NEUROSCIENCE

Perfecting ChR2

Two new reports describe variants of channelrhodopsin 2 with improved properties.

Channelrhodopsin 2 (ChR2) has been a godsend tool to study brain function. This protein—originally found in tiny algae—is a membrane-ion channel that opens up in response to pulses of light, producing a change in the membrane potential of charged cells. Algae use ChR2 to signal the presence of light and trigger their swimming away or toward it in the pond; neuroscientists, after 'transplanting' ChR2 into neurons, use it to provoke light-triggered action potentials in cells embedded deep in brain tissue. Not surprisingly, some of ChR2's natural properties are not exactly ideal for this purpose.

In particular, the channel's small currents and slow kinetics still limit the potential applications of ChR2 in neuroscience. Improving these properties would enable researchers to more reliably induce action potentials ('spikes') in cells located farther away from the applied light source, use lower light powers to stimulate them or get away with weaker transgene expression. One way to improve ChR2's performance is by mutagenesis. Although this strategy has already yielded several ChR2 variants that exhibit faster kinetics or larger currents, so far one thing has always come at the expense of the other.

The search for the 'perfect ChR2' continues in many laboratories around the world, and two independent teams have now reported several improved ChR2 variants. A joint three-laboratory team composed of the labs of Peter Hegemann at Humboldt University, Karl Deisseroth at Stanford University and Thomas Oertner at the Friedrich Miescher Institute have developed a ChR2(T159C) mutant, the 'TC' mutant, which, when expressed in neurons, elicits photocurrents almost twofold larger than those of wild-type ChR2 (Berndt *et al.*, 2011). Researchers can use the TC mutant to spike neurons with dimmer light pulses, which will be handy when performing experiments *in vivo*.

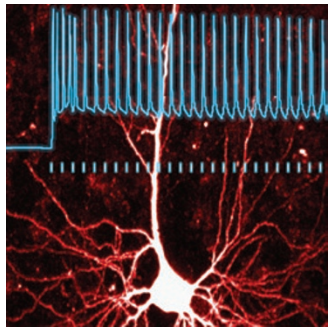

Image of a neuron expressing the TC mutant, and its spiking trace. Image courtesy of T. Oertner.

As with previous higher-current ChR2 mutants, however, the closure of the TC mutant's ion channel after a light stimulus is slightly slowed down. "A fast closure is very important because after an action potential you want to repolarize the neurons to their initial, baseline membrane potential; if the channelrhodopsin is still open, you get all sorts of problems," explains Oertner. To tackle this, the group decided to combine the TC mutation with a mutation already known to accelerate the closure of wild-type ChR2, the E123T mutation or 'ChETA'. The double E123T,T159C (ET-TC) mutant exhibits both increased photocurrents and faster kinetics compared to wild-type ChR2.

When expressed in neurons, the ET-TC mutant elicits spikes with high fidelity across a wide range of light intensities and stimulation frequencies. During these studies, the group also found a previously unknown role for the ET mutation. "We found that [the mutation that created ChETA] is the voltage-sensing position for ChR2, so by introducing this mutation the channel no longer slows down at low voltage," explains Hegemann. This property enables rapid repolarization of the membrane after a spike.

A second study, by members of Ernst Bamberg's lab at the Max Planck Institute of Biophysics, used mutagenesis to alter ChR2's preference for certain ions over others. ChR2 is a nonselective cation channel, but in a typical neurobiology experiment the channel is thought to transport mostly sodium ions. If one were to slightly increase the number of calcium ions transported, the group reasoned, this could result in improvements in the channel's performance for neuronal activation.

By modifying one residue in wild-type ChR2, the group generated a mutant with higher calcium permeability, called 'CatCh' (Kleinlogel *et al.*, 2011). In nonneuronal cells, CatCh's modest preference for calcium ions elicits approximately three times higher currents and a slight slowdown of its kinetics compared to wild-type ChR2. But surprisingly, when expressed in neurons, the group saw a nearly 70-fold increase in the cell's light sensitivity and a surprisingly rapid and complete repolarization of its membrane after each spike.

Behind these properties, Bamberg explains, are the indirect effects triggered by the local increases in calcium produced by CatCh at the neuron's membrane. "CatCh can be seen as a light-gated membrane-bound calcium source," he says. For one, local increases in calcium result in the activation of voltage-gated sodium channels and result in the fact that you need much less light to get a depolarization event. Secondly, calcium activates channels that are responsible for the membrane's repolarization, accelerating this process. CatCh could also be used to modulate calcium levels in subcellular compartments in response to light in any kind of cell.

One exciting lesson from these studies is the potential of combining mutations to refine the properties of channelrhodopsin, promising yet better tools to come for optogenetics.

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RESEARCH PAPERS

Berndt, A. *et al.* High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels. *Proc. Natl. Acad. Sci. USA* **108**, 7595–7600 (2011).

Kleinlogel, S. *et al.* Ultra light-sensitive and fast neuronal activation with the Ca^{2+}-permeable channelrhodopsin CatCh. *Nat. Neurosci.* **14**, 513–518 (2011).