

































## Marine Metagenomics and the Discovery of Proteorhodopsin

Although surface waters in the open ocean receive ample sunlight to fuel photosynthetic growth, concentrations of dissolved organic carbon, a photosynthetic byproduct, typically are very low (less than 200  $\mu\text{mol C}$  per liter). Heterotrophic members of the bacterioplankton community thus are forced to contend with severe carbon substrate limitation, yet the specific metabolic strategies employed by these marine oligotrophs to grow under these conditions are just being discovered. As in many environments, the lack of insight arises primarily from difficulties inherent in cultivating relevant organisms in a laboratory setting. New metagenomic approaches, however, are overcoming this challenge by characterizing microbial DNA directly from the environment.

Metagenomic sampling of microbial communities in the open ocean has revealed a surprising new pathway for energy conservation by marine heterotrophs. Proteorhodopsin, a protein functioning as a light-driven proton pump in cell membranes, has been detected in a wide range of ocean habitats. Previously thought to exist only in archaeal extremophiles living in salt ponds, genes encoding these proteins are ubiquitous in marine bacterioplankton such as the SAR cluster, which was originally isolated from samples taken in the Sargasso Sea. In those samples alone, more than 782 rhodopsin-like photoreceptors were identified (Venter et al. 2004). The common occurrence of bacterioplankton harboring this protein in surface waters worldwide suggests a potential mechanism for widespread mixotrophic energy conservation in marine environments. Mixotrophy is a form of growth in which two methods of energy generation are used simultaneously. In this form, bacteria would augment energy derived from the consumption of organic substrates and conserve carbon resources by creating an additional proton gradient using light energy to drive synthesis of ATP, a multifunctional nucleotide responsible for cellular energy transfer and storage (see figure above).

The initial observation of proteorhodopsin in metagenomic samples sparked a series of experiments to test the mixotrophic-growth hypothesis. Expression of the proteorhodopsin gene in *Escherichia coli* confirmed the protein was involved in light-dependent ATP formation. Preparations of bacterial cell membranes collected from ocean surface waters reveal not only high levels of proteorhodopsin, but various types of the protein tuned to absorb different wavelengths of light. This variation suggests ecological specialization for different niches and depths in the water column. Furthermore, experiments using recently cultivated marine heterotrophs equipped with proteorhodopsins have shown that at least some types grow more efficiently under substrate-limited conditions when exposed to light.

Though further field studies are needed to assess the role of proteorhodopsin in marine ecosystems, initial results suggest this novel mode of growth could represent an important new pathway affecting carbon flow and energy conservation in ocean-surface habitats. Proteorhodopsin discovery also represents an important early success story for using metagenomic approaches to detect previously untapped metabolic capabilities, facilitate development of new hypotheses and experiments, and reveal significant components of the global carbon cycle.

### References

Beja, O., et al. 2000. "Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea," *Science* **289**(5486), 1902–06.

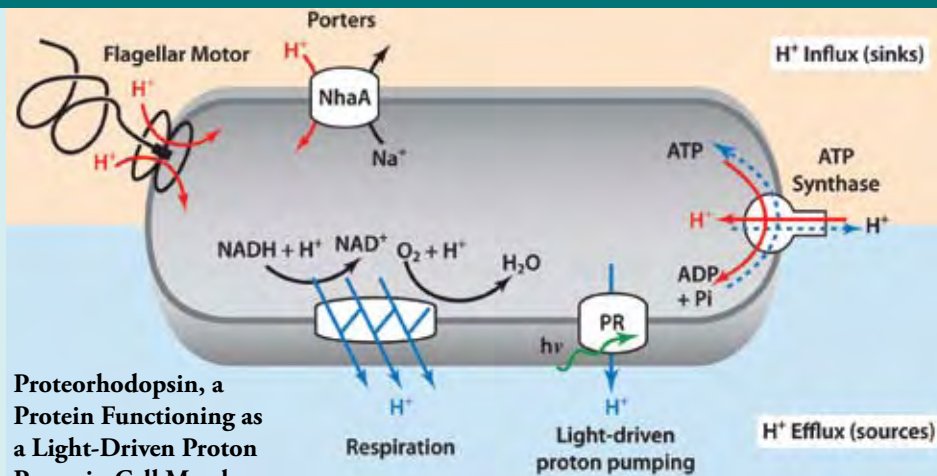
Beja, O., et al. 2001. "Proteorhodopsin Phototrophy in the Ocean," *Nature* **411**(6839), 786–89.

Giovannoni, S. J., et al. 2005. "Proteorhodopsin in the Ubiquitous Marine Bacterium SAR11," *Nature* **438**(7064), 82–85.

Gomez-Consarnau, L., et al. 2007. "Light Stimulates Growth of a Proteorhodopsin-Containing Marine Flavobacterium," *Nature* **445**(7124), 210–13.

Martinez, A., et al. 2007. "Proteorhodopsin Photosystem Gene Expression Enables Photophosphorylation in a Heterologous Host," *PNAS* **104**(13), 5590–95.

Venter, J. C., et al. 2004. "Environmental Genome Shotgun Sequencing of the Sargasso Sea," *Science* **304**(5667), 66–74.



**Proteorhodopsin, a Protein Functioning as a Light-Driven Proton Pump in Cell Membranes.**

[Source: Lawrence Berkeley National Laboratory. 2007. "Research News: Shedding New Light on Proteorhodopsin," <http://www.lbl.gov/Science-Articles/Archive/PBD-proteorhodopsin.html>. Image used with permission and adapted from original figure in Walter, J. M., et al. 2007. "Light-Powering *Escherichia Coli* with Proteorhodopsin," *Proceedings of the National Academy of Sciences of the USA* **104**(7), 2408–12. doi: 10.1073/pnas.0611035104. Copyright (2007) National Academy of Sciences, USA.]