YALE JOURNAL OF BIOLOGY AND MEDICINE 92 (2019), pp.561-564.

INTERVIEW

YALE JOURNAL OF BIOLOGY AND MEDICINE
YJBM
CELEBRATING 90 YEARS 1928–2018

Insights from Algae on Engineering High Efficiency Plants

An Interview with Martin Jonikas, PhD

Halbert Bai[a,*] and Ian J. Gonzalez[b,*]

[a]*MPH Candidate, Yale School of Public Health, New Haven, CT;* [b]*PhD Candidate, Department of Cell Biology, Yale School of Medicine, New Haven, CT*

HB: Would you mind telling us a bit about your personal and professional background?

I had a somewhat unconventional path coming into plant biology. Growing up I was sure I was going to be an engineer, so in college I majored in aerospace engineering. My world was turned upside-down in my junior year when as part of a graduation requirement I was forced to take a molecular biology class. In that class I had a really fascinating professor who blew my mind about biology. Her name was JoAnne Stubbe, and the class explored ribosomes, fatty acid synthase, and polyketide synthases. Learning about those molecular machines got me very excited about biology.

I became very interested in aging research. I went to U.C. San Francisco to pursue a PhD where I wanted to work with Cynthia Kenyon, an inspiring leader in aging research. UCSF had a mandatory rotation system where I got to explore several fields before choosing a lab for my PhD. I ended up doing my PhD in yeast high-throughput genetics working with two amazing scientists, Jonathan Weissman and Peter Walter. We used high-throughput genetics to discover new genes with roles in protein folding in the endoplasmic reticulum.

Towards the end of grad school I again had a change in career direction when I became very excited about photosynthetic organisms. I think this was in part because UCSF is a medical school where we had no exposure whatsoever to photosynthetic organisms. I became excited by how important they are to how the planet works, and I wanted to learn more about them.

And then, something incredible happened: I was given the opportunity to start my own lab directly out of grad school at the Carnegie Institution for Science's Department of Plant Biology. This opportunity was totally crazy, especially considering that I didn't know a thing about photosynthetic organisms. I am forever grateful to the Carnegie faculty and their director Wolf Frommer for taking that chance on me.

Given that I didn't know anything about photosynthesis, the first challenge was figuring out what my lab would do. The basic idea I came up with was to apply yeast-style high-throughput genetics approaches to photosynthetic organisms. In this case, it was using the model eukaryotic unicellular green alga Chlamydomonas (known colloquially as "Chlamy") as a "green yeast."

*To whom all correspondence should be addressed: Halbert Bai, Yale School of Public Health, New Haven, CT; Email: halbert.bai@yale.edu; Ian J. Gonzalez, Department of Cell Biology, Yale School of Medicine, New Haven, CT; Email: i.gonzalez@yale.edu.

†Abbreviations: O_2, oxygen; CO_2, Carbon Dioxide; Chlamy, Chlamydomonas; FRAP, fluorescence recovery after photobleaching; HB, Halbert Bai; IG, Ian Gonzalez.

Keywords: Algae, Chlamy, Carbon Dioxide, Condensates, Photosynthetic, Pyrenoid, Rubisco

That turned out to be a good approach because we could immediately start contributing, and at the same time it gave me a little time to learn about photosynthesis and figure out what particular aspect we would pursue in molecular detail. Along the way we got interested in pyrenoids[1], and this became the second focus of the lab.

HB: Your lab has made an incredible resource in the groundbreaking algal mutant library. How do you envision other researchers will use this resource?

I think the vast majority of the use of the library so far has been to enable the study of individual genes in Chlamy. Half of the most highly conserved genes in photosynthetic organisms are completely uncharacterized. People are interested in these and other uncharacterized genes, but it has been very hard to obtain mutants for them in a single-celled model organism. That's where a lot of the mutants will be useful.

Chlamy is also a great model organism for cilia, and it has made a number of fundamental contributions to that field. A lot of our mutants are being used to study the structure and function of cilia. For example, our colleagues often want to know which proteins correspond to which part of a particular cilia structure. To figure this out, they can compare the wild type structure to the structure from a mutant that lacks a candidate protein. Often, a portion of the structure will be absent in the mutant, which suggests that the missing protein may be present in that portion of the structure or is required for the formation of the structure.

Before this resource was available, it was very difficult to study the function of any gene of interest. Now that there is a library available, it's easy to order up a mutant in almost any gene. The resource is also synergistic with the recent advent of CRISPR, which allows generation of mutants in genes that are not currently covered by the library.

HB: Could you discuss how your research breakthroughs in algae could be implemented to increase crop yields?

I think our work on algae could someday contribute to engineering crops that make more food with fewer resources. It turns out that many of the major global crops like rice and wheat are starving for carbon. Algae have figured out a mechanism to assimilate carbon more effectively, and we think that transferring this mechanism into crops could improve crop yields.

Plants get their carbon from atmospheric Carbon Dioxide (CO_2), which the plants turn into sugar. The enzyme that fixes CO_2, Rubisco, runs very slowly for an enzyme in central carbon metabolism. In most plants, it catalyzes only about three reactions per second. We think this slow activity is due to its very interesting evolutionary history. It probably worked quite well when it evolved about 3 billion years ago, because at that time CO_2 was much more abundant and there was no oxygen in the atmosphere. Since then, photosynthetic organisms have changed our atmosphere dramatically. They sucked out almost all the CO_2, to the point where CO_2 is now quite scarce, only about 400 parts per million, and also they produced a massive amount of oxygen. In today's atmosphere, the low abundance of CO_2 makes Rubisco run very slowly.

Martin Jonikas, PhD, Assistant Professor of Molecular Biology, Princeton University

It turns out that algae have evolved a mechanism to assimilate CO_2 more efficiently than many land plants. Essentially, they take CO_2 at a low concentration outside the cell and pump it to generate a locally high concentration of CO_2 around Rubisco in a structure called the pyrenoid, and this allows the Rubisco to run faster. As a result of their Rubisco running faster, the algae grow faster and also require less nitrogen per unit of carbon biomass. We think that if we could transfer this mechanism from algae into higher plants, this might help crops grow better and also require less water and less nitrogen fertilizer per unit biomass produced.

IG: You mentioned Rubisco and how it developed and came into biology billions of years ago. If it's had billions of years to become a better enzyme, why do you think Rubisco is still a poor-functioning enzyme? Is there no pressure for it to be better because of the

[1]Pyrenoids, as Dr. Jonikas will discuss in more detail later in the interview, are structures found within algae that locally concentrate CO_2 and increase the enzymatic rate of Rubisco, an essential enzyme in carbon fixation/assimilation during photosynthesis.

abundance of sunlight or water?

I think that's a very good question. People have dedicated whole careers on trying to design a better Rubisco, without success. Nature has been working on the same problem for much longer than us, and also has not been successful. So it looks like there is some fundamental challenge that is not easily overcome.

One of the interesting things is that you can actually make a faster Rubisco, and there are Rubiscos out in nature that run faster. The problem is that Rubisco pays a heavy price for running faster, and the price is that the Rubisco reacts with oxygen more. It turns out that molecular oxygen (O_2) looks a lot like CO_2, so Rubisco sometimes mistakenly catalyzes a reaction with O_2 instead of with CO_2. In most plants today, Rubisco makes this mistake quite often, about one in four reactions catalyzed by the enzyme are with O_2. And every time that Rubisco reacts with O_2, it produces an unwanted product that must be metabolized in a process that results in the loss of CO_2. If the Rubisco reacted with O_2 much more than it does already, the plants would lose more carbon through Rubisco than they gain. So, it looks like in all photosynthetic organisms the Rubisco has been optimized to be as fast as possible while still being net selective for CO_2 over O_2 in the environment in which that Rubisco operates.

It looks like this tradeoff is inherent to Rubisco's catalytic mechanism. To get away from the tradeoff, plants would have to switch to a different enzyme, which they can't do because Rubisco is so central to carbon metabolism. Instead, the way that most plants have found to deal with Rubisco's slow catalytic rate is that they just make tons of Rubisco. So much that it's estimated that half the protein in the leaves of many plants is Rubisco, and this has led to the estimates that Rubisco is the most abundant enzyme on the planet.

Rubisco's abundance explains why we think that if we could enhance CO_2 uptake of plants, we would reduce their nitrogen requirements. Rubisco makes up so much of the protein in the plant that a lot of the nitrogen that the plant takes up goes to making Rubisco. If you could enhance CO_2 uptake by putting Rubisco in a pyrenoid and feeding it with concentrated CO_2 like algae do, you'd need less Rubisco and thus less nitrogen per plant.

IG: Why don't all plants have a pyrenoid – is this a stochastic event that happened to algae?

Nearly all algae have a pyrenoid, but only one group of land plants, the hornworts, has them. It's not clear why this is the case.

My personal bet is that this is more of an evolutionary history issue: CO_2-concentrating mechanisms – of which the algal pyrenoid-based mechanism is an example – may not have been necessary or valuable for land plants until very recently in evolution. Atmospheric CO_2 levels are thought to have been high in the early Earth's atmosphere and then they've dropped over time to present day levels. It's only about 50 million years ago that atmospheric CO_2 levels dropped down to levels so low where operating a CO_2-concentrating mechanism becomes worthwhile in a land plant. 50 million years is a very short time evolutionarily, so it's possible that most plants don't have a CO_2-concentrating mechanism simply because they haven't had time to evolve one.

In contrast, in the aquatic environment like in the oceans, because CO_2 diffuses more slowly in water, it's much easier for an organism to deplete its local environment of CO_2. So we think that aquatic organisms would have experienced a shortage of CO_2 much earlier in their evolutionary history and would have benefited from a CO_2-concentrating mechanism much earlier, giving them more time to evolve one. Additionally, much of the photosynthesis in the oceans is done by unicellular organisms, which appear to be able to share evolutionary innovations through horizontal gene transfer more rapidly than multicellular organisms like land plants. The ocean is one giant vat of liquid so if you have an evolutionary innovation like the pyrenoid it can take over the entire ocean pretty easily.

Another thing to consider is that there might be a lot of constraints on how CO_2-concentrating mechanisms evolve. Evolving such a mechanism involves tinkering with carbon assimilation, which is essential to growth. There may not be many workable evolutionary paths where each iteration is slightly more fit than the previous one. In land plants, we see that a different kind of CO_2-concentrating mechanism called the C4 mechanism has evolved many times. But it appears that only certain species can evolve this C4 mechanism. These species start out with a particular leaf vein structure that is unrelated to CO_2 assimilation but is a pre-requisite for evolving a C4 mechanism. It's possible that this kind of mechanism evolves because this is one of the few evolutionary paths to CO_2 concentration available to plants. With synthetic biology, we have the opportunity to transfer evolutionary innovations across species in new ways that are not constrained by the fitness of intermediate species or the recalcitrance of plants to natural horizontal gene transfer. All of this gives me hope that a pyrenoid might be engineered into higher plants to make crops that produce more food with fewer resources.

IG: Your work interfaces with the really hot topic in cell biology of phase separation and biomolecular condensates. Can you tell us a little bit about the background of this field and how your findings are intersecting with this field as well?

I learned of the field of biomolecular condensates when I was teaching a graduate class at Stanford. One of the papers that we read for this class was a now-classic paper from 2009 by Cliff Brangwynne, which introduced the concept of phase-separated liquid-like structures within the cell. The authors looked at *C. elegans* P granules and found that they showed behaviors that were similar to liquid droplets, mixing internally, fusing, and undergoing phase transitions. That paper was really exciting to me and led us to hypothesize about the possibility that the pyrenoid may be such a phase-separated organelle because we knew that it was spherical and not surrounded by a membrane.

We and the field had previously assumed that the pyrenoid was solid, probably in large part because everyone had been looking at it in electron micrographs of fixed cells. There was also some literature suggesting that the pyrenoid is crystalline, because one could see crystalline arrays of particles in the pyrenoid of some species of algae.

A really talented postdoc in the lab Luke Mackinder – who's now running his own lab at the University of York – was the first to do a key experiment that suggested to us that the Chlamy pyrenoid is indeed one of these liquid-like phase-separated structures. If the pyrenoid behaved as a liquid, we would expect it to mix internally. Rubisco is the main protein component of the pyrenoid, and Luke had made some Chlamy that were expressing fluorescently-tagged Rubisco. To test whether the pyrenoid mixes internally, Luke did a fluorescent recovery after photobleaching (FRAP) experiment, where he bleached half the fluorescence of the pyrenoid and he observed that the remaining fluorescence mixed throughout the pyrenoid on a timescale of seconds. That's the classical behavior of these phase-separated organelles, so that was a really good hint that we were onto something.

Liz Freeman Rosenzweig, who was a graduate student in my lab at the time, went on to confirm these results and really develop the story into something we could publish. She found that pyrenoids divide by fission, which is quite unusual for phase-separated organelles and might be the first example of a phase-separated structure that divides by being pinched in half during division of the surrounding compartment. Also, she discovered that the pyrenoid dissolves into the surrounding chloroplast stroma during every cell division. We then collaborated with Ben Engel's group at the Max Planck Institute who looked by Cryo-EM tomography at the pyrenoid in Chlamy and saw that the arrangement of Rubiscos in the pyrenoid was not crystalline. Also, with Ned Wingreen here at Princeton, we explored some ideas of the polymer biophysics that underlie the pyrenoid's phase separation.

The realization that the pyrenoid is a phase-separated organelle has helped us both in thinking about how to engineer this organelle into higher plants and also in thinking about how it works from a basic biology perspective. We're hoping that some of things that we learn about how the pyrenoid works might also contribute more broadly to the field of phase-separated organelles. One example of this was our discovery that cells can dissolve a phase-separated organelle throughout a compartment to increase the chances of even inheritance of its components as that compartment divides, the organelle being the pyrenoid and the compartment being the chloroplast in this case. We think that this could be a general principle that might be at work in other biological systems or could be leveraged in an engineered system. There may also be other surprises that the pyrenoid holds, which could inform us more generally on how phase-separated organelles work or how we could engineer them in the future.