Scientists capture 'redox moments' in living cells

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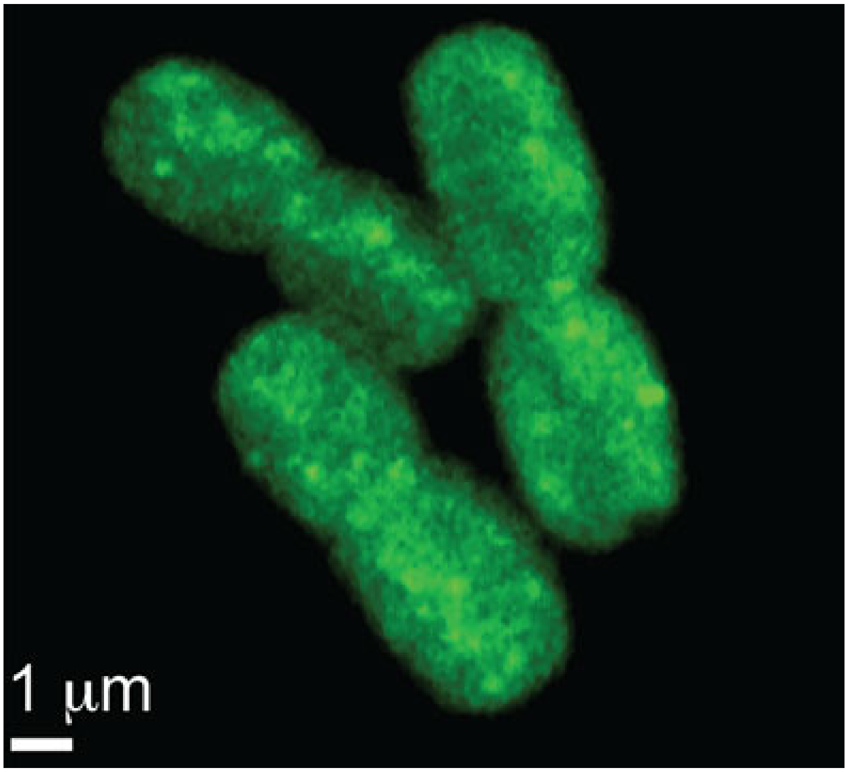

Green fluorescence shows redox reactions in living *Synechococcus* cells. Credit: Aaron Wright/PNNL

Scientists have charted a significant signaling network in a tiny organism that's big in the world of biofuels research. The findings about how a remarkably fast-growing organism conducts its metabolic business bolster scientists' ability to create biofuels using the hardy microbe

Synechococcus, which turns sunlight into useful energy.

The team at the Department of Energy's Pacific Northwest National Laboratory glimpsed key chemical events, known as redox reactions, inside living cells of the organism. The publication in *ACS Chemical Biology* marks the first time that redox activity, a very fast regulatory network involved in all major aspects of a cell's operation, has been observed in specific proteins within living cells.

The findings hone scientists' control over a common tool in the biofuels toolbox. At a more basic level, the work gives researchers the newfound ability to witness a basic biological process that occurs every moment in everything from bacteria to people.

"Redox activity tells us where the action is going on within a cell," said chemist Aaron Wright, the leader of the PNNL team whose project was funded by DOE's Office of Science. "We've been able to get a look at the redox system while it's still operating in a living cell, without destroying the cell first. This allows us to tell who the players are when the cells are engaged in the activity of our choice, like making components for biofuels."

Redox activity is one of the most powerful tools an organism has to sense and adapt to a changing environment; it's particularly active in plants that must respond constantly to changing conditions, such as light and dark.

The PNNL study was aimed at ferreting out proteins that are potential redox players in the cyanobacterium *Synechococcus*. Cyanobacteria absorb light energy from the sun and use it to convert carbon dioxide into food and other molecules, while also giving off oxygen. Redox reactions play a role in directing where the harvested energy goes.

This is *Synechococcus* in a bioreactor. Credit: Aaron Wright/PNNL

Scientists believe the organism and its plant-like cousins, including algae, were responsible for producing the first oxygen on Earth, more than 2.5 billion years ago. It's a sure bet that you have inhaled oxygen molecules produced by *Synechococcus*, which today contributes a significant proportion of the oxygen available on Earth.

The organism is attractive to scientists for a number of reasons. It's adept at converting carbon dioxide into other molecules, such as fatty acids, that are of interest to energy researchers. *Synechococcus* is easy for scientists to change and manipulate as they explore new ideas. And it grows quickly, doubling in approximately two hours. A patch just two feet wide by seven feet long – roughly the area of a typical dining room table – could blossom into an area the size of a football field in just one day.

Biofuels makers and other scientists are trying to exploit this ability to churn out quantities of materials that might serve as biofuel. *Synechococcus* is also remarkably hardy, capable of tolerating the stress caused by intense sunlight, which kills many other cyanobacteria. Redox reactions that take place throughout the organism are at the core of this ability, and understanding them gives scientists a treasured global view of how the cell lives and responds to change.

Some researchers are working to get the bacteria itself to create biofuel, growing an organism with more fatty acids that could be converted to diesel fuel. Others, like Wright, are working to understand the organism more completely, to direct the organism to create fuels using light and carbon dioxide.

Wright's team found the signals by keeping the bacteria hungry, then suddenly flooding it with food – a massive, immediate change in environment. Within 30 seconds, the team detected redox activity, which changes the way proteins operate by adding or subtracting electrons.

His team uncovered an extensive network of redox activity, identifying 176 proteins that are sensitive to signaling in this manner. Before this study, just 75 of those proteins were known to be part of a redox signaling network. The scientists found that the system is involved in all the major processes of a cell – which genes are turned on and off, for example, as well as how the cell maintains its molecular machinery and converts energy into fuel.

Central to the work are the chemical probes Wright developed that are able to cross the cell membrane and get into the cytoplasm of the cell. The probes flag redox events by binding to certain forms of the amino acid cysteine, which is a known player in many of these interactions.

Then the probes and the interactions they flag are subjected to scrutiny at EMSL, the DOE's Environmental Molecular Sciences Laboratory on the PNNL campus, where instruments detect redox activity through various means, such as through fluorescent imaging and mass spectrometry. The analysis tells scientists about when and where within the cell redox activity occurred.

"Knowing the proteins that are sensitive to redox signaling lets us know where to look as we test out new methods for working with this organism," said Wright. "We can tinker with a specific protein, for instance, and then watch the effects immediately.

"This is the type of information we really must have if we want organisms like this to produce substances that make a difference, like biofuels, chemicals or potential medicines," he added.

More information: Natalie C. Sadler, Matthew R. Melnicki, Margrethe H. Serres, Eric D. Merkley, William B. Chrisler, Eric A. Hill, Margaret F. Romine, Sangtae Kim, Erika M. Zink, Suchitra Datta, Richard D. Smith, Alexander S. Beliaev, Allan Konopka, and Aaron T. Wright, Live Cell Chemical Profiling of Temporal Redox Dynamics in a Photoautotrophic Cyanobacterium, *ACS Chemical Biology*, October 29, 2013, DOI: 10.1021/cb400769v

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