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Light-Powered Bacteria Spin Off a New Discovery

Researchers have demonstrated that a protein that captures light in ocean-dwelling bacteria is able to put that light's energy to work inside cells. The researchers, who inserted the gene for the protein proteorhodopsin into the common gut bacteria *E. coli* and watched as it powered their twirling flagella, say that proteorhodopsin's ability to harness energy from light could have a variety of practical uses.

According to one of the senior authors of the research, Howard Hughes Medical Institute investigator Carlos Bustamante, the molecule might be used to power microscopic robots composed of biological molecules. It could also render yeast or other organisms able to harness light for commercial tasks, such as producing ethanol as a biofuel, he said.

The researchers published their findings February 2, 2007, in the online Early Edition of the *Proceedings of the National Academy of Sciences*. Senior authors on the paper were Jan Liphardt and Bustamante, both at the University of California at Berkeley. First author of the paper was Jessica Walter, and the other co-author was Derek Greenfield, also both at UC Berkeley.

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The gene for proteorhodopsin was originally isolated from a sample of seawater, as part of a "community sequencing" of organisms in the world's oceans. Researchers had established that the molecule closely resembled light-capturing proteins found in the eye and in some bacteria, and that it harnessed light to pump protons across membranes, thereby storing energy.

“However, there was one key element missing,” said Liphardt. “That was to show that cells can actually obtain a benefit by expressing proteorhodopsin. Retrospectively, it sounds relatively obvious, but it wasn't clear for a long time,” he said. For example, other researchers' experiments had found that simply illuminating cells containing proteorhodopsin did not enhance growth.

To fill in that gap, Liphardt proposed inserting the gene for proteorhodopsin into *E. coli*, then treating the engineered bacteria with the chemical azide to shut down the normal respiratory energy pathway. The researchers could then measure the bacteria's energy state by observing changes in the speed at which their flagella rotated. If the researchers illuminated the engineered bacteria, theorized Liphardt, energy produced by proteorhodopsin should accelerate its flagellar rotation.

When Walter conducted the experiments, she found that the bacteria's flagella did spin faster under light. What's more, the cells became more light-responsive as she increased the amount of azide or the light intensity.

As an independent test, Walter also measured the effects of depleting the bacteria of oxygen, which should also deplete their energy and render them light-responsive. Indeed, she found oxygen-depleted bacteria showed light-dependent energy production.

Also significant, said Liphardt, was Walter's finding that proteorhodopsin's ability to supply energy saturated at about the maximum light intensity of sunshine at sea level. “That finding makes perfect sense, because in nature the bacteria wouldn't experience higher intensities,” he said.

Other researchers have found evidence that proteorhodopsin does function in marine bacteria, Liphardt pointed out. Scientists led by Jarone Pinhassi of the University of Kalmar in Sweden have reported that light stimulates growth of proteorhodopsin-containing marine flavobacteria, he said. Those findings were reported in a paper published in the January 11, 2007, issue of the journal *Nature*.

According to Bustamante, the discovery of proteorhodopsin and its installation in *E. coli* could energize efforts to develop what he calls “biobots”—or biological robots. “We've been thinking for a long time about the possibility of recapitulating biological functions in objects that are not necessarily alive,” he said. Bustamante said biobots might consist of saclike liposomes loaded with biological machinery specifically designed to produce energy or carry out another useful function.

Bustamante added that the proteorhodopsin gene might also be inserted into microbes already used commercially, to light-supercharge their capacity to produce the energy molecule ATP. “Maybe it's a bit crazy, but we're considering whether we could endow such cells as yeast with the ability to capture light and create ATP,” he said. “Yeast are being widely used to

produce ethanol as a biofuel, but there are reports that their ability to produce ATP may be limited. But if you have yeast cells that can also use light, they might be more efficient in converting carbon sources into biofuels,” he said.

Liphardt added that proteorhodopsin's rapid response to light would make it attractive to a variety of researchers. “For purposes of synthetic biology or nanotechnology, researchers are always looking for new ways of providing signal inputs to cells and have them respond quickly,” he said. “Organisms with proteorhodopsin respond to light on timescales of seconds, whereas responses to changes in gene expression take place over scales of twenty or thirty minutes.”

Liphardt also noted that proteorhodopsin-equipped bacteria could constitute valuable research tools. “They would be very useful to study energy fluxes inside cells, because we could create a system whereby changing light intensity would let us precisely tune the proton motive force in single cells. We could change illumination intensity and immediately see the cell respond,” he said.