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Interview

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Molecular Time Travel to the Dawn of Photosynthesis

In this interview, Lead Scientists Prof. Dr. Tobias Erb and Dr. Georg Hochberg together with First Author Luca Schulz explain why knowing the history of proteins might help create alternatives for the future.

In order to understand how photosynthesis adapted to the presence of oxygen billions of years ago, you resurrected long-extinct enzymes in the lab. What was the starting point for this research?

Tobias Erb: Studying the mechanisms that allow enzymes to capture and convert CO_2 is at the heart of research in our lab. But how did these powerful CO_2-converting catalysts emerge and adapt to dramatic changes in the environment over billions of years? In paleontology we can make use of fossilized bones to understand the evolution of species, like us humans. However, there is no "molecular fossils" of proteins that we could use to learn about their evolution. Meeting Georg [Hochberg], who is an expert in reconstructing the history of proteins over billions of years opened the exciting possibility to finally go back in time to reconstruct ancient Rubisco versions. Through this molecular time travel, we can now retrace the key steps in the history of Rubisco and understand how this enzyme became the dominant CO_2 capturing machine on earth that converts more than 1.25 billion tons of CO_2 per day.

Georg Hochberg: For me, it was one of those career changing moments when Tobi and I first met. It became immediately clear to me that there was a unique opportunity to do something significant on the evolution of these very important CO_2 fixing enzymes. It was as if I found the perfect nail for my methodological hammer. Everything else just fell into place afterwards, not least because we could convince a very talented graduate student to work on this question.

Luca Schulz: I was immediately hooked on the idea of re-tracing the evolution of Rubisco, when Tobi and Georg first pitched the project to me. A combination of Georg's methodologies and Tobi's knowledge of carbon-fixing enzymes seemed like the perfect opportunity for me to conduct cutting-edge research on a topic I cared about. Focusing on Rubisco seemed like the perfect model system for trying to understand the mechanisms of protein evolution, given that the protein has been under immense selective pressure for billions of years. In the end, I was in the fortunate position to be co-supervised by two great mentors that gave me all possible freedoms to explore project ideas and pursue any idea I could come up with.

The proteins you are studying have long since disappeared on earth. Why is this relevant for us humans today?

Tobias Erb: Well, every bite of food that you take, every carbon atom that you eat is basically CO_2 that was fixed through Rubisco. This enzyme literally feeds all life on this planet. I believe

that there is some value in understanding how this essential process on earth evolved. One lesson is that evolution apparently tends to build more complex machines with time. Understanding the molecular principles behind this process tells us about the potential, but also the limitations of evolution as an optimization force. There is another angle to the story: we can try to use this knowledge to replay evolution and guide it into another direction. So unlike the famous quote of Robert Penn Warren who said: “History cannot give us a program for the future”, there is at least some tools we can use to create an alternative future starting from these ancient proteins.

Georg Hochberg: Even if the past may hold no plan for the future, it does help us understand the present. All the biology we see around us, including enzymes like Rubisco, are the result of an eons long evolutionary process. Just in the same way that all organisms or also human societies, enzymes have accumulated historical idiosyncrasies.

Understanding their histories can help us sort features of enzymes that are actually useful to their functions from those that are simply coincidental.

Luca Schulz: In this context, let me give you an example from our work: We were only able to measure the impact that a new protein component had on Rubisco, because we looked at it in ancestral proteins. In extant proteins, such an analysis is near impossible, because Rubisco does not function properly anymore as soon as you remove the novel component. Over the course of evolution, Rubisco started to depend on its novel component. As such, studying long-gone proteins can help us circumvent confounding factors that complicate the analysis of individual aspects of a protein.

How do you resurrect an ancient protein?

Georg Hochberg: It’s a bit like studying nieces and nephews to learn about grandparents: Information about the sequences of extinct proteins is still encoded in the sequences of their descendants that are still alive today. Practically, we first build a family tree for whatever protein we are interested in. This tree is based on the dissimilarities and similarities of the protein sequences of different Rubiscos. Ones with a more similar sequence are closer to each other on that family tree than more dissimilar ones. Once we have the tree, calculating the ancestral sequences is almost trivial: We effectively run the tree inference algorithm in reverse to infer sequences of ancestral proteins. But I’m making this sound a bit easier than it really is: The real challenge for this kind of ‘molecular paleontology’ is to get an accurate protein family tree, which is by no means trivial.

Luca Schulz: What made it possible in our case is that Rubisco is a very widespread protein. Many sequenced organisms encode Rubisco genes, which means that many Rubisco sequences are available in public databases. That allowed us to build a very large family tree for this protein, which meant that we could be more certain in the sequences of extinct Rubiscos than we would be for many other enzymes.

Tobias Erb: The last step of this process is to recreate these ancient proteins in the lab. This ‘resurrection’ of ancient proteins technically very simple and uses synthetic biology techniques that are very much routine by now: you produce the ancient protein according to a genetic “blueprint” in bacteria. It is almost like 3D printing an ancient object with a biological “printer”. Once you have that, you can purify this protein and measure its various biochemical properties.

What was the greatest challenge here?

Luca Schulz: I think one important challenge was to resurrect a form of Rubisco that can already interact with its new subunit but doesn’t yet need it to be active at all. This result

allowed us to actually figure out exactly how Rubisco's new protein component influenced its function. To do this, we had to identify which historical mutations caused Rubisco to interact with the subunit and which made it become more specific for CO2. This was very difficult, because these mutations were only a small subset of the many changes that happened in Rubisco around the time this new subunit appeared. Finding them was a bit of a combinatorial nightmare: we had to try dozens of different combinations of mutations before we zeroed in on just the crucial mutations. But it was worth it: we found that the small subunit functions as an "evolutionary modulator" that awakens otherwise inconsequential mutations, which have no effect on their own!

Georg Hochberg: This was a project with many moving parts and challenges. On top of Luca's points, another great challenge was to understand the mechanisms by which important mutations changed Rubisco's properties. Rubisco is an enigmatic beast: Structurally, Rubiscos with wildly different biochemical properties look almost the same. That means that all biochemical differences between them must be caused by very subtle mutations. Understanding how just a few mutations make Rubisco completely dependent on its new interaction partner, for example, was a difficult challenge that took us two years to overcome. In the end, the mechanism we discovered is very elegant, but for a long time we were chasing nothing more than a hunch.

How could we theoretically use this knowledge to boost photosynthesis?

Georg Hochberg: Evolution is a tinkerer. It uses what parts it has available to improve what's already there, rather than building its solutions from the ground up like an engineer would. That means that its solutions aren't always optimal. But we can still learn from this erratic process. In this particular case, we learned a new principle for how the properties of Rubisco can be changed: through addition of an evolutionary modulator like its new subunit. I would wager that there are other modulators that we could add to this protein that might change Rubisco's properties even more drastically than the one it acquired historically. This is where we evolutionary biologists must look to synthetic biologists to turn our insights into useful technologies.

Tobias Erb: We know that plants suffer from the very slow CO_2 conversion speed of Rubisco and the enzyme's poor discrimination against O_2. This makes Rubisco a prime target for any efforts to improve photosynthesis and crop yield. So far, scientists mainly focused on modifying Rubisco itself by changing the enzyme's core to improve its catalysis. However, this has only met with limited success. The surprising discovery of our study is that you can improve Rubisco by simply adding additional protein components that 'activate' otherwise silent mutations in the protein. This "evolutionary modulation" of Rubisco through the small subunit gives a completely different perspective on future engineering efforts. Imagine that we do not have to modify the core of the enzyme itself, but screen for new modulators that allow us to unleash the full catalytic power that is already present, but hidden in the enzyme's core. This is a completely novel road that no one has taken so far.

Luca Schulz: I think our work also has an interesting implication about how we might reverse engineer Rubisco to be simpler and more efficient: Our research shows that adaptations that evolve as a response to environmental changes can quickly become essential and then cannot be lost anymore. Rubisco is famously "cluttered", in that many organisms require a whole army of accessory proteins for correct Rubisco assembly, regulation, and maintenance. It is a genuine possibility that at least some of these accessory proteins may have evolved and become essential as a response to an ancestral environmental change that no longer reflects current selection pressures. Our line of research can help us understand the contributions and

adaptive values of this complexity and may help improve photosynthetic organisms by de-cluttering the “Rubisco-some” that is currently required for proper function of this vital enzyme.

Why is this research important in the context of climate change?

Tobias Erb: Our research has two important aspects. First it explains, how Rubisco, the biocatalyst that captures 95% of the CO_2 on earth, 400 billion tons CO_2 per year, has evolved and adopted towards environmental changes over the billions of years of evolution. One dramatic challenge was the rise of oxygen on ancient earth, which has put enormous pressure on the enzyme. We now know, that from the beginning the enzyme was prepared to deal with oxygen because it had already recruiting its new interaction partner even before there was any oxygen around. Knowing these principles at work, we can try to make predictions about future scenarios and answer the question whether we can overcome the current limitations of Rubisco. Remember, that the enzyme is (still) surprisingly slow and can tolerate, but not fully discriminate against oxygen. And this brings me also to the second aspect of our research, which is more applied. We can now think about replaying the tape of evolution with our ancestral proteins provide it with completely novel interaction partners to improve the enzyme’s catalysis and eventually overcome the natural limitations.

Georg Hochberg: I think there is another dimension to our findings: Rubisco’s ability to tolerate oxygen so early was most likely accidental. For now, it looks like it did not evolve in response to rising oxygen levels, but rather it was a lucky coincidence that it already existed in a microbe that probably lived a relatively obscure life at the time - until its accidental capacity to fix CO_2 better in the presence of oxygen suddenly became an asset when the atmosphere changed. If we extrapolate to today, it may very well be that the biochemistry we need to tackle present and future environmental change already exists out there, in an obscure habitat where we perhaps least expect it. I think that is a very powerful argument for explorative biochemistry and microbiology.

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