

## New and Notable

### **Bacteriorhodopsin: A Spectroscopic Intermediate with Two Conformations and Three Relay Events Is Voltage Sensitive**

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Bacteriorhodopsin in the purple membrane of halobacteria is a prominent prototype of an integral membrane protein, especially of the family of seven membrane-spanning  $\alpha$ -helical proteins. At present, this light-driven proton pump is one of the best characterized active ion-translocating proteins. Soon, it will be the first membrane protein whose vectorial transport mechanism is understood at the molecular and even atomic level. To unravel the mechanism of a catalytic protein, such as bacteriorhodopsin, the link between the structure, its dynamics, and the function has to be established. The concerted application of both powerful biophysical methods and tailor-made bacteriorhodopsin samples shall make it possible to reach this goal. The report by Nagel and co-workers (1997) in this issue is a marvelous example of that; indeed, it is a successful way to approach the goal.

Tracking the individual steps of charge translocation through bacteriorhodopsin obviously requires knowledge of its structure. Fortunately, by high-resolution electron crystallography on natural, two-dimensional purple membrane crystals (Kimura et al., 1997) as well as by x-ray diffraction on

three-dimensional (3D) crystals (Pe-  
bay-Peyroula et al., 1997) the 3D  
structure of bacteriorhodopsin has  
been recently determined at a resolu-  
tion of 3.0 and 2.5 Å, respectively,  
supporting the pioneering work of R.  
Henderson and co-workers. Soon, we  
can expect a bacteriorhodopsin struc-  
ture at 2.0 Å. However, to pinpoint the  
elementary steps of proton transloca-  
tion, the bacteriorhodopsin structure of  
the ground state is not sufficient. The  
structure of the photocycle intermedi-  
ates, at least of the functionally rele-  
vant intermediates is also required. Al-  
though controversial at the beginning,  
the light-induced appearance of ter-  
tiary structural changes in the protein,  
predominantly at helix F and G, has  
now been established by various inves-  
tigations using electron crystallogra-  
phy and neutron and x-ray diffraction.  
The photocycle and the proton pump-  
ing cycle of bacteriorhodopsin are ac-  
companied and most probably pro-  
pelled by structural changes in both the  
chromophore, the protonated Schiff  
base of retinal, and the protein (Hauss  
et al., 1994). Recent structural studies  
on wild type and mutant bacteriorho-  
dopsin have revealed that the tertiary  
structural changes in the protein moi-  
ety occur in the M intermediate and  
persist also in the N intermediate. The  
onset of these tertiary structural  
changes is correlated with the transi-  
tion between two structurally distinct  
substates of the spectroscopic interme-  
diate M, designated M<sub>1</sub> and M<sub>2</sub> (Sass  
et al., 1997; Nagel et al., 1997). These  
large changes in the structure take  
place after the transfer of the proton  
from the Schiff base to the amino acid  
Asp-85 and after the release of the  
proton to the extracellular surface.  
This indicates that these structural  
changes follow a redistribution of  
charges. (It should be noted that a dif-  
ferent view has been published in some  
Fourier transform infrared studies, i.e.,  
the onset of the largest structural  
changes, as solely defined by differ-

ence bands in the amide I and II re-  
gions, in the transition from M to N.)

How are these conformational  
changes in bacteriorhodopsin coupled  
to the vectorial, electrogenic proton  
transfer from the cytoplasmic side to  
the extracellular side? The kinetics, lo-  
cation, and stoichiometry of proton  
transfer reactions, such as proton ejection  
to and migration along the mem-  
brane surface as well as into the aqueous  
water phase, have been successfully de-  
termined by the application of laser ab-  
sorption spectroscopy with pH probes  
either covalently bound to bacteriorho-  
dopsin or residing in the bulk (Heberle  
et al., 1994). In contrast, charge transloca-  
tion through the core of the protein is  
best studied by electrical measurements.  
During the past 25 years, several ap-  
proaches to monitor light-induced  
charge translocation across the purple  
membrane have been applied, which are  
marked by their extreme sensitivity and  
often by their high time resolution. How-  
ever, the interpretation of the data of  
most studies dealing with the light-  
induced charge translocation across bac-  
teriorhodopsin and of all studies exam-  
ined the voltage dependence was se-  
verely hampered by the undefined and  
non-unidirectional orientation of the cur-  
rent generator bacteriorhodopsin. In this  
respect, the study by Nagel et al. (1997)  
is unique. For the first time, the voltage  
dependence of charge translocation by  
the proton pump could be unequivocally  
determined, i.e., under well defined volt-  
age clamp conditions, by expressing  
bacteriorhodopsin from *Halobacterium  
salinarum* in the plasma membrane of  
oocytes from *Xenopus laevis* and by  
measuring the current voltage behavior  
of this unidirectionally oriented bacterio-  
rhodopsin population. According to the  
interpretation of the voltage dependence  
of the pump current and blue light  
quenching effect, the externally applied  
electrical field changes the ratio of the  
M<sub>1</sub> and M<sub>2</sub> states. This should also occur  
during light-induced generation of an  
electrochemical gradient in the halobac-  
teria. The observation that negative po-

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tentials arrest the protein in  $M_1$  is of functional relevance, yet it could also be an experimental tool to study this substate, e.g., by diffraction techniques or Fourier transform infrared spectroscopy. The voltage dependence of the ratio between  $M_1$  and  $M_2$  is a strong hint for the functional importance of these two  $M$  substates in controlling the vectoriality of the proton pump. During formation of  $M_1$ , a proton is transferred from the Schiff base to Asp-85 and at the same time, one proton is ejected to the extracellular surface of bacteriorhodopsin. In the  $M_2$  to  $N$  transition, the Schiff base becomes reprotonated, but via the cytoplasmic side, receiving its proton from Asp-96, whose  $pK$  is lowered. Thus, the spectroscopic intermediate  $M$  is, in fact, composed of two structural different substates that differ in the accessibility of the Schiff base for protons and its  $pK$  (intermediate  $M_1$ : Schiff base accessible to the extracellular proton pathway,  $pK$  shifted from originally  $>13$  to  $<4$ ;  $M_2$ : Schiff base accessible to the cytoplasmic proton pathway,  $pK >10$ ). The impor-

tant finding by Nagel et al. (1997) is that the electrical potential regulates the ratio between  $M_1$  and  $M_2$  in this key transition, which might be the sole or at least a "molecular switch" in the pumping mechanism (another molecular switch could be the observed positional change of the retinal polyene chain close to the Schiff base; Hauss et al., 1994). The electrical measurements on membranes with perfectly oriented bacteriorhodopsin molecules can bridge the gap between proton translocation steps through this pump and the light-triggered tertiary structural changes. This experimental system would be the perfect one to unravel the elementary steps of proton translocation if spectroscopic and structural investigations would be feasible. However, because the density of bacteriorhodopsin expressed in the plasma membrane is relatively low, not even the photocycle can be studied at present. Presumably bacteriorhodopsin is in the monomeric state and highly mobile as opposed to the trimeric state in the crystalline purple membrane. This might

be even advantageous for data interpretation, as the bacteriorhodopsin monomer is the functional unit for proton pumping.

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