Biophys. Struct. Mechanism 3, 69–77 (1977)

Biophysics of Structure and Mechanism

What Does *Halobacterium* Tell us About Photoreception?*

E. Hildebrand

Institut für Neurobiologie, Kernforschungsanlage Jülich,
Postfach 1913, D-5170 Jülich, Federal Republic of Germany

Abstract. A photosensory mechanism is proposed for *Halobacterium halobium* based on the observation of light-induced motor responses. Possible mechanisms of signal transduction in *Halobacterium* are discussed. Bacteriorhodopsin and the visual pigment rhodopsin are compared with respect to their structural and functional properties. The conclusion is drawn that *Halobacterium* may help to understand primary photochemical events of rhodopsin rather than the transduction mechanism of visual photoreceptors.

Key words: Bacteria – Photophobic response – Rhodopsin – Membrane – Sensory transduction.

Introduction

Since the discovery of bacteriorhodopsin (BR) by D. Oesterhelt and W. Stoeckenius (1971) it has been expected that *Halobacterium* could serve as a photoreceptor model which would help to understand the process of vision. Several experiments brought strong evidence that BR functions as an energy transducer converting light by means of a light-driven proton pump into chemical energy which is finally stored in the form of ATP (Oesterhelt and Stoeckenius, 1973; Oesterhelt and Hess, 1973; Danon and Stoeckenius, 1974). Thus the main function of BR is quite different from that of rhodopsin in visual photoreceptors which act as signal transducers transmitting information from the environment to the nervous system.

However, the bacteria show also light-induced behavioral responses and one could presume that BR, besides its energy converting function, might be involved in a photosensory mechanism. First experiments done by H. Berg and K. Foster using a three-dimensional tracking microscope indicated the existence of two photosystems in *Halobacterium* which trigger motor responses. One of them seemed indeed

* Presented at the EMBO-Workshop on Transduction Mechanism of Photoreceptors, Jülich, Germany, October 4–8, 1976

to be related to BR (Berg, personal communication, 1973). Thus the question became again interesting whether *Halobacterium* could be regarded as a model system suitable to study the transduction mechanism of photoreceptors, and N. Dencher in our laboratory started to analyse the photoresponses in detail (Hildebrand and Dencher, 1975).

Halobacterium represents a cyclindrical cell, 3–10 μm in length and about 0.5 μm in diameter. Because of its small size it seems hopeless to insert a microelectrode for intracellular voltage recording, so that the application of this classical electrophysiological method is out of the question. Experiments can be performed, however, by use of recording techniques, or simple observation, of light-dependent motor responses of the whole organism. From this kind of system analysis, recording the output and manipulating mainly the input, one could expect to get some information about the sensory mechanism.

Two Photosystems Controlling the Motor Response

Halobacterium swims more or less on a straight course in the direction of its long axis, while slowly rotating around its long axis. The mean swimming rate is 2.3 $\mu m \cdot s^{-1}$ at 24° C. Upon light stimulation the organism carries out a characteristic behavioral reaction: After approx. 1 s it stops and after a short phase of tumbling it starts again swimming, but in nearly the opposite direction and reversely rotating. This response can be triggered as well by a sudden increase as by a decrease of the light intensity. We call the former "on"-response (or step-up photophobic response, formerly direct photophobic response) and the latter "off"-response (or step-down photophobic response, formerly inverse photophobic response). Both mechanisms exhibit different action spectra. Apparently two photosystems are involved in the sensory capacity of the organism. Photosystem 370 (PS 370) shows maxima of sensitivity at 370 nm and 280 nm and is responsible for the on-response. Photosystem 565 (PS 565), which exhibits only a single peak at 565 nm in the action spectrum, triggers the off-response. PS 565 is obviously based on the presence of BR, which forms distinct patches of purple membrane within the surface membrane (Fig. 1) (Blaurock and Stoeckenius, 1971). The nature of the photopigment underlying PS 370 is not yet clear. Our first results seem to favour a flavoprotein (Hildebrand and Dencher, 1975). Recent results, however, support the assumption of a retinylidene protein, probably a precursor of BR. For example, nicotine, which is known to inhibit the synthesis of retinal, abolishes both the on-response and the off-response. Both can be restored by adding trans retinal (Dencher, unpublished results). In any case PS 370 seems to be located in the membrane areas adjacent to the purple membrane. According to the view of Sumper et al. (1976) the retinal protein complex of PS 370 should be expected in the so-called brown membrane which is considered as the site of BR synthesis. The assumption of the photopigment of PS 370 as a precursor of BR is also supported by the fact that the on-response occurs approximately 10 h earlier than the off-response in the growing culture.

Both photosystems differ with respect to their sensitivity. To elicit a standard response the light intensity has to be decreased by $2 \cdot 10^{12}$ $h\nu \cdot mm^{-2} \cdot s^{-1}$ at 565 nm whereas an intensity increase of $1.5 \cdot 10^{11}$ $h\nu \cdot mm^{-2} \cdot s^{-1}$ at 370 nm and of

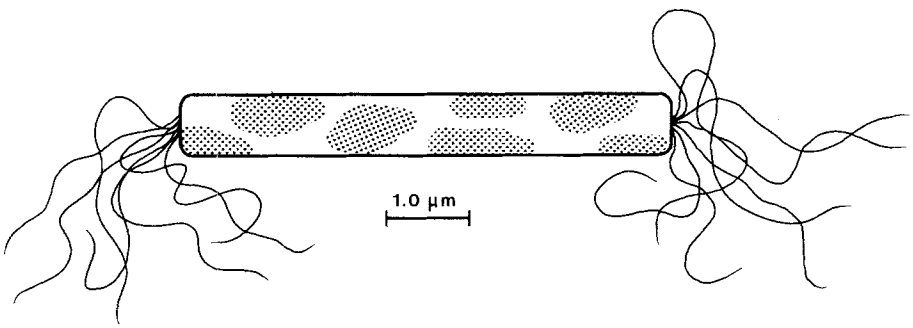

Fig. 1. Scheme of *Halobacterium halobium* showing patches of purple membrane and bipolar flagellation

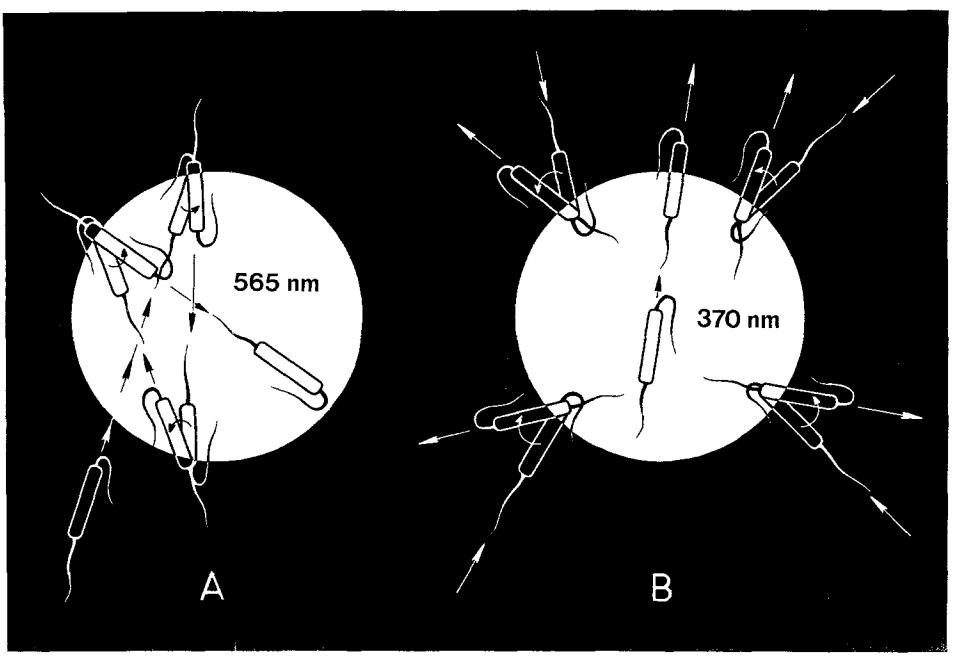

Fig. 2A and B. "Phototactic" behavior of *Halobacterium halobium* as a consequence of photophobic responses. **A.** Accumulation of bacteria in a light spot of orange (565 nm) resulting from step-down (inverse) photophobic responses (off-responses) carried out at the border to the dark. **B.** Avoiding of uv light (370 nm) as a result of step-down (inverse) photophobic responses (on-responses) at the border to the light.

$5 \cdot 10^9$ h$\nu \cdot$ mm$^{-2} \cdot$ s^{-1} at 280 nm is sufficient. In PS 565 the stimulus threshold was found to be independent of the intensity of background illumination, but in PS 370 background illumination decreased the sensitivity. Thus adaptation seems to be present only in the latter case. PS 370 obeys the law of reciprocity whereas PS 565 fails to do so.

Repetitive photophobic responses result in a fictitious phototactic behavior. Although the direction of the photophobic response has no relation to the direction of

the incident light – the bacteria thus maintaining their random movement – they will accumulate after some time in an area of orange light and avoid uv light (Fig. 2). In this way the photoresponses render possible a kind of orientation in the way of trial and error.

Motor Organelles

Flagella of *Halobacterium* could not be detected by light microscopy even under dark field illumination. Electron micrographs published by Houwink (1956) show polar tufts of 5–10 filaments. But it was not clear whether only one or both poles carry flagella. From electron micrographs of negative stained preparations (kindly performed by H. Falk) we know, however, that both poles of the cell are flagellated. As known from other bacteria the filaments are 120–140 Å in diameter representing a polymeric protein (flagellin) without enzymatic activity (for review see Berg, 1975). According to experiments of Silverman and Simon (1974) the filaments rotate with respect to the cell body. The motor must be located on their basis. But how does the motor work? The basis of the filaments shows certain structures which were interpreted as a wheel-like apparatus (De Pamphilis and Adler, 1971a). This led to the hypothesis that the basal body together with a certain abutment in the cell envelope forms the motor (De Pamphilis and Adler, 1971b). Experimental evidence was obtained that the rotary direction can be altered and this obviously occurs when bacteria change their direction of movement (Silverman and Simon, 1974; Larsen, Reader et al., 1974).

Filaments of each pole form a helical-shaped flagellum which propels the organism forward. From *Spirillum volutans* which carries flagella on both ends, as *Halobacterium* does, it is known that the posterior flagellum is always in the "tail" position thus pushing the cell, whereas the anterior organelle shows a "head" position. When the filaments reverse their rotation the flagella change from a head-tail to a tail-head configuration and the organism backs up (for review see Berg, 1974). Although we have no evidence at all at the present time, there is little doubt that the same behavior will be true also for *Halobacterium*. This implies a coordinated reversal of filament rotation on both ends resulting in a sudden change of the position of flagella during the phobic response (Fig. 3).

Receptor Effector Coupling

Although our present view concerning the coordination of the flagella is rather hypothetical we must claim a mechanism which spreads information from the sites of light absorption to both poles of the cell. The problem is: How are the photosystems linked to the motor apparatus? Diffusion of a transmitter seems probable only if the photopigments responsible for motor responses are located close to the basis of the flagella and if both ends are stimulated simultaneously. Stimulation of one end, however, is sufficient to elicit a response (see Fig. 2). We hope that partial irradiation of the cell will further help to elucidate this problem. From the fact that BR is used by the cell to synthesize ATP one could conclude that a sudden decrease of the ATP

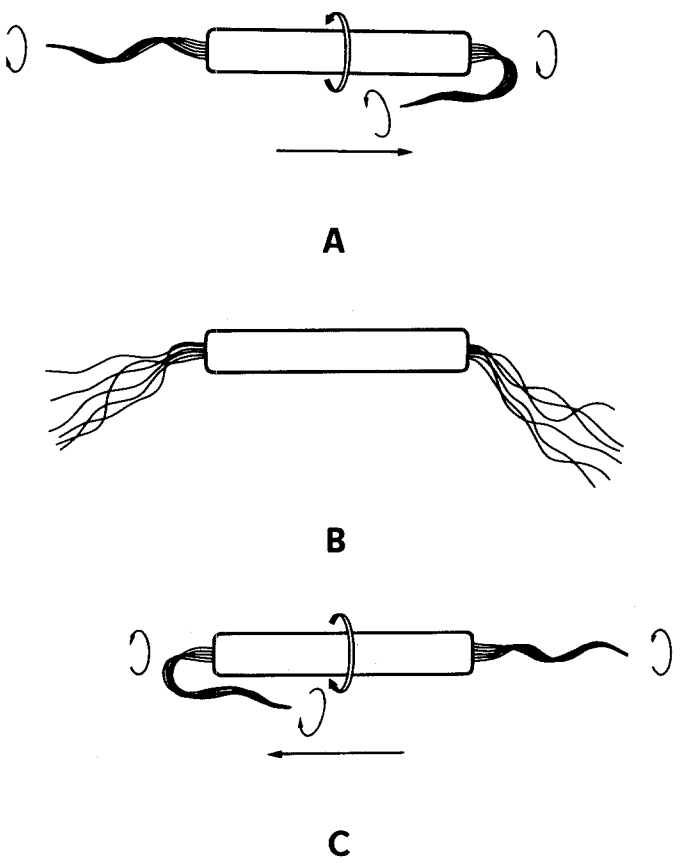

Fig. 3A–C. Scheme illustrating the hypothetical mechanism of the reversal of the swimming direction during the motor response of *Halobacterium halobium*. **A.** Swimming direction prior to photic stimulation. Anterior bundle of flagella in head position, posterior flagella in tail position. **B.** Stop phase during photophobic response exhibiting uncoordinated flagella. **C.** Reversed movement resulting from a reversal of flagellar rotation and reestablished coordination. Arrows indicate the rotational direction of the single flagella and the cell body, respectively

level may be detected by the motor apparatus. But (1) ATP seems not to be the energy source for flagellar motility in bacteria, rather another high energy intermediate of oxidative phosphorylation (Larsen, Adler et al., 1974). (2) The off-response can be evoked at a considerably high level of background illumination which should keep the amount of cellular energy nearly on the level of saturation. (3) How can the transduction in the case of PS 370 be explained which is coupled to qualitatively the same response of the effector organelle? The discussion of a direct coupling of the proton motive force to the motor apparatus is also restricted to PS 565.

It seems more likely to assume that in analogy to the ciliary reversal in Protozoa the flagellar response in bacteria is controlled by electrochemical events, i.e. by sudden changes of the membrane potential (Eckert, 1972). Such a signal would immediately spread over the entire surface of the bacterium and could trigger the motor

response at both ends of the cell simultaneously. The intracellular accumulation of K^+ ions (Christian and Waltho, 1962) as well as the electrogenic H^+ pump (Racker and Stoeckenius, 1974; Kayushin and Skulachev, 1974; Drachev et al., 1974) could well serve as the generator of a membrane potential.

Preliminary experiments with a reduced level of ionized calcium indicate that Ca^{2+} may be essential for the photophobic response (Dencher, unpublished results), but up to now no decision can be made whether Ca^{2+} acts on the transducer mechanism or at the flagellum itself. Also Mg^{2+} has been reported to be essential for excitation of bacteria (Ordal, 1976). Detailed studies on the effects of several cations on the excitability of *Halobacterium* are necessary.

It is also an open question which step in the photochemical cycle of BR triggers the motor response. Is the interruption between BR^{568} and the subsequent intermediates responsible or a certain back reaction or even the trans-cis transition of BR? The latter seems improbable because of the long half time which is about 20 min.

Bacteriorhodopsin and Visual Rhodopsin

As compared with photoreceptor membranes of invertebrates and vertebrates the purple membrane (BR) of *Halobacterium* is different with respect to structural and functional properties. Whereas BR forms an extremely rigid crystalline structure (Blaurock and Stoeckenius, 1971; Henderson, 1975) the mobility of rhodopsin in the lipid phase of vertebrate disc membranes is relatively high (Cone, 1972; Liebman and Entine, 1974). Photoreceptor membranes of invertebrates are less "fluid" and in so far more similar to the purple membrane.

The most striking similarity between BR and visual pigments is the presence of retinal as a chromophore which in all cases is covalently linked to the protein moiety forming a Schiff base with the ε-amino group of a lysine. In BR as well as in vertebrate rhodopsin the Schiff base was found to be protonated (Lewis et al. 1974; Lewis, 1976). Further linkages between retinal and the protein pocket have to be assumed in order to explain the red shift in the absorption spectrum of all the retinal protein complexes in question. Retinal in BR has either 13-cis or trans configuration (Oesterhelt et al., 1973) whereas visual pigments contain the 11-cis or trans isomers, respectively. For a long time the cis-trans isomerization has been considered as the primary event in the chain of light-induced reactions of rhodopsin (Hubbard and Kropf, 1959; Wald, 1968). Trans-BR, however, exhibits similar absorption changes as visual rhodopsin after illumination: The spectrum is first shifted to the red in about 10 ps and subsequently back towards the blue (Hubbard and St. Georges, 1958; Matthews et al., 1963; Hubbard et al., 1965; Hamdorf et al., 1973; Lozier et al., 1975; Dencher and Wilms, 1975; Busch et al., 1972; Kaufmann et al., 1976). Although this parallelism indicates that the initial photochemical reactions of BR may be identical with those of rhodopsin, the correspondence with the entire sequence of rhodopsin reactions is weak. This may result in part from the different ability of the molecules to undergo conformational changes. Resonance Raman spectroscopy indicate that a deprotonation of the Schiff base occur both in BR and the rhodopsin molecule (Lewis et al., 1974; Lewis, 1976). Cis-trans isomerization seems not to play

an essential role in the function of BR and probably it can no longer be regarded as the first step which leads to visual excitation.

As demonstrated here recent experiments on the light-induced reactions of BR renewed the discussion about the possible primary photochemical events in photoreception. We can expect that further comparative studies will help to understand the initial steps in vision.

Despite the chemical similarity of all known retinal protein complexes their occurrence in largely separated groups of organisms indicates that rhodopsin was created by nature independently at different stages of evolution. It was modified and embedded in the membrane in different ways adapted to different functions. The rigid structure of BR, preventing large conformational changes, allows fast reversibility making it favourable for energy conversion (Lewis, 1977). Signal transduction, however, needs irreversibility and this is realized by the cis-trans isomerization and the complete splitting of retinal from opsin as a consequence of excitation of a vertebrate rhodopsin molecule. Such a mechanism which guarantees a high degree of safety can be maintained only by a steady metabolic resynthesis of rhodopsin. Certain circumstances of behavior and metabolism may have favoured bistability and photoreversibility of rhodopsin in some arthropods.

Conclusions

The statement that BR besides its energy converting function is also involved in sensory transduction may be surprising, and one should perhaps check whether all important criteria of a sensory system are fulfilled in the case of *Halobacterium*. As demonstrated before the photoresponse apparently serves for a kind of orientation which has positive consequences for the organism. Light interacts with a receptor molecule present in the membrane and, although the nature of the receptor effector coupling is not known, there must be a certain kind of signal transmission in the cell. This may, of course, differ very much from the excitation mechanism of receptor membranes. As a direct action of light on the motor organelle can be excluded, there is no doubt that the carrier energy for the signal is changed during the process of transduction. Thus the system fulfils an important criterion of a sensory system: The stimulus controls a process which is provided with energy from metabolic sources. Adaptation at the receptor level, i.e. depending on background illumination, seems to be present only in PS 370. Both the on-response and the off-response, however, show refractoriness indicating some change of sensitivity of the transducer mechanism. From the high stimulus threshold one can calculate that amplification, which is believed to be an important property of sensory systems, is negligible or even absent. One may, however, ask whether amplification is really an essential criterion. Clayton (1958) calculated that in the case of *Rhodospirillum rubrum* one quantum of light has more energy than is expended in the response. The same seems to be true for *Halobacterium* and therefore it may become senseless to claim amplification for such a system.

But could *Halobacterium* serve as a model for visual photoreceptors? Concerning the mechanism of excitation photoreceptor cells have obviously many properties in common with nerve cells and Protozoa although the photoreceptor membrane is

not electrically excitable. Excitation in *Halobacterium* may be completely different and is at least not accessible for classical electrophysiological methods. In so far it seems doubtful that the study of *Halobacterium* could considerably help to understand the visual transduction in its restricted sense. One can, however, have good hope that the study of photochemical reactions of BR will further on contribute to the elucidation of early processes in photoreception.

Although *Halobacterium* may not be considered as a model for studying the transducer mechanism in visual photoreceptors it might tell us very much about the sensory mechanism of bacteria; for the light stimulus has an irreplaceable advantage: It can be suddenly turned on and removed and easily applied locally.

Acknowledgements. The work on the sensory mechanism of *Halobacterium* reported here has been done by Norbert Dencher who was supported by the Deutsche Forschungsgemeinschaft (SFB 160).

References

Berg, H. C.: Bacterial behaviour. Nature (Lond.) **254**, 389–392 (1973)

Blaurock, A. E., Stoeckenius, W.: Structure of the purple membrane. Nature, New Biol. (Lond.) **233**, 152–154 (1971)

Busch, G. E., Applebury, M., Lamola, A. A., Rentzepis, P. M.: Formation and decay of prelumirhodopsin at room temperature. Proc. nat. Acad. Sci. (Wash.) **69**, 2802–2806 (1972)

Christian, J. H. B., Waltho, J.: Solute concentrations within cells of halophilic and nonhalophilic bacteria. Biochim. biophys. Acta (Amst.) **65**, 506–508 (1962)

Clayton, R. K.: On the interplay of environmental factors affecting taxis and motility in *Rhodospirillum rubrum*. Arch. Mikrobiol. **29**, 189–212 (1958)

Cone, R. A.: Rhodopsin – Rotational diffusion in the visual receptor membrane. Nature, New Biol. (Lond.) **236**, 39–43 (1972)

Danon, A., Stoeckenius, W.: Photophosphorylation in *Halobacterium halobium*. Proc. nat. Acad. Sci. (Wash.) **71**, 1234–1238 (1974)

Dencher, N., Wilms, M.: Flash photometric experiments on the photochemical cycle of bacteriorhodopsin. Biophys. Struct. Mechanism **1**, 259–271 (1975)

Drachev, L. A., Kaulen, A. D., Ostroumov, S. A., Skulachev, V. P.: Electrogenesis by bacteriorhodopsin incorporated in a planar phospholipid membrane. FEBS-Lett. **39**, 43–45 (1974)

Eckert, R.: Bioelectric control of ciliary activity. Science **176**, 473–481 (1972)

Hamdorf, K., Paulsen, R., Schwemer, J.: Photoregeneration and sensitivity control of photoreceptors of invertebrates. In: Biochemistry and Physiology of Visual Pigments (ed. by H. Langer), p. 155–166. Berlin-Heidelberg-New York: Springer 1973

Henderson, R.: The structure of the purple membrane from *Halobacterium halobium*: Analysis of the X-ray diffraction pattern. J. molec. Biol. **93**, 123–138 (1975)

Hildebrand, E., Dencher, N.: Two photosystems controlling behavioural responses of *Halobacterium halobium*. Nature (Lond.) **257**, 46–48 (1975)

Houwink, A. L.: Flagella, gas vacuoles and cell-wall structure in *Halobacterium halobium*; an electron microscope study. J. gen. Microbiol. **15**, 146–150 (1956)

Hubbard, R., Kropf, A.: Molecular aspects of visual excitation. Ann. N.Y. Acad. Sci. **81**, 388–398 (1959)

Hubbard, R., St. Georges, R. C. C.: The rhodopsin system of the squid. J. gen. Physiol. **41**, 501–528 (1958)

Hubbard, R., Bownds, D., Yoshizawa, T.: The chemistry of visual photoreception. Cold Spr. Harb. Symp. Quant. Biol. **30**, 301–315 (1965)

Kaufmann, K. J., Rentzepis, P. M., Stoeckenius, W., Lewis, A.: Primary photochemical processes in bacteriorhodopsin. Biochem. biophys. Res. Commun. **68**, 1109–1115 (1976)

Kayushin, L. P., Skulachev, V. P.: Bacteriorhodopsin as an electrogenic proton pump: Reconstitution of bacteriorhodopsin proteoliposomes generating $\Delta\Psi$ and Δ pH. FEBS-Lett. **39**, 39–42 (1974)

Larsen, S. H., Adler, J., Gargus, J. J., Hogg, R. W.: Chemomechanical coupling without ATP: The source of energy for motility and chemotaxis in bacteria. Proc. nat. Acad. Sci. (Wash.) **71**, 1239–1243 (1974)

Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W., Adler, J.: Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*. Nature (Lond.) **249**, 74–77 (1974)

Lewis, A.: Tunable laser resonance Raman spectroscopic investigations of the transduction process in vertebrate rod cells. Fed. Proc. **35**, 51–53 (1976)

Lewis, A.: Primary photophysical and photochemical processes in visual excitation. Biophys. Struct. *Mechanism* **3**, in press (1977)

Lewis, A., Spoonhover, J., Bogomolni, R. A., Lozier, R. H., Stoeckenius, W.: Tunable laser resonance Raman spectroscopy of bacteriorhodopsin. Proc. nat. Acad. Sci. (Wash.) **71**, 4462–4466 (1974)

Liebman, P. A., Entine, G.: Lateral diffusion of visual pigment in photoreceptor disk membranes. Science **185**, 457–459 (1974)

Lozier, R. H., Bogomolni, R. A., Stoeckenius, W.: Bacteriorhodopsin: A light-driven proton pump in *Halobacterium halobium*. Biophys. J. **15**, 955–962 (1975)

Matthews, R. G., Hubbard, R., Brown, P. K., Wald, G.: Tautomeric forms of metarhodopsin. J. gen. Physiol. **47**, 215–240 (1963)

Oesterhelt, D., Hess, B.: Reversible photolysis of the purple complex in the purple membrane of *Halobacterium halobium*. Europ. J. Biochem. **37**, 316–326 (1973)

Oesterhelt, D., Stoeckenius, W.: Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nature, New Biol. (Lond.) **233**, 149–152 (1971)

Oesterhelt, D., Stoeckenius, W.: Functions of a new photoreceptor membrane. Proc. nat. Acad. Sci. **70**, 2853–2857 (1973)

Oesterhelt, D., Meentzen, M., Schuhmann, L.: Reversible dissociation of the purple complex in bacteriorhodopsin and identification of 13-cis and all-trans-retinal as its chromophores. Europ. J. Biochem. **40**, 453–463 (1973)

Ordal, G. W.: Control of tumbling in bacterial chemotaxis by divalent cation. J. Bact. **126**, 706–711 (1976)

De Pamphilis, M. L., Adler, J.: Fine structure and isolation of the hook-basal body complex of flagella from *Escherichia coli* and *Bacillus subtilis*. J. Bact. **105**, 384–395 (1971a)

De Pamphilis, M. L., Adler, J.: Attachment of flagellar basal bodies to the cell envelope: Specific attachment to the outer, lipopolysaccharide membrane and the cytoplasmic membranes. J. Bact. **105**, 396–407 (1971b)

Racker, E., Stoeckenius, W.: Reconstitution of purple membrane vesicles cytalyzing light-driven proton uptake and adenosine triphosphate formation. J. biol. Chem. **249**, 662–663 (1974)

Silverman, M. R., Simon, M.: Flagellar rotation and the mechanism of bacterial motility. Nature (Lond.) **249**, 73–74 (1974)

Sumper, M., Reitmeier, H., Oesterhelt, D.: Biosynthesis of the purple membrane of halobacteria. Angew. Chem. Int. Ed. Engl. **15**, 187–194 (1976)

Wald, G.: The molecular basis of visual excitation. Nature (Lond.) **219**, 800–807 (1968)

Received December 17, 1976