FINAL PROGRESS REPORT

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Folding and Function of Proteorhodopsins in Photoenergy Transducing Membranes

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Overall research goals: The overall research objectives are to develop proteorhodopsin (PR) proteins as a model system for α -helical membrane protein insertion and folding, and to advance understanding of the diversity and mechanisms of PRs, a large family of photoenergy transducers (~4000 identified) abundant in the world's oceans. Specific aims are: (1) To develop a high-efficiency genetic selection procedure for light-driven proton-pumping in *E. coli* cells. Such a procedure would provide a positive selection method for proper folding and function of PRs in the *E. coli* membrane. (2) Characterize flash-induced absorption changes and photocurrents in PR variants in organisms from various environments, and their expression level and function when expressed in *E. coli*. Subaims are to: (a) elucidate the relationship of the transport mechanism to mechanisms of other microbial rhodopsins, some of which like PRs function as ion transporters and some of which use light energy to activate signaling pathways (sensory rhodopsins); and (b) identify important residues and chemical events in light-driven proton transport by PRs. In addition to their importance to the energy of the biosphere PRs have attracted interest for their potential for use in making photoenergy-transducing membranes for bioengineering applications.

Significant achievements 2007-2012:

1. We characterized photochemical reaction cycles and photocurrents in 20 PR variants from organisms from various environments. The PRs differ greatly in expression level of functional pigments which correlates neither with photocycle rate, nor with absorption maximum. Proteorhodopsin from the Antarctic bacterial species *Psychroflexus torquis* exhibits two unusual properties: (i) A very fast photocycle with halftime at 20°C of 2.6 ms, ~3-fold faster than the most rapid previously measured proton-pumping rhodopsin. We attribute this fast rate to its adaptation to sea-ice temperature. (ii) Flash-induced absorption difference spectra reveal fine structure, which may be from polyene stretching vibrational modes as in sensory rhodopsin II, or may possibly be a first indication of Förster resonance energy transfer from a bound carotenoid in this carotenoid-rich bacterium, a process which has been proven to occur in the proton pump xanthorhodopsin. Therefore Psychroflexus PR may be the fastest retinylidene proton pump and also may have an enhanced absorption range and efficiency of energy capture, properties potentially useful for bioengineering applications.
2. New key residues in PRs were identified and their roles in the transport mechanism assessed (publications 1-5). We identified two new residues (His75 and Glu142) participating in proton transfers.
3. We discovered a deprotonation/reprotonation subcycle of His75 within the pumping cycle as a novel mechanism in rhodopsins that appears to be ubiquitous in PRs (1).

4. We identified a shared fundamental process, the Schiff base “connectivity switch”, in the mechanisms of proton transport and signaling by the rhodopsin family (8) and clarified the relationship between energy and sensory transduction by microbial rhodopsins (6-12).

5. Our extensive characterization of *E. coli* physiological responses to light-driven proton pumping in this project argued for a selection protocol based on designing conditions in which light-driven proton ejection protects against an abrupt short-term stress in non-growing conditions, i.e. a “shock”. We now have optimized and finalized development of a highly efficient genetic selection procedure for light-driven proton-pumping in *E. coli* cells based on low pH shock. We developed the method using co-expression of two microbial rhodopsins that have opposite effects on cell survival during a low pH shock: the chloride pump halorhodopsin (HR) and a proton pump rhodopsin HTDR. Shock conditions are 45-min incubation at pH4 in the presence of azide and a low concentration of CCCP, and intense illumination with broad-band yellow light. The rationale is that HR, which hyperpolarizes the membrane by inwardly directed electrogenic chloride transport, facilitates acidification of the cytoplasm eliminating cells without functional PRs, which rescue the cells by pumping protons out. We assessed the selection efficiency (i.e. extent of enrichment) of this procedure by adding cells expressing both HR and HTDR to cell suspensions containing cells expressing only HR in various proportions. The most dramatic result was that when the HTDR-expressing cells were introduced in a ratio of 1:10⁶, after selection 100% of the viable population consists of HTDR-expressing cells, i.e. an enrichment factor of $\geq 10^6$ -fold in a single pass of the selection, excellent for our purposes (M. Ntefidou, O.A. Sineshchekov, and J.L. Spudich, unpublished).

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Research articles on the relationship of the mechanism of proteorhodopsin and homologous light-driven proton pumps to the mechanism of signal transduction by light-sensing rhodopsins:

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