentration and become fast-growing crops. Perhaps, someday, this story will come true.

Researchers grow algae for experiments aimed at discovering how the single-celled aquatic plants are able to concentrate carbon dioxide and thus grow more quickly than most land plants.

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