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Engineering the Outputs

Microbial Rhodopsins as Versatile Phototransducing Modules

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Abstract:

Microbial rhodopsins are a large family of membrane proteins found throughout the microbial world in both prokaryotes and eukaryotes and in diverse environments. In ocean plankton alone >800 different microbial rhodopsin genes have been identified. We are studying the structure and function of members of this family to understand how their common protein design, consisting of seven transmembrane helices forming a membrane-embedded pocket for the chromophore retinal, has been adapted to different physiological functions. Nature has used the rhodopsin domain as a versatile component in diverse phototransducing systems. Some of these proteins are light-driven ion pumps (transport rhodopsins, which carry out electrogenic transport of protons or chloride across the membrane), whereas others are photosensory receptors (sensory rhodopsins). The sensory rhodopsins use a variety of signaling mechanisms, including interaction with membrane-embedded transducers to control a phototaxis phosphorylation cascade in haloarchaea, interaction with a soluble cytoplasmic transducer in cyanobacteria, and control of calcium channels in algae. In many of the algal proteins, the 7-helix photoactive rhodopsins are present as photoactive domains of larger proteins.

In the course of our studies, we have engineered *E. coli* to express retinal biosynthetic enzymes and several microbial rhodopsin apoproteins. Two new functions have been conferred on the organism: (i) The marine light-driven proton pump proteorhodopsin enables *E. coli* cells to use green light to eject protons from their cytoplasm to the medium thereby generating proton electrochemical potential across the cytoplasmic membrane. The transformed cells exhibit robust light-driven proton transport activity. (ii) We constructed a fusion-chimera gene which encodes a “photosignaling module” from haloarchaea consisting of the rhodopsin protein connected by a flexible linker to the interacting N-terminal portion of its transducer, in turn fused to an enteric chemotaxis methyl-accepting receptor’s cytoplasmic domain. This protein mediates phototaxis responses by *E. coli* cells. Both the transporters and photosignaling fusion-chimera exhibit their phototransducing activities also in purified states when incorporated into proteoliposomes.