Shedding New Light on Proteorhodopsin（图）

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3 摘要 [Abstract]February 9, 2007, BERKELEY, CA — New light has been shed on proteorhodopsin, the light-sensitive protein found in many marine bacteria. Researchers at the U.S. Department of Energy's Lawrence Berkeley National Laboratory (Berkeley Lab) and the University of California at Berkeley have demonstrated that when the ability to respire oxygen is impaired, bacteriaan equipped with proteorhodopsin will switch to solar power to carry out vital life processes.
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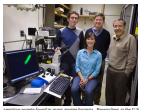

February 9, 2007, BERKELEY, CA —
New light has been shed on proteorhodopsin, the light-

sensitive protein found in many marine bacteria. Researchers at the U.S. Department of Energy's Lawrence Berkeley National Laboratory (Berkeley Lab) and the University of California at Berkeley have demonstrated that when the ability to respire oxygen is impaired, bacteriaan equipped with proteorhodopsin will switch to solar power to carry out vital life processes.

"Our research shows that proteorhodopsin contributes to a bacterial cell's energy balance only under certain environmental conditions, namely when the cell's ability to respire has been impaired," said Jim Liphardt, a biophysicist who holds a joint appointment as a Divisional Fellow in Berkeley Lab's Physical Biosciences Division (PBD) and the Physics Department of UC Berkeley (UCB). "By harvesting light, proteorhodopsin enables bacterial cells to supplement respiration as a cellular energy source.

Liphardt said that the solar power option represents a potentially significant boost for efforts to develop alternatives to fossil fuel energy sources. Microbes that can simultaneously harvest energy from several different sources may be better at producing biofuels than microbes that can only utilize a single energy source.

The results of this study appear in a paper published by the Proceedings of the National Academy of Sciences (PNAS), entitled: Light-

powering Escherichia coli with proteorhodopsin. Co-

authoring the paper with Liphardt were UCB graduate students Jessica Walter and Derek Greenfield, and Carlos Bustamante, who also holds a joint Berkeley Lab

-UCB appointment and is a Howard Hughes Medical Institute (HHMI) investigator.

There was a great deal of excitement in the biology community in 2000 when proteorhodopsin was first discovered encoded within the genomes of uncultivated marine bacteria. The discovery implied that such bacteria possessed phototrophic as well as respiratory capabilities. This would be a critical adaptation for outlasting microbes because the world's oceans are permeated with "dead zones," areas that lack sufficient oxygen to sustain life.

Subsequent studies established that proteorhodopsin is a light-

driven proton pump, able to transport protons across cellular membranes in order to create stored electrochemical energy. In this respect, it is similar to another protein, bacteriorhodopsin, that's used by bacteria in salt ponds to supplement respiration. However, in experiments in which marine bacteria endowed with proteorhodopsin were exposed to light, there was no response. This begged the question: What does proteorhodopsin actually do?

A recent study out of the University of Kalmar in Sweden, led by Jarmo Pinkoss, showed that light could be used to stimulate the growth of some types of marine bacteria carrying proteorhodopsin. This indicated that such bacteria can use a form of photosynthesis to supplement respiration as an energy source, but the extent to which light could be used to replace respiration was still unknown.

"Our thinking was that if you had a system that could harvest energy from two different sources and you knocked out one of those sources then you would probably maximize the alternative energy source," Liphardt said. "Think of it like a capacitor. If a capacitor is already fully charged and you connect a battery to it nothing happens. However, if you drain the capacitor and then connect a battery, a current will flow."

To observe proteorhodopsin in action and measure its effects, Liphardt and his co-

authors genetically engineered a strain of Escherichia coli that would express the light-sensitive protein.

Said Walter, "The energy metabolism of E. coli is well understood so it served as an excellent testbed for observing proteorhodopsin activity when the microbe's ability to respire is suddenly impaired. We impaired respiration through either oxygen-depletion or the respiratory poison azide."

The Berkeley researchers monitored single cells of E. coli and observed the response to light of the proton motive force (pmf), the electrochemical potential of protons across cellular membranes that bacteria use as the energy source to, among other functions, power the rotary flagellar motor which enables them to swim.

"We found that if we shined light on our E. coli cells when their respiration was impaired, they would swim or stop depending on the light's color," said Walter. "Proteorhodopsin has an absorption spectrum that peaks in the green wavelengths, so the cells swam when they were exposed to green light, but stopped when they were exposed to red light."

In the absence of the azide-respiratory poison, green light had no effect on the flagellar motors of these proteorhodopsin-

equipped E. coli. By measuring the pmf of individual illuminated cells under different concentrations of azide or various degrees of lighting, the Berkeley researchers were able to quantify the coupling between light

-driven and respiratory proton currents. At the highest azide concentrations, the average cell velocity increased 70-

percent upon green light illumination. In the control study, normal E. coli cells, which do not not express proteorhodopsin, had no response to the green light.

The next step in this work, Liphardt said, is to optimize the amount of light that can be collected in cells enhanced with proteorhodopsin. For this the researchers will need to identify the most efficient forms of the protein, then manipulate microbial genomes through the addition or deletion of key genes.

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