E-DRIVE FOR LIFE

TEXT: CATARINA PIETSCHMANN

Every living creature has to gather materials from the environment and convert them into the materials it needs to live. Without metabolism, there would be no life on Earth. Tobias Erb, Director at the Max Planck Institute for Terrestrial Microbiology in Marburg, wants to reprogram metabolic pathways so that raw materials can be produced more sparingly and efficiently. His latest coup? A metabolic cycle driven by electrical energy.

Up to a thousand enzymes are working simultaneously in a single cell, manufacturing countless substances in parallel. However, evolution has not always taken the fastest and simplest path. Many metabolic processes occur in stages, with byproducts and waste products emerging at each step. Max Planck researcher Tobias Erb wants to simplify the winding paths. In the future, complex and expensive chemical plants might be replaced by genetically modified microorganisms and plants that use new metabolic pathways to produce important raw materials for humans. As with any journey, the main thing is planning the route. Where do I start, where do I want to go, and what is the shortest path to my destination?

Erb and his team have already found a shorter pathway for photosynthesis – the researchers have paid special attention to this process that is so fundamental to life on Earth. It consists of two reaction cycles. In the first, the "light reaction," energy from sunlight is converted into chemical energy. The second, known as the Calvin cycle, then uses this energy to form carbohydrates from carbon dioxide in the environment. In 2016, Erb and his team recreated the Calvin cycle in a lab. The artificial cycle of 17 enzymes, which the researchers dubbed "Cetch," does the same as its natural model. It converts carbon dioxide into sugar compounds, but is around twice as effective at it. "Furthermore, depending on the choice of enzymes, our metabolic pathway can manufacture not only sugar and starch, but other substances as well, such as antibiotics and proteins," says Erb. Apart from the Cetch cycle, he and his team have since developed additional alternatives to the Calvin cycle. They include the Hopac and Theta cycles, which form different raw materials from carbon dioxide.

PHOTO: TOBIAS ERB/MPI FOR TERRESTRIAL MICROBIOLOGY

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Artificial chloroplasts could someday provide cells with energy and nutrition. Researchers have filled these 0.09 millimeter droplets with chloroplast membranes from spinach plants (green). They implement the light reaction and drive an artificial reaction cycle that binds carbon dioxide.

Shanshan Luo and Tobias Erb with the reaction chamber for the AAA cycle.

PHOTO: VIRGINIA GEISEL/MPI FOR TERRESTRIAL MICROBIOLOGY

SUMMARY

Researchers are now able to recreate the metabolic cycles of cells in the lab and improve their efficiency.

The Cetch cycle is an artificial metabolic cycle that can fix carbon dioxide and convert it into carbohydrates. Cetch works much more efficiently than the natural Calvin cycle and can provide other raw materials besides sugar.

The AAA cycle can convert electricity into biochemical energy. In the future, the cycle could serve as the interface between electricity and biology and, for example, drive the Cetch cycle for carbon dioxide fixation.

Tailor-made reaction cycles such as these could therefore be used to produce a host of different raw materials. An important ingredient is still lacking, though, without which the enzyme mix cannot do its work: energy! The Sun provides it for the Calvin cycle. In the light reaction cycle, enzymes use energy from sunlight to manufacture the fuel that keeps most life processes running: an energy-rich chemical compound named adenosine triphosphate. Called ATP for short, the molecule provides energy for the Calvin cycle.

Like many reaction cycles in nature, it takes several intermediate steps to form ATP. In the light reaction cycle, an electrochemical voltage builds up along a membrane, and is then used for a protein motor, which generates ATP with the help of mechanical energy. "Electricity therefore flows in photosynthesis, but the charge separation on a membrane is very cumbersome. So, my employee Shanshan Luo and I asked ourselves whether we couldn't power our synthetic metabolism directly with electrical energy, thus linking electricity and biological systems," says Erb.

ATP from electrical current

In the summer of 2023, Erb, Shanshan Luo, and the team were able to declare success. They had developed an artificial metabolic pathway that extracts the biochemical energy source ATP from electrical current. The AAA cycle combines electricity with energy generation in living cells. The cycle consists of four enzymes derived from different microorganisms. The first enzyme, aldehyde ferredoxin oxidoreductase (AOR), is the "adapter," so to speak. The electrons are transferred from an electrode to a soluble carrier molecule, which passes them on to the AOR. At a voltage of 0.6 volts, AOR absorbs two electrons. It can use these to reduce a low-energy acid molecule to an energy-rich aldehyde. The other three

enzymes of the cycle then handle the reconversion, that is, the oxidation of the aldehyde into acid. The energy released in the process can be used to form ATP from ADP.

The central enzyme of the cycle, AOR, comes from the little-known bacterium Aromatoleum aromaticum. Researchers at the Center for Synthetic Microbiology of the Philipps-Universität Marburg were the first to successfully cultivate this petroleum-degrading microbe in a lab. Along the way, they stumbled across AOR, which now serves as the central energy converter in the AAA cycle. "It was really a pure accident that colleagues at the neighboring institute were studying precisely the enzyme we needed for the AAA cycle," says Erb.

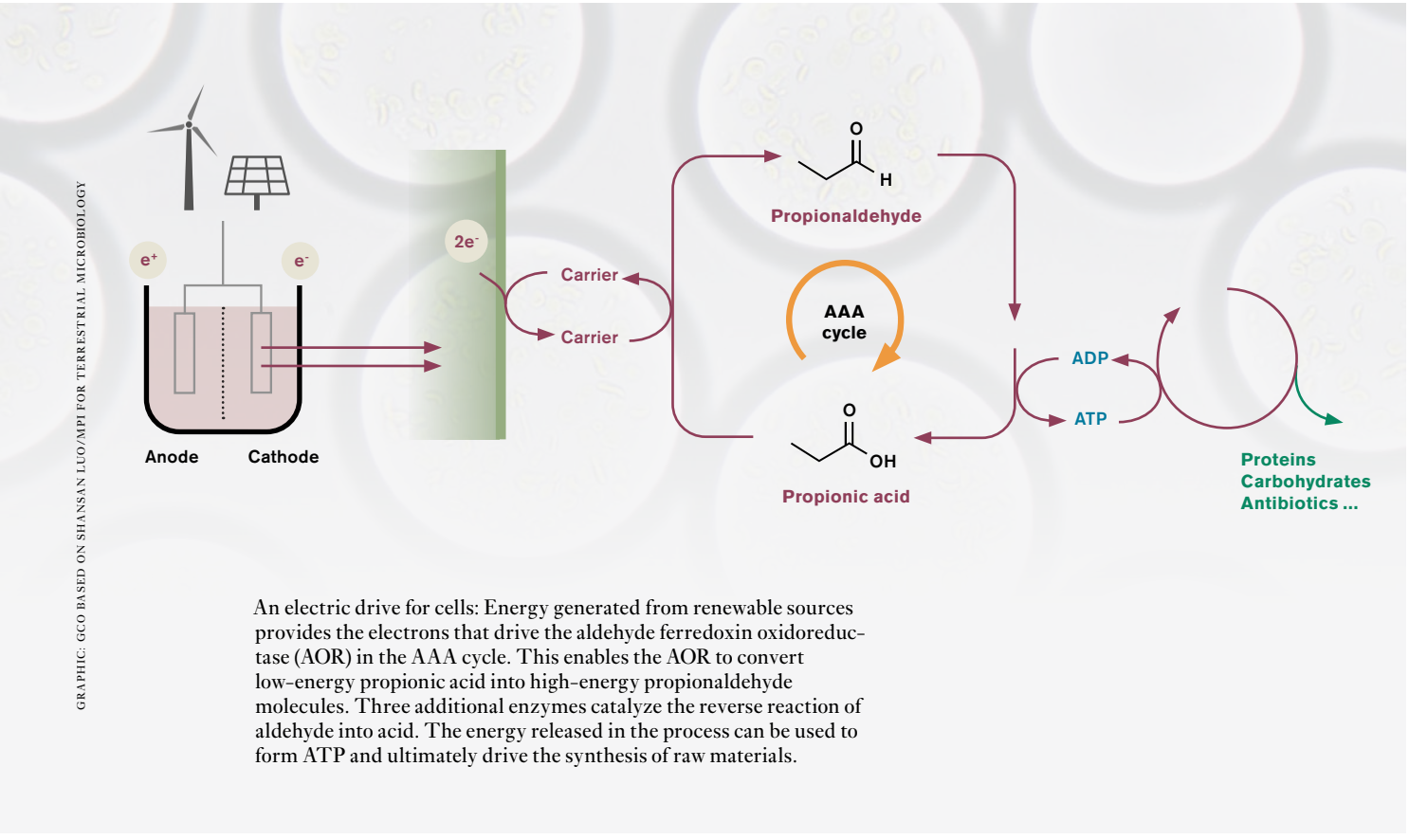
The researcher's success in converting electrical current directly into biochemical energy and then using the latter for chemical reactions is a breakthrough. To demonstrate that they can use it to manufacture more than just sugar molecules, they put the components of the cycle in a reaction vessel together with the necessary building blocks for a protein. "The whole machine rested until the current was switched on," reports Erb. "Then the AAA cycle really started driving production of the protein."

One day it might be possible to use the AAA cycle to drive the production of drugs and other raw materials, but right now it is only possible in small quantities. To make large-scale production possible in the future, the Max Planck researchers are working with colleagues from the Fraunhofer Institute for Interfacial Engineering and Biotechnology in Stuttgart to develop a prototype they can use to manufacture a perfume. Erb sees this as "an example of the interlinking of basic science and applied research."

As in the case of AOR, Erb often finds suitable enzymes in nature. "We start by checking whether there are examples in nature of the reactions we need." Microorganisms in particular are masters of an abundance of all types of metabolic processes. All multicellular animals from worms to humans are alike in terms of metabolism. In microbes, by contrast, completely different types of biochemical process are commonplace. As a result, they can survive in extreme habitats, such as the deep sea or permanent ice.

Enzymes made to measure

When researchers fail to find what they need in the kingdom of microorganisms, they fall back on existing ones, taking enzymes that fulfill tasks similar to what they are looking for and adapting them to their purposes.



GRAPHIC: GCO BASED ON SHANSAN LUO/MPI FOR TERRESTRIAL MICROBIOLOGY

An electric drive for cells: Energy generated from renewable sources provides the electrons that drive the aldehyde ferredoxin oxidoreductase (AOR) in the AAA cycle. This enables the AOR to convert low-energy propionic acid into high-energy propionaldehyde molecules. Three additional enzymes catalyze the reverse reaction of aldehyde into acid. The energy released in the process can be used to form ATP and ultimately drive the synthesis of raw materials.

Mutations change the blueprint of an enzyme in such a way that it can take on a new function. However, it often happens that the researchers seem to have all the ingredients for a functioning metabolic pathway, yet the components get along poorly and inhibit each other. "It's like when a soccer coach has to form a new team out of famous superstars. If the players don't work well together, the team won't win, despite their individual abilities," says Erb. And so it takes a lot of fine-tuning before the perfect new team is standing on the field. In the meantime, the researchers have managed to build enzyme teams of up to 70 "players" into functional metabolic pathways.

Optimization, not invention

Nevertheless, it is often easier to optimize an existing team; that is, to spice up the natural original of a metabolic cycle, such as the Calvin cycle in photosynthesis. The central molecule in the Calvin cycle is the enzyme rubisco. It binds carbon dioxide, making it usable for the metabolism of plants. "But rubisco works very inefficiently; it only absorbs five carbon dioxide molecules per second. That's really modest for an enzyme. It also makes a lot of mistakes. In every fifth reaction, it snaps up a molecule of oxygen rather than carbon dioxide," says Erb. For that reason, he decided against using rubisco in his Cetch cycle and instead replaced it with the enzyme Crotonyl-CoA carboxylase/reductase. The latter comes from a purple bacteria and binds carbon dioxide a hundred times faster than rubisco.

The researchers use automated procedures to find out where a cycle is still stumbling. This means they can analyze the material conversion of 300 variants of a cycle at the same time. A computer varies the concentrations of the different enzymes and evaluates which variants are the most efficient. These are then optimized further.

In principle, the Cetch cycle functions like its natural model during photosynthesis, but none of its 17 enzymes are identical with the ones plants use for carbon fixation in the Calvin cycle. Erb compares this with the invention of aviation. "By studying birds, people learned what laws govern flight and how a wing works. But airplanes don't look like birds. They don't have feathers and their wings don't flap. By the same token, the Cetch cycle is inspired by its biological model, but its structure is fundamentally different."

But then why didn't nature come up with the cycles that Erb and his team invented in the lab? One obvious reason is that life depends not just on maximum yield, but also on resistance to environmental influences. The Cetch cycle runs primarily in a lab. On the tundra or in tropical rainforests it would almost certainly have problems. Furthermore, nature is conservative and often tends to develop existing systems further rather than break new ground.

Erb and his team are now hoping to get the artificial metabolic pathways running in real cells. To that end, they are reprogramming the genes of microorganisms and single-celled algae to enable them to produce the necessary enzymes. The Cetch cycle could boost photosynthesis in an algae cell, for example. In photovoltaics, 20 to 30 percent of the Sun's energy can be converted into electricity, while the cell's own chloroplasts use only one percent. However, replacing the Calvin cycle entirely with the artificial Cetch cycle would be very challenging, according to Erb. "To do that we would have to remove the key component of plant metabolism, which would massively affect the way the cell functions. A much more promising approach, in my opinion, is not to swap out the entire 'operating system' at first, but to help along the natural process with a handful of optimized enzymes." The team has

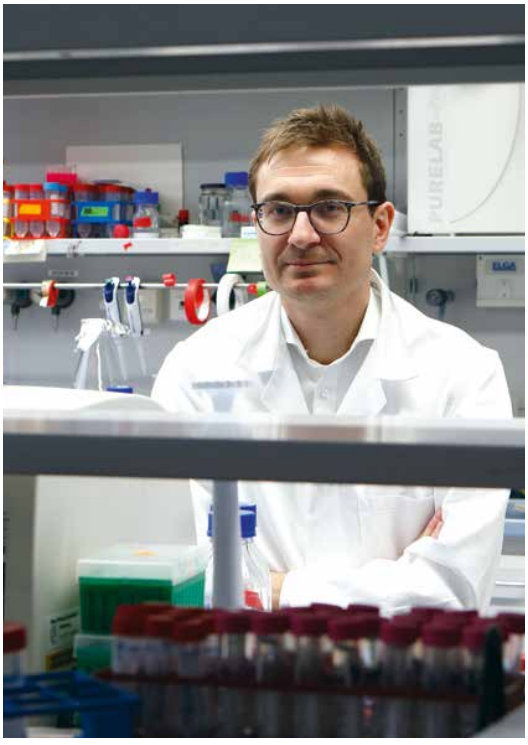


PHOTO: THOMAS HARTMANN

Tobias Erb wants to create raw materials from simple molecules. To do so, the biochemist relies on metabolic cycles based on models found in nature. They are more efficient and better for the environment than traditional chemical manufacturing processes.

PHOTO: MPI FOR TERRESTRIAL MICROBIOLOGY

Algae bind carbon dioxide with the enzyme rubisco. But, because the latter works relatively slowly and repeatedly makes mistakes, Tobias Erb's team is experimenting with other enzymes and metabolic pathways to make the reaction more efficient. Equipped with artificial reaction cycles, algae cultures could one day be used as bioreactors for converting carbon dioxide into organic molecules.

already managed to show that this form of optimization works in algae.

In Erb's view, biology today is at the same point chemistry was a hundred years ago. Back then, the world of atoms and molecules had been so thoroughly studied that researchers were able not only to recreate natural molecules, but to synthesize new ones as well: fertilizers, drugs, plastics, and much more. Today's researchers understand the material cycles in cells so well that they are able develop new ones. Biology could now take over the production of chemicals.

Photosynthesis could therefore become central to the future of humanity. Eventually, crop plants with more efficient photosynthesis would form more biomass from carbon dioxide, removing greenhouse gas from the atmosphere in the process. The result would be bigger harvests, with the additional benefit of helping protect the climate. "We need both more urgently than ever. Around 10 billion people will inhabit our planet by the year 2050. Feeding them all would require not only changing our diets and reducing food waste, but also increasing agricultural productivity. And if we can do something to help protect the climate while we're at it, all the better," says Erb.