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Editorial

Bacteriorhodopsin

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In the last three decades, few single biological objects have attracted as much general interest as the purple protein, bacteriorhodopsin. Although it occurs in the membranes of an obscure organism that lives in the hypersaline lakes found only in remote and unusual locations, and until recently had no evolutionary connection with any other known eubacterial or eukaryotic protein, it rapidly became the subject of intense research, and has remained at the center of attention ever since. The reason is that this small retinal-containing protein has long promised to be the Rosetta stone for deciphering how ion pumps work.

As a very large number of investigators realized soon after its discovery, in many respects, bacteriorhodopsin is an ideal transport protein for study. It is available in large quantities, can be purified with trivial effort, is extraordinarily stable, and most important of all, the simple process of photoisomerization of the all-*trans* retinal to 13-*cis* is able to drive the efficient translocation of a proton across the membrane. The desire to understand bacteriorhodopsin brought out the best in all the technologies either available or waiting to be developed. It was one of the first membrane proteins in which extensive mutagenesis was carried out, first in a heterologous then in a homologous expression system. It was one of the first proteins to be studied with infrared, Raman and solid-state nuclear magnetic resonance spectroscopy, methods of extraordinary specificity and exquisite sensitivity. Instrumentation for time-resolved measurements at visible wavelengths has been developed expressly for bacteriorhodopsin, with resolutions that reach into the fs range. Electron diffraction, now widely used, cut its teeth on solving the structure of this protein in its naturally occurring two-dimensional crystalline lattice. More recently, three-dimensional bacteriorhodopsin crystals have been grown that diffract unusually well, uniquely for an integral membrane protein, otherwise notoriously difficult to crystallize. In short, the over 5000 publications on bacteriorhodopsin have helped directly, or inspired by example, the entire field of membrane proteins.

Research on bacteriorhodopsin has many facets, including interesting and important questions concerning the photochemistry of the retinal, protein folding, insertion, assembly into a naturally occurring hexagonal crystalline array, stability, conformational fluctuations and substates, and others, but the most urgent question probably concerns its function as a transmembrane pump. There have been times in the past when the solution to how bacteriorhodopsin transports protons seemed to be near, only to be frustrated by contradictions and controversies. With the rising expectations in the field of all membrane proteins, it is now clear for bacteriorhodopsin also that a full and comprehensive mechanism will not be available until we have a detailed structural understanding of the protein, as well as its many intermediate states in the transport cycle. To many, this stage seems tantalizingly near. Given the recent rapid progress in this direction, from both crystallographic and spectroscopic studies, are we being too optimistic when we expect that the main questions of the bacteriorhodopsin problem will soon be solved?

We now know that the extracellular half of the protein contains many water molecules that form,

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together with polar protein residues, a three-dimensional hydrogen-bonded network. A water molecule hydrogen-bonded to the protonated retinal Schiff base and to two anionic aspartates stabilizes the separated charges at the active site. The cytoplasmic half, in contrast, contains only a few water molecules, and acts as a hydrophobic barrier. Photoisomerization of the retinal to 13-*cis* initiates changes that begin near the Schiff base and spread to the rest of the protein over the about 10 ms duration of the photocycle. The result of these first local, and then later larger-scale, conformational changes is to change the p*K* of acidic residues, in a sequence now being described in detail. As a consequence of the changes in proton affinities, the proton occupancies of these residues change in a way that generates the step by step travel of a proton across the protein, from the cytoplasmic to the extracellular side. The most important of these events, that impart a direction to the transport, are the protonation of Asp-85 to the extracellular side of the Schiff base and the deprotonation of Asp-96 to the cytoplasmic side. Understanding these photocycle events has provided profound insights into the transport process, but it should be evident from the collection of articles in this issue that important questions are still unanswered. It should be evident also from the articles, however, that the most searching questions are, or can be, asked more specifically than ever before. In the following, I give my impressions of the controversies of today (and tomorrow).

Many of the articles in this issue contain novel and original suggestions concerning the nature of the 'protonation switch', that decides the direction of proton transfers and ultimately accounts for the vectorial transport. I am intrigued that, even at this stage of our understanding of the protein, all of these proposed models are different. This raises profound questions as to how much we really know about this crucial aspect of the transport cycle. Does the switch reside in a single bond rotation of the retinal, that allows it to relax from a strongly distorted configuration once the electrostatic constrains are removed when a proton passes from the Schiff base to Asp-85? Does it need to be an unidirectional reaction? Is the switch function performed, alternatively, by a mobile water at the Schiff base, carrying a proton across the barrier? Is the direction of the switch, extracellular to cytoplasmic and back, decided only at the Schiff base, or do the varying proton affinities and proton transfer pathways make a greater difference? Does it make sense, in this case, to distinguish a single switch step, separate and independent of other photocycle steps, or is the 'switch' simply the directional aspect of all photocycle reactions?

The articles in this issue raise many other, more mechanistic, questions as well. Neither the proton release to the extracellular surface nor the proton uptake from the cytoplasmic surface is well understood. As to the former, where does the released proton originate? As to the latter, how is the p*K* of the recipient of the proton, Asp-96, modulated over what amounts to at least 5 pH units? Does bound water dissociate and yield a mobile hydroxyl ion, at any step of the transport cycle? Given the fact that it is difficult to dissociate H_2O (in water less than one out of 10^8 molecules is dissociated), at which site in the protein would dissociation be most likely? If a hydroxyl ion instead of a proton is moved at the Schiff base, should bacteriorhodopsin be called a hydroxyl pump, analogous to the chloride ion pump, halorhodopsin? What is the role of the observed large-scale conformational change at the cytoplasmic surface? Indeed, does it have any functional role or is it simply a (not even inevitable) consequence of more subtle conformational shifts of direct functional significance? Does the analogy of bacteriorhodopsin with sensory rhodopsins reflect mainly the requirement of a large-scale conformational change for signaling, but then how do we explain that when the means for signaling are removed, some sensory rhodopsins transport protons?

In the last years, much progress has been made in the study of other transmembrane pumps, such as the cytochrome *c* oxidase and the mitochondrial ATPase, as well. In a larger sense, it is legitimate to ask whether, in the light of what we know now, the questions of bacteriorhodopsin are still of general interest. I suggest that bacteriorhodopsin is regarded as a stripped-down ion pump, where complications from the chemistry of the driving reaction, and a proton stoichiometry greater than one, are absent. Although the problems that remain in such a simplified protein still loom large, the vitality and imagination displayed in the articles in this issue promise that they will be attacked with vigor. Once the controversies and contradictions now evident in the bacteriorhodopsin field are settled, the acid/base chemistry and the participation of hydrogen-bonded water, that underlie the translocation of protons, will finally be understood. This seems like a worthy goal, and in the general interest.

There are many contributors to the bacteriorhodopsin field. My unpalatable duty as an editor was to limit the number of reviews to something managable, and many investigators with considerable accomplishments could not be included. Some of them have promised articles but could not contribute them in the end, others overlapped too much with newer-generation researchers who were given preference where possible, and still others have research directions not exactly in line with the focus on structural issues in this issue. Their contributions should be acknowledged, nevertheless. They include: U. Alexiev, P.A. Anfinrud, G.H. Atkinson, E. Bamberg, D. Bashford, R.R. Birge, R.A. Bogomolni, M.S. Braiman, S.E. Braslavsky, R.H. Callender, R.K. Crouch, W.J. De Grip, H.J.M. De Groot, A.K. Dioumaev, A. Dér, R. Diller, L.A. Drachev, T.G. Ebrey, M.A. El-Sayed, M. Engelhard, D. Engelman, Y. Fujiyoshi, W. Gärtner, K. Gerwert, R. Govindjee, F.M. Goñi, G. Groma, M. Gutman, S. Hashimoto, J. Heberle, R.W. Hendler, B. Honig, W. Hubell, T. Kakitani, L. Keszthelyi, H.G. Khorana, D. Kliger, Y. Kimura, T. Kitagawa, T. Kouyama, K. Koyama, R.S.H. Liu, J. Lugtenburg, A. Maeda, R.A. Mathies, Y. Mukohata, K. Nakanishi, R. Needleman, D. Oesterhelt, P. Ormos, H. Otto, M. Ottolenghi, E. Padros, R. Pomes, R. Renthal, J.-L. Rigaud, K.J. Rothschild, H.J. Sass, C. Scharnagl, M. Sheves, Y. Shichida, Y.-K. Shin, F. Siebert, V.P. Skulachev, S.O. Smith, H.-J. Steinhoff, M. Stockburger, W. Stoeckenius, H. Takei, T.E. Thorgeirsson, J. Tittor, Zs. Tokaji, F. Tokunaga, A. Watts, G. Zaccai, L. Zimányi, their coworkers, and others.