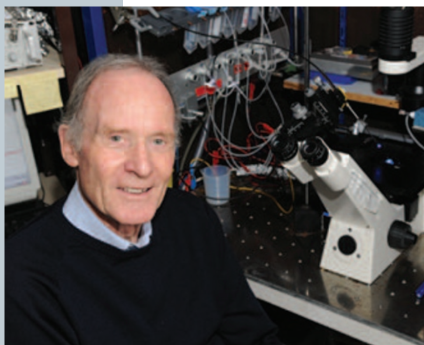
THE AUTHOR FILE

# Ernst Bamberg

Fusing light-activated proteins for precise optogenetic control

Ernst Bamberg has grown used to surprises from microbial rhodopsins. About a decade ago, he, together with Georg Nagel and Peter Hegemann, characterized the proteins that help green algae move toward light. Unlike previously known light-sensing proteins, such as light-activated bacterial ion pumps, algal rhodopsins are light-gated ion channels that operate on a much faster time scale.



P. Lastrico, MPI Biophysics

Ernst Bamberg

Scientists had trouble believing that a rhodopsin-like molecule can act as a cation channel. "We had a hard time trying to convince people that it was true," Bamberg recalls. However, Bamberg says he'd already realized the potential of these light-gated channels, or channelrhodopsins. "Before we published the first papers [showing that algal proteins could generate currents in eukaryotic cells], we applied for a patent where we gave to our fantasy a free run about the possible applications of channelrhodopsins on electrically excitable cells, including some biomedical applications." (The patent was recently licensed by a large pharmaceutical company for a project on gene therapy to restore vision in the eye.)

The scientific community is now convinced: channelrhodopsins have become a central part of a new technology called optogenetics. Researchers across the world have reported using channelrhodopsins to control electrical signals in neurons as well as cardiomyocytes, sometimes within living mice and other animals. These applications came as yet another surprise, says Bamberg. Few expected these microbial membrane proteins to function in animal cells. "This is still for me a little miracle," says Bamberg, at the Max-Planck-Institut für Biophysik in Frankfurt.

In fact, researchers who want to use light to control processes in animal cells now have several kingdom-crossing proteins to choose from. The proteins even perform different tasks. Stimulated by blue light, channelrhodopsin-2 allows cations to move across the cell membrane, depolarizing cells and causing neurons to fire. Stimulated by orange light, halorhodopsin pumps chloride ions across the cell membrane, causing hyperpolarization and preventing neurons from firing.

Optogenetic tools could be even more powerful if two proteins were expressed in the same ratio in one cell—one would activate a neuron, and another would silence it. Such control could allow incredibly precise experiments about what happens when certain neurons fire as well as when they are prevented from firing.

In practice, such experiments have been very challenging. When cells are made to express two optogenetic proteins, they tend to make too many copies of one and too few of another, and ratios vary from cell to cell. Even when genes for both proteins are placed on the same construct, differences in how proteins are processed make it impossible to control how many functional copies of the hyperpolarizing molecules exist for every depolarizer.

To solve this problem, Bamberg and colleagues attempted an apparently simple solution: they created a fusion protein in which the two optogenetic tools remained physically attached. Not only would they be expressed together, they would be placed into the plasma membrane together.

The details were harder to deal with. If channelrhodopsin and halorhodopsin were physically linked together directly, one rhodopsin would turn the other upside down. "You need another helix in between," explains Bamberg. To link the proteins, Bamberg chose a section of a rat gastric proton ATPase as well as a fluorescent protein.

> "This is still for me a little miracle"

The very first construct looked good, at least initially. The fusion construct seemed to be appearing at the cell membrane. "We saw something and we were happy," says Bamberg. "Then we analyzed the data and we saw that it didn't work properly." Setbacks are to be expected, says Bamberg. "Science is such. You cannot make a plan and say this has to work."

They kept at it: changing the length of the linker, switching which side of the linker each rhodopsin was on. Finally, after creating some two dozen constructs, the researchers obtained convincing results. The paired proteins could be used to precisely control membrane potential in cultured neurons.

As a bonus, this linker promises to work for several optogenetic tools. In addition to combining channelrhodopsin-2 and halorhodopsin, the researchers were able to join the blue light–absorbing channelrhodopsin-2 with the yellow light–absorbing channelrhodopsin-1, allowing the activation of nerve cells over a wide range of the spectrum of visible light. "Now we have a cassette where you can put all kinds of rhodopsins together," says Bamberg.

**Monya Baker**

Kleinlogel, S. *et al.* A gene-fusion strategy for stoichiometric and co-localized expression of light-gated membrane proteins. *Nat. Methods* **8**, 1083-1088 (2011).

© 2011 Nature America, Inc. All rights reserved.
npg