Hypothesis

The dual role of rhodopsin in vision: light-driven charge translocation and formation of long-lived photoproducts

Vladimir P. Skulachev

Department of Bioenergetics, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

Received 14 July 1982

Photoreception *Charge translocation, light driven* *Rhodopsin* *Transducin* *Cyclic GMP pathway* *Electrogenesis* *Ca^{2+} pathway*

1. INTRODUCTION

Three striking features are inherent in the mechanism of vision:

(i) Excitation of a photoreceptor cell can be induced by such a minimal quantity of light energy as a single photon;

(ii) An extremely fast rate of excitation, which develops in a millisecond time scale can be achieved by increasing light;

(iii) There is a system of adaptation allowing small changes in the illumination to be sensed in dim as well as in bright light, although in bright light the amount of photons exceeds the minimal amount by several orders of magnitude.

The recent studies on the structure and function of animal and bacterial rhodopsins open up the prospect of explaining these unique properties of photoreception. Two observations seem to be especially important, namely:

(1) Identification of a special protein, called transducin that mediates activation of phosphodiesterase by a long-lived rhodopsin photoproduct (review [1]);

(2) Resolution of the fast kinetics of the rhodopsin-driven photoelectrogenesis, which proved to resemble greatly that of bacteriorhodopsin (review [2]).

Observation (1) was interpreted as evidence in favour of the cGMP concept of vision [3], whereas (2) was discussed in terms of the calcium scheme [4]. Usually, the cGMP and calcium hypotheses are considered as alternatives. Here, I review recent data on the molecular mechanism of vision showing the two concepts as complementary. A suggestion will be developed that accounts for the relationship between two functions of rhodopsin, i.e., electrogenic and transducin-activating.

2. THE cGMP PATHWAY

It is commonly believed that absorption of a photon by rhodopsin localized in photoreceptor discs induces a chain of events resulting in closing Na^+ channels in the outer membrane of a rod cell. According to the cGMP scheme [3], this effect is mediated by a decrease in the cGMP level in the rods.

There are several indications that illumination does result in a decrease in cGMP. On the other hand artificial lowering of [cGMP] in the dark mimicks the action of light (reviews, [1,5–8]).

The major steps involved in light-initiated splitting of cGMP were elucidated in [1,9,10]. These steps are shown in the right-hand branch of the flow diagram (fig.1). A long-lived intermediate of the rhodopsin photolysis, most probably metarhodopsin II [11,12], affects a GDP-binding protein, transducin, in a fashion resulting in substitution of GTP for GDP. Transducin · GTP complex activates a cGMP-specific phosphodiesterase

Published by Elsevier Biomedical Press

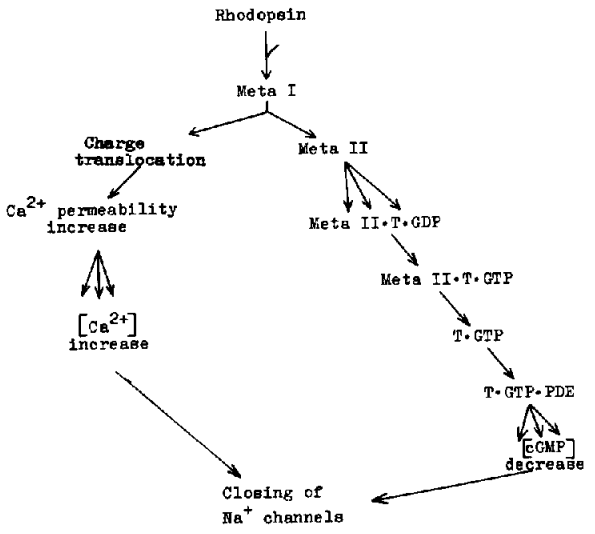

Fig.1. 'Dualistic' concept of vision. Absorption of a photon by rhodopsin results in photolysis which is accompanied by the formation of a local electric field at the step of conversion of metarhodopsin I (Meta I) to metarhodopsin II (Meta II). The field induces a fast increase in the permeability of a rhodopsin-containing membrane, allowing a messenger, e.g., Ca^{2+}, to reach the cytoplasm and to close the Na^+ channels in the plasma membrane. This pathway is simple, fast and involves only one step of amplification shown by triple arrows. The other pathway is initiated by Meta II which interacts with transducin (T) containing GDP. As a result, GDP is substituted by GTP and Meta II is released to attack a new molecule of T · GDP. Thus T · GTP accumulates. The latter combines with a cGMP-specific phosphodiesterase (PDE), activating this enzyme. PDE splits cGMP. The fall in the concentration of cGMP, an activator of the Na^+ channels, induces the closing of these channels. cGMP-pathway is slower, more complicated, and involves two, instead of one, steps of amplification.

(PDE). This is most likely to occur due to the formation of a ternary complex, transducin · GTP · PDE. The splitting of cGMP by PDE decreases the cGMP level and induces the closing of Na^+ channels, which according to the scheme, require cGMP to be open.

There are two steps of amplification of the signal in the cascade described above:

(i) A single metarhodopsin molecule activates many (~500, see [1]) molecules of transducin, replacing transducin-bound GDP by GTP.

(ii) Each PDE, when activated by transducin · GTP, hydrolyzes many cGMP molecules.

In the dark, cGMP hydrolysis ceases since both metarhodopsin and the active PDE · transducin · GTP complex are unstable. Indeed, metarhodopsin spontaneously decomposes to opsin and free retinal. GTP in the ternary complex also decomposes to PDE · transducin · GDP and phosphate. The the former dissociates to free inactive PDE and transducin · GDP. Inactivation of metarhodopsin and PDE are rather slow processes taking seconds or even minutes.

The flaw in the cGMP scheme as the only mechanism of visual transduction is that it can hardly explain the fast rate of photoreceptor cell excitation developing within 5 ms if light intensity is sufficiently high [13,14]. The studies performed on living perfused retinas showed that much longer illumination was required to lower substantially the cGMP level [8,15]. It may of course be speculated that the system responding to decrease in cGMP is so sensitive that even a very small fall in [cGMP] is sufficient to induce excitation of a visual cell. Calculation showed, however, that such sensitivity would be too high to be realistic.

The slow rate is not surprising if we recall that the cGMP pathway is a multistep process involving interactions of several proteins and nucleotides as well as a hydrolytic reaction. This mechanism looks like being meant for high amplification rather than fast rate of response.

3. FAST ELECTROGENESIS AS ONE OF THE FUNCTIONS OF THE VISUAL PIGMENT

The first indication of an electrogenic activity of visual pigments was obtained in 1964 when the so-called early receptor potential (ERP) was described [16]. It was a very rapid small biphasic photoelectric response preceding the late receptor potential, i.e., excitation of the visual cell. ERP develops too fast to be associated with post-rhodopsin steps of the visual process. The maximal amplitude of ERP was not more than several millivolts on an intracellular recording. This value was so small that one had to regard ERP as a side-effect that cannot play a functional role in visual excitation (review [17]).

In recent years, interest in the rhodopsin-linked photoelectric activity was stimulated by the discov-

ery of bacteriorhodopsin which proved to be an electrogenic light-dependent H^+ pump [18–21]. In 1977, Trissl et al. [22] revealed a small (0.5–3 mV) photoelectric effect, induced by illumination a solid Teflon film covered on one side with rhodopsin (see also [23,24]). In 1978, Ostrovsky et al. [25] demonstrated a photopotential across a photoreceptor disc membrane (the disc interior positive), using a suspension of discs and phenyldicarbaundecaborane anion as a $\Delta\Psi$ probe. In a review published in 1979, Montal [24] mentioned the results of Takagi and Kishimoto who had recorded an ERP-like signal in a system consisting of squid outer-segment vesicles adsorbed onto a thick monoolein–decane film separating two water solutions. A similar photoelectric effect ($\Delta\Psi \leq 0.5$ mV) was observed in 1980 by Lindau et al. [26] when they studied rod outer segments adsorbed on a membrane filter.

A direct and universal method developed in our group to measure membrane protein-generated electrogenic activity [27–30] was recently applied to visual rhodopsin. As was found by Bolshakov et al. [31–33] and Drachev et al. [2,34], illumination of cattle photoreceptor discs associated with the surface of a phospholipid-impregnated millipore filter or collodion film results in a rather large (up to 43 mV) potential difference being generated. Independently, this technique was used by Chapron [35,36]. However, under the conditions of his experiment, the magnitude of $\Delta\Psi$ was <1 mV. A small photopotential also was described by Bamberg et al. [37] who studied rhodopsin proteoliposomes attached to a planar phospholipid membrane. Cafiso and Hubbell [38] obtained some indications of the rhodopsin-generated $\Delta\Psi$ in discs by means of hydrophobic spin-labelled ions.

My group together with Ostrovsky's and Rubin's laboratories have performed a systematic comparison of the photoelectric activity of 3 light-driven $\Delta\Psi$ generators: bacteriorhodopsin; animal rhodopsin; and bacterial photosynthetic reaction centers [2,33,34,39,40]. To resolve the fast kinetics of $\Delta\Psi$ we used the following system: The membranes containing the proteins being studied were incorporated into lipid-impregnated collodion film separating two electrolyte solutions. The film was illuminated with a 15 ns laser flash inducing a single turnover of the incorporated $\Delta\Psi$ generators. The photoelectric effect was measured with two electrodes immersed in the solutions separated by film.

It was revealed that the two rhodopsins generate $\Delta\Psi$ in a strikingly similar fashion, which strongly differs from that of the reaction centers (fig.2). In both rhodopsins:

(i) The first laser flash induces the generation of a rather large (>30 mV) $\Delta\Psi$;
(ii) The electrogenic process consists of 3 major phases, the first being oppositely directed to the second and the third;
(iii) The kinetics of the first phase is faster than 50 ns, whereas the second phase develops in microsecond and the third in millisecond timescales;
(iv) The amplitudes of the 3 phases are first < second < third;
(v) If we take into account that the N- and C-ends of the polypeptide chain of both animal and bacterial rhodopsin are located on the opposite sides of the membrane, we can conclude that both rhodopsins generate photopotentials of the same direction (the C-side negative);
(vi) A blue flash added after the green produces an oppositely-directed photoeffect (the C-side positive);
(vii) The decay of the photoresponse to the first flash occurs in a second time-scale.

In fact, the only difference in the kinetics of electrogenesis mediated by the two rhodopsins was found to consist in a smaller time distance between the 2nd and 3rd phases, since the former was somewhat slower and the latter somewhat faster in animal rhodopsin.

At the same time, most of the above features proved to be quite different in a 'non-rhodopsin' photoelectric generator, i.e., bacterial photosynthetic reaction center. Here, all of the electrogenic phases were of the same direction, the first being the largest, in the photopotential decay there was a fast (millisecond) phase, etc.

Decreasing the resistance of the disc–collodion film system (by an external conductance) down to the resistance of the outher membrane of the photoreceptor cell, we succeeded in demonstrating a photoelectric response that was quite similar to the early receptor potential of the retinal cell [2].

Returning to the comparison of the two rhodopsins, I would like to emphasize that in the activity of bacteriorhodopsin there is nothing 'electrical'

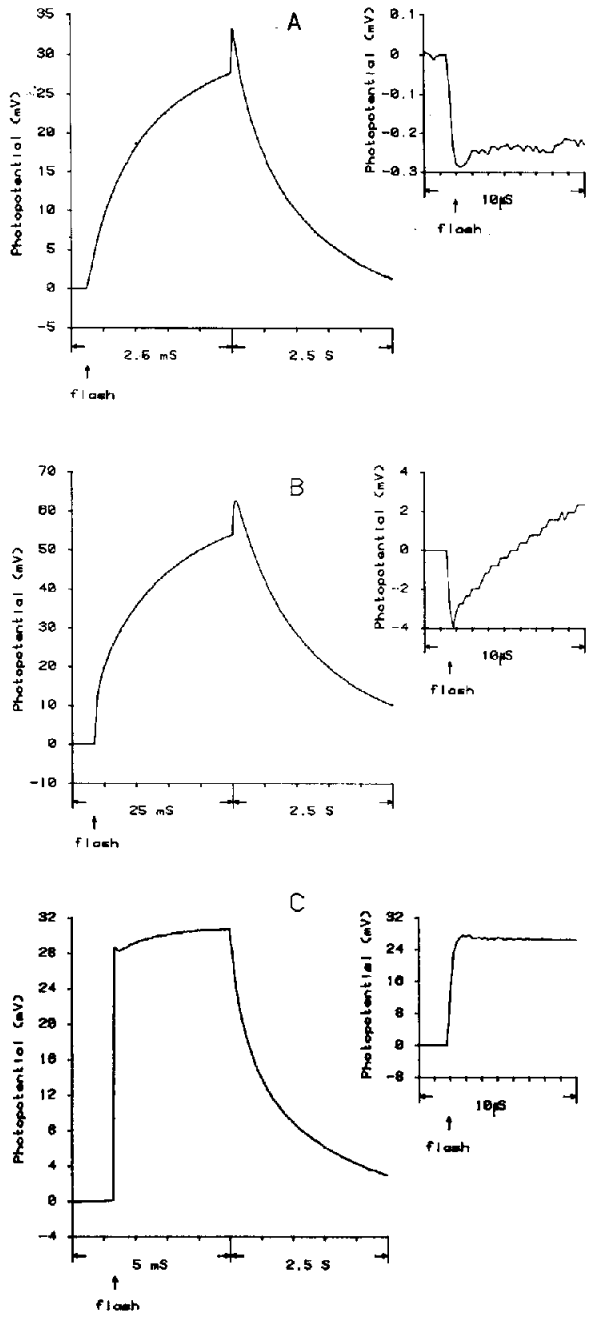

Fig.2. Generation of photoelectric potentials by animal rhodopsin (A), bacteriorhodopsin (B) and photosynthetic reaction centers of *Rhodospirillum rubrum* chromatophores (C). Cattle photoreceptor discs, bacteriorhodopsin membrane sheets or chromatophores were incorporated into a collodion film impregnated with a decane solution of phospholipids: (→) 15 ns laser flash; from Drachev et al. [2,39,40].

except for the described photoresponse which proves to be the only manifestation of the electrogenic function of this H^+-pump. As almost identical photoelectric response is inherent in visual rhodopsin, the minimal hypothesis is that this rhodopsin, like its bacterial analog, has a function as a photoelectric energy transducer.

The electrogenic function of visual rhodopsin is quite distinct from its other function described above, i.e., the ability to give a long-lived intermediate of photolysis, which stimulates the GDP–GTP exchange in transducin. This was directly proven by the observation [11,12] that addition of an instantaneously photo-bleached preparation of purified rhodopsin to crude rod outer segments causes activation of phosphodiesterase in the dark, while all-*trans* retinal and opsin do not. Under such conditions, rhodopsin electrogenesis developing faster than in 10 ms could not have been involved in the phosphodiesterase activation.

Now the problem arises what is the role of rhodopsin electrogenesis in the process of vision? Since the cGMP mechanism is of no avail here I shall turn to the rival hypothesis of visual excitation, i.e., the Ca^{2+} scheme of Hagins [4].

4. Ca^{2+} PATHWAY

According to Hagins' hypothesis, illumination of a rhodopsin-containing membrane results in increase of its Ca^{2+} permeability and hence in a Ca^{2+} release to cytoplasm from the intradiscal and/or extracellular space. A rise of the cytoplasmic Ca^{2+} is assumed to induce the closing of Na^+ channels in the outer cell membrane [4].

In [8], Uhl and Abrahamson reviewed many pieces of evidence in favour of, as well as against, the Ca^{2+} scheme. In favour they mentioned the observations:

(i) Externally added Ca^{2+} rapidly (< 1 s) and reversibly suppress the dark Na^+ current in rods.

(ii) A Ca^{2+} ionophore greatly potentiates the above (i) effect. It reduces the external $[Ca^{2+}]$ required to suppress 95% of the dark current from 20 mM down to 0.01 mM.

(iii) Lowering of the external Ca^{2+} for a short time immediately depolarizes the cell.

(iv) The light response is inhibited by introduction of a Ca^{2+} chelator into the rods.

(v) The light response of cones vanishes if the external Ca^{2+} proves to be $< 10^{-9}$ M, the effect being reversible.

The Ca^{2+} hypothesis, however, has its own pitfalls:

(i) Whether the observed increase in the cytoplasmic [Ca^{2+}] is sufficient to account for the magnification of the light signal?

(ii) What is the source of the Ca^{2+} which should appear in the cytoplasm of rod cells in the light?

(iii) Why the light response of cones does not disappear right after removal of extracellular Ca^{2+} and a rather long time proves to be necessary to inhibit illumination-induced excitation of these cells completely? [8].

5. THE 'DUALISTIC' SCHEME

In this section I would like to consider a concept postulating that there are at least two pathways of visual excitation: one, extremely sensitive to light but rather slow; the other, extremely fast and not so sensitive. The simplest version of this hypothesis is shown by fig.1. The scheme represents a combination of the Ca^{2+}- and the cGMP-concepts. It is assumed that:

(i) The cGMP–pathway is sensitive and slow;

(ii) The Ca^{2+} pathway is less sensitive and fast;

(iii) The electrogenic function of rhodopsin is involved in the Ca^{2+}–pathway.

It is not surprising that such a 'dualistic' scheme, like any compromise, succeeds in explaining more experimental observations than any of the two competing hypotheses of vision. The gain is that the scheme takes into account existence of two quite distinct functions of rhodopsin, i.e., electrogenic and transducin-activating. It is assumed that these two functions are responsible for solution of the two 'superproblems' inherent in photoreception, i.e.:

(i) *Maximal rate* of the response;

(ii) *Maximal sensitivity* to light.

In all probability, highly sensitive and very fast sensory mechanisms must have entirely different parameters. The calculation made in this group by Dr V.S. Markin has shown that a single-step system of amplification (see the Ca^{2+} pathway, fig.1), can be switched on right after addition of a stimulus (light) whereas a double-step mechanism (the cGMP pathway) is bound to have a lag-phase [41]. Even for this reason alone, a more sensitive system should be less rapid. Moreover, the cGMP mechanism involves several interactions of large molecules and a hydrolytic reaction, the events which would hardly allow the top rate of the overall process to be achieved.

Moreover, Markin's calculations clearly showed that an increase in the light intensity must soon result in saturation of the light-induced response if such a cascade of amplification as cGMP pathway is involved. This means that cGMP system can sense changes in illumination only at very low intensities of light. On the other hand, systems organized like the Ca^{2+} pathway, require a much higher intensity of light to be saturated.

Besides, the Ca^{2+} pathway seems to be perfect for fast response. Indeed, the cytoplasmic Ca^{2+} level in the dark is very low owing to activity of the Ca^{2+} pumps. This means that substantial increase in the cytoplasmic Ca^{2+} occurs when Ca^{2+} release to cytoplasm starts. As to the light-induced rise of Ca^{2+} permeability of a rhodopsin-containing membrane, it is apparently very rapid. In our experiments on photoreceptor discs incorporated into a collodion film [2], we not only confirmed observations on the light-dependent increase in disc membrane permeability [24,42–50] but also revealed that such an effect requires < 50 ms. (A faster measurement is impossible because of a conductivity increase due to operation of rhodopsin as a photogenerator of much lower internal resistance than that of the disc membrane.)

The suggestion was put forward [2] that this fast permeability increase is due to electric break-down of the membrane induced by a rhodopsin-generated field. This should, apparently, be a local, rather than delocalized, field.

Such an assumption seems to be necessary if we consider the outer membrane of cones. This membrane contains iodopsins, analogues of the rod pigment rhodopsin with different absorption maxima. In iodopsins, as in rhodopsin, there is 11-*cis* retinal which is isomerized to all-*trans* retinal by light. The mechanism of photolysis of iodopsins strongly resembles that of rhodopsin. The end-products are free retinal and opsins. Most probably, iodopsins are also competent in generation of photopotential since ERP of retina, which seems to be mainly due to the activity of cones, is

very similar to the photoelectric response of photoreceptor discs, measured in our experiments [2]. Calculation showed that iodopsin-produced $\Delta\Psi$, if delocalized all over the outer membrane of a cone, proved to be much lower than the dark $\Delta\Psi$ across the same membrane. This undermines any idea assuming delocalized ERP as a working component. This may be overcome by suggesting that the light-driven mechanism of the permeability increase operates sufficiently fast to utilize the energy of the local electric field produced by iodopsin or rhodopsin.

As was found by Benz and Zimmermann [51,52], a 1 V $\Delta\Psi$ across a bilayer phospholipid membrane induces an increase in the membrane conductance due to the formation of pores of a size similar to that of light-induced pores in rhodopsin-containing membranes. Formation of pores in planar membrane was found to occur faster than in 10 ns.

A potential difference as high as 1 V across a photoreceptor membrane is hardly probable even for a local field. However, there are several reasons to suggest that the electric break-down voltage for a photoreceptor membrane is much lower than that for a model one:

(i) In photoreceptor membrane phospholipids, there is a high percentage of extremely unsaturated fatty acids (up to 6 double bonds).
(ii) There is a Ca^{2+} asymmetry across this membrane due to a much higher $[Ca^{2+}]$ on the side which charges positively due to rhodopsin photoelectrogenesis.
(iii) Photoreceptor membrane contains proteins besides phospholipids.

All these factors were shown to be favourable for electrical breakdown [2,53,54], the latter being especially important if not only the lipid but also protein components are organized so as to facilitate the electric field-induced permeability increase.

The local field effect would be stabilized in time if light triggers a process resulting in a higher permeability state which exists longer than the field. The process in question may be aggregation of long-lived products of rhodopsin photolysis [43,50].

The above scheme postulating local-field induced permeability increase is only one of possible versions of a more general concept assuming that light-induced charge translocation is a common function of animal and bacterial rhodopsins. Other tentative schemes based on the same concept may also be considered.

For instance, one may speculate that rhodopsin translocates H^+ from water to a Ca^{2+}-binding site inside the membrane so that H^+ substitutes for Ca^{2+} at this site and Ca^{2+} releases to water. If mechanisms of the light-induced H^+ translocation in two rhodopsins are similar it can explain similarities of their structural and electrical properties.

6. INTERRELATIONS OF FAST AND SLOW PATHWAYS

An interesting possibility arises if one analyzes the conditions favourable for the manifestation of each of the two functions of rhodopsin. Under very low levels of illumination when excitation of a photoreceptor is induced by several photons or even by a single photon, the cGMP pathway should be operative since it involves a cascade of signal amplification. Increasing the light, we can switch on the less sensitive but more rapid Ca^{2+} pathway. When the intensity of illumination reaches a certain critical level, the Ca^{2+} pathway should prove to be dominant. This does not mean, however, that the cGMP pathway becomes indifferent to the events taking place in the photoreceptor cell. Being too slow to compete with the Ca^{2+} pathway for an immediate response to the light, the cGMP system can prepare the photoreceptor for the next portion of photons, by decreasing the photoreceptor sensitivity. Indeed, accumulation of metarhodopsin II must result in the activation of phosphodiesterase and, hence, in a cGMP level decrease. The latter effect that develops after the generation of the Ca^{2+}-mediated late receptor potential, proves to be favourable for the maintenance of the Na^+ channels in the closed state during the period between two light stimuli. This results in a situation when each next photon absorbed by a rod has a lower probability of inducing closure of the Na^+ channel than the previous one. In this way, adaptation of retina cells to light may occur.

The 'dualistic' concept of vision may account for both similarities and differences in the structural and functional properties of bacterial and animal rhodopsins. The equal parameters of photoelectric

responses of the two rhodopsins proves to be a consequence of the fact that the light-driven charge translocation which is the only function of bacteriorhodopsin is also one of the functions of the animal pigment. In the same way we may explain the similarities of all the series of light-induced spectral shifts as well as of many structural features of rhodopsins. Among them the arrangement of rhodopsins in the membrane should be mentioned.

In the primary structure of bacteriorhodopsin, Ovchinnikov [55–58] has revealed alternating hydrophilic and hydrophobic amino acid sequences that correspond to the 7 α-helical columns seen in Henderson and Unwin's model of this protein [59–61]. A similar array of hydrophilic and hydrophobic sequences pointing to the existence of 7 transmembrane columns was found by the same group for animal rhodopsin. In both rhodopsins, retinal was found to be bound to a lysine localized in the middle part of the last (closest to the C-end) column. The main structural feature which distinguishes the animal from bacterial protein is that some of the hydrophilic sequences proved to be larger in the visual pigment, the fact explaining its higher M_r-value. One may speculate that it is this part of the molecule that is involved in the interaction with transducin.

The existence of the additional (transducin-linked) function of animal rhodopsin can explain why this pigment undergoes irreversible photolysis instead of the reversible photocycle of bacteriorhodopsin. Apparently, there is a need to have a long-lived transient, say metarhodopsin II, which has time to deal with many molecules of transducin, facilitating substitution of GTP for GDP. The bifunctional character of the photosensitive protein forming $\Delta\Psi$ and a long-lived intermediate may well be an evolutionary gain of higher animals, that solves the same problem as, at the cellular level, the differentiation of photoreceptor cells into rods and cones involved in the dusk-light vision and bright-light vision, respectively.

It is interesting that in some primitive photoreceptor systems, the light-sensitive mechanism proves to be less intricate and less effective. For example, in light-sensitive neurons of marine mollusc *Aplysia californica*, Ca^{2+}-loaded membrane vesicles were found [62–64]. The vesicles named lipochondria were shown to contain pigments with β-carotene and a 'retinol-like' compound as chromophores [65]. Illumination induces release of Ca^{2+} from lipochondria [63,64]. The same could be initiated by addition of a Ca^{2+} ionophore [65]. In neurons, Ca^{2+} release was found to result in a rise of K^+ conductance and excitation [66,67]. Comparison of this system with the retinal photoreceptor showed that the latter has a much lower threshold and a much better amplification. Perhaps, the lipochondrial type of photosensing exemplifies a 'monistic' (in this case, calcium) mechanism. To verify this speculation, an analysis of the electrogenic activity of lipochondrial pigment as well as of the transducin cycle in *Aplysia* neurons seems to be necessary.

In principle, a system mediated by cyclic nucleotides with no Ca^{2+} involved, may represent another type of the 'monistic' mechanism. A light-sensitive system of *Phycomyces blakesleeanus* seems to be a candidate for this role [6]. In this organism, blue light absorbed by a flavoprotein was found to activate a cAMP-specific phosphodiesterase. The effect is potentiated by GTP [68]. Dibutyryl cAMP added to the fungus triggered a negative growth response which was abolished by blue light [69].

It is obvious that both visual rhodopsin and light-sensitive flavoprotein systems are two particular cases of a more general mechanism responsible for the amplification of a signal from an extracellular effector and its transmission to cyclic nucleotides in cytoplasm [1]. It is shown that the action of hormones, such as catecholamines, is mediated by a membrane-linked receptor which, in combination with the hormone, greatly facilitates the substitution of GTP for GDP bound to the so-called G protein [70]. G protein • GTP complex activates adenylate cyclase until bound GTP splits to GDP and phosphate [71]. This system, like the transducin mechanism, has a rather slow rate but involves at least two steps of amplification [72].

As to the Ca^{2+} pathway of photoreception, it resembles certain light-independent regulatory systems mediated by Ca^{2+}. In this respect, the role of Ca^{2+} in contraction of fast muscles may be especially interesting: I mean the chain of events initiated by a change in $\Delta\Psi$ across the outer muscle cell membrane, which might involve a Ca^{2+} influx from intercellular space to cytoplasm, a release of Ca^{2+} from sarcoplasmic reticulum and interaction

of this ion with the actomyosin system.

Interaction of Ca^{2+}- and cyclic nucleotide-mediated pathways can be exemplified by the effect of adrenaline on cardiac muscle. It is known that the hormone modulates contractile activity of this muscle by means of two distinct mechanisms: one results in a rise of $[Ca^{2+}]$ in cytoplasm; the other includes an increase in the cAMP level [78]. Such a situation is similar to that postulated in our scheme of vision.

The strongest argument against the Ca^{2+} hypothesis of vision, according to Uhl and Abrahamson [8] is the observation of Szuts [73] that only 10% of the Ca^{2+} content of the photoreceptor discs is substituted by $^{45}Ca^{2+}$ during a 30 min illumination of intact retina perfused in a pure $^{45}Ca^{2+}$-containing medium. In the context of the 'dualistic' hypothesis, this observation may indicate that it is intercellular, rather than intradiscal, space that should be regarded as the Ca^{2+} reservoir. If this were the case, rods might be considered as largely cGMP photoreceptors, whereas cones largely Ca^{2+} photoreceptors. According to this point of view, a cone should absorb Ca^{2+} in response to the excitation of any rhodopsin molecules; in a rod it is only the rhodopsins which are localized in the plasma membrane that should be competent in Ca^{2+} permeability increase, the major pool of rhodopsin in discs being ineffective in this respect. The possibility of a fundamental difference in the mechanisms of reception of dusk light in rods and of bright light in cones is considered by Botchkin, Zak and Ostrovsky [77].

There are some indications that rhodopsins in the plasma membrane differ in some features from disc membrane rhodopsins. In particular, it was noted that rhodopsin phosphorylation, a slow light-dependent process apparently involved in the adaptation of the photoreceptor, occurs only in plasma membrane and in freshly formed discs at the basal end of the rod outer segment (review [8]). Phosphorylation results in the addition of 8 phosphate groups causing strong increase in the total negative charge of the protein. This effect should, most probably, prevent rhodopsin aggregation and induce decomposition of pre-formed aggregates. In this way, the slow Ca^{2+} mechanism (stable channels) can be abolished without inhibition of the fast Ca^{2+} pathway mediated by electrical breakdown. Besides, phosphorylation might prevent interaction of metarhodopsin with transducin.

When analyzing the possible relationship of the Ca^{2+}-pathway and the cGMP-pathway, we should take into account that a certain minimal level of Ca^{2+} may be necessary for the light-induced cGMP decrease. This effect may be due to Ca^{2+} activation of the transducin–phosphodiesterase system [7] and/or Ca^{2+} inhibition of guanylate cyclase [74]. In particular, it was found that in crude frog rod outer segment preparations, decrease in external $Ca^{2+} < 10^{-7}$ M resulted in desensitization of phosphodiesterase to light [7].

One cannot rule out that the Ca^{2+} pathway does not reach the Na^{+} channel, and the role of Ca^{2+} in vision is confined to their effect on the cGMP system (e.g., [7,43]). In this case, however, more than one step of amplification seems to be involved in the Ca^{2+} pathway, so that a stimulus and the response prove to be separated by a lag-phase [41]. If so, the Ca^{2+} mechanism loses its kinetic advantages.

Another possibility is that the cGMP-linked mechanism deals exclusively with a special system of light and dark adaptation rather than with signal transmission mediated by Ca^{2+} only. In this case, however, we shall have to face all the 'anti-calcium' arguments (e.g., [8]).

The concept developed in this paper differs in two respects from the rather diffuse earlier concepts postulating a Ca^{2+}- and cGMP-containing black box (e.g., [75]). It takes into account the electrogenic function of rhodopsin and allows several ways of experimental verification to be indicated.

7. CONCLUSIONS AND PREDICTIONS

The above concept of vision rests on the assumption that visual rhodopsin can initiate at least two separate processes of different time scale and molecular mechanism. They are:

(i) Fast charge translocation which results in the release of a transmitter, say Ca^{2+}, into the cytoplasm of the photoreceptor cell.

(ii) Slower process(es) mediated by a long-lived product(s) of rhodopsin photolysis, activating cGMP hydrolysis via transducin.

Item (i) explains the striking similarities of bacterial and animal rhodopsins in: photoelectric responses, initial steps of photolysis and the structure of the membranous parts of protein. Moreover, it accounts for fast light-induced increase in the ion permeability of photoreceptor membrane.

Item (ii) deals with the features specific for visual rhodopsin only, such as: activation of transducin; irreversibility of photolysis; existence of the additional hydrophilic cytoplasm-exposed parts of the protein, absent from bacteriorhodopsin.

The 'dualistic' scheme allows the following predictions to be made:

(1) Two modes of the photoreceptor cell excitation can be distinguished:
(i) A fast one, mediated by a charge translocation in rhodopsin rather than by accumulation of metarhodopsin II;
(ii) A slow one, linked to the accumulation of products of rhodopsin photolysis.
Amplification of a light signal in the fast mechanism should be lower than in the slow one.

(2) If Ca^{2+} are not necessary for the slow mechanism, it can be studied in Ca^{2+}-free systems. If, however, Ca^{2+}-requiring steps are involved in both pathways, one may hope that the half-maximal $[Ca^{2+}]$ are different in the fast and slow mechanisms.

(3) Acceleration of metarhodopsin II decay should specifically inhibit the cGMP-pathway but not the Ca^{2+} pathway.

(4) Rhodopsin derivatives may be obtained lacking one of the functions of this protein. For example, the transducin-activating ability of rhodopsin should be more sensitive to proteinases than the $\Delta\Psi$-generating function if the former is due to the hydrophilic, and the latter to the hydrophobic part of the molecule. It is appropriate to mention here that papain-treated bacteriorhodopsin deprived of its hydrophilic terminal sequences and split between the second and the third α-helical columns retains its photoelectric activity [76].

ACKNOWLEDGEMENTS

The author expresses his gratitude to Professor Yu.A. Ovchinnikov, Professor M.A. Ostrovsky, Dr L.M. Botchkin, Dr L.A. Drachev, Dr G.R. Kalamkarov, Dr A.D. Kaulen and Dr V.S. Markin for valuable discussion and very useful advice. This study was carried out within the framework of research program 'Rhodopsin' organized by the USSR Academy of Sciences and Moscow State University, and supervised by Vice-President of the USSR Academy of Sciences, Professor Yu.A. Ovchinnikov.

REFERENCES

[1] Stryer, L., Hurley, J.B. and Fung, B.K.-K. (1981) Trends Biochem. Sci. 6, 245–247.
[2] Drachev, L.A., Kalamkarov, G.R., Kaulen, A.D., Ostrovsky, M.A. and Skulachev, V.P. (1981) Eur. J. Biochem. 117, 471–781.
[3] Bitensky, M.W., Gorman, R.E. and Miller, W.H. (1971) Proc. Natl. Acad. Sci USA 68, 561–562.
[4] Hagins, W.A. (1972) Annu. Rev. Biophys. Bioeng. 1, 131–158.
[5] Honig, B. (1978) Annu. Rev. Phys. Chem. 29, 31–57.
[6] Shichi, H. and Rafferty, Ch.N. (1980) Photochem. Photobiol. 31, 631–639.
[7] Bownds, M.D. (1980) Photochem. Photobiol. 32, 487–490.
[8] Uhl, R. and Abrahamson, E.W. (1981) Chem. Rev. 81, 291–312.
[9] Fung, B.K.-K. and Stryer, L. (1980) Proc. Natl. Acad. Sci. USA 77, 2500–2504.
[10] Fung, B.K.-K., Hurley, J.B. and Stryer, L. (1981) Proc. Natl. Acad. Sci. USA 78, 152–156.
[11] Fukada, Y., Kawamura, S., Yoshizawa, T. and Miki, N. (1981) Biochim. Biophys, Acta 675, 188–194.
[12] Fukada, Y. and Yoshizawa, T. (1981) Biochim. Biophys. Acta 675, 195–200.
[13] Cone, R.A. (1964) J. Gen. Physiol. 47, 1089–1105.
[14] Ostroy, S.E. (1977) Biochim. Biophys. Acta 463, 91–125.
[15] Kilbride, P. and Ebrey, T.G. (1979) J. Gen. Physiol. 74, 415–426.
[16] Brown, K.T. and Murakami, M. (1964) Nature 201, 626–628.
[17] Cone, R.A. and Pak, W.L. (1972) in: Handbook of Sensory Physiology (Loewenstein, W.R. ed) vol. 1, pp. 345–367, Springer, Heidelberg, New York.

[18] Kayushin, L.P. and Skulachev, V.P. (1974) FEBS Lett. 39, 39–42.
[19] Drachev, L.A. Kaulen, A.D., Ostroumov, S.A. and Skulachev, V.P. (1974) FEBS Lett. 39, 43–45.
[20] Racker, E. and Stoeckenius, W. (1974) J. Biol. Chem. 249, 662–663.
[21] Belyakova, T.N., Kadzyauskas, Yu.P., Skulachev, V.P., Smirnova, I.A., Chekulaeva, L.N. and Jasaitis, A.A. (1975) Dokl. Akad. Nauk SSSR 223, 483–486.
[22] Trissl, H.W., Darszon, A. and Montal, M. (1977) Proc. Natl. Acad. Sci. USA 74, 207–210.
[23] Trissl, H.W. (1979) Photochem. Photobiol. 29, 579–588.
[24] Montal, M. (1979) Biochim. Biophys. Acta 559, 231–257.
[25] Bolshakov, V.I., Kalamkarov, G.R. and Ostrovsky, M.A. (1978) Dokl. Akad. Nauk SSSR 240, 1241–1244.
[26] Lindau, M., Hochstrate, P. and Ruppel, H. (1980) FEBS Lett. 112, 17–20.
[27] Drachev, L.A., Kaulen, A.D. and Skulachev, V.P. (1977) Mol. Biol. SSSR 11, 1377–1387.
[28] Drachev, L.A., Kaulen, A.D. and Skulachev, V.P. (1978) FEBS Lett. 87, 161–167.
[29] Drachev, L.A., Kaulen, A.D., Semenov, A.Yu., Severina, I.I. and Skulachev, V.P. (1979) Anal. Biochem. 96, 250–262.
[30] Skulachev, V.P. (1979) Methods Enzymol. 55, 586–603; 751–776.
[31] Bolshakov, V.I., Kalamkarov, G.R. and Ostrovsky, M.A. (1980) in: Frontiers of Bioorganic Chemistry and Molecular Biology (Ananchenko, S.N. ed) pp. 433–438, Pergamon, Oxford, New York.
[32] Bolshakov, V.I., Kalamkarov, G.R. and Ostrovsky, M.A., (1979) Dokl. Akad. Nauk SSSR 248, 1485–1488.
[33] Bolshakov, V.I., Drachev, A.L., Drachev, L.A., Kalamkarov, G.R., Kaulen, A.D., Ostrovsky, M.A. and Skulachev, V.P. (1979) Dokl. Akad. Nauk SSSR 248, 1462–1466.
[34] Drachev, L.A., Kalamkarov, G.R., Kaulen, A.D., Ostrovsky, M.A. and Skulachev, V.P. (1980) FEBS Lett. 119, 125–131.
[35] Chapron, Y. (1979) CR Hebd. Seances Acad. Sci Paris, 288, 155–158.
[36] Chapron, Y. (1980) Photobiochem. Photobiophys. 1, 297–304.
[37] Bamberg, E., Bauer, P.J. and Fahr, A. (1980) Annu. German Biophys. Soc. Meet. Abstr. B (7).
[38] Cafiso, D.S. and Hubbell, W.L. (1980) Biophys. J. 30, 243–264.
[39] Drachev, L.A., Kaulen, A.D., Khitrina, L.V. and Skulachev, V.P. (1980) Eur. J. Biochem. 117, 461–470.
[40] Drachev, L.A., Semenov, A.Yu., Skulachev, V.P., Smirnova, I.A., Chamorovsky, S.K., Kononenko, A.A., Rubin, A.B., and Uspenskaya, N.N. (1980) Eur. J. Biochem. 117, 483–489.
[41] Markin, V.S. and Skulachev, V.P. (1982) Biofizika USSR 27, in press.
[42] Fesenko, E.E. and Lyubarsky, A.L. (1977) Nature 268, 562–563.
[43] Montal, M., Darszon, A. and Schindler, H. (1981) Quart. Rev. Biophys. 14, 1–79.
[44] Shevchenko, T.F. (1976) Biofizika USSR 21, 321–323.
[45] Ostrovsky, M.A. (1978) in: Membrane Transport Processes (Tosteson, D.C. et al. eds) pp. 217–243, Raven Press, New York.
[46] Hubbell, W.L., Fung, B.K.-K., Hong, K. and Chen, Y.S. (1977) in: Vertebrate Photoreception (Barlow, H.B. and Fatt, P. eds) pp. 41–59, Academic Press, London, New York.
[47] O'Brien, D.F., Zumbulyadis, N., Michaels, F.M. and Ott, R. (1977) Proc. Natl. Acad. Sci. USA 74, 5222–5226.
[48] Darszon, A., Montal, M. and Zarco, J. (1977) Biochem. Biophys. Res. Commun. 76, 820–827.
[49] Shevchenko, T.F., Kalamkarov, G.R. and Ostrovsky, M.A. (1980) Biofizika USSR 25, 462–468.
[50] Montal, M. and Borochov-Neori, H. (1981) 7th Int. Biophys. Congr. Mexico City, Abstr. p.195.
[51] Benz, R. and Zimmermann, U. (1980) Biochim. Biophys. Acta 597, 637–642.
[52] Benz, R. and Zimmermann, U. (1981) Biochim. Biophys. Acta, 640, 169–178.
[53] Pilwat, G., Hampp, R. and Zimmermann, U. (1980) Planta (Berl.) 147, 396–404.
[54] Chernomordik, L.V. (1979) Thesis, Electrochem. Inst. USSR Acad. Sci, Moscow.
[55] Ovchinnikov, Yu.A. (1979) Eur. J. Biochem. 94, 321–336.
[56] Ovchinnikov, Yu.A., Abdulaev, N.G., Feigina, M. Yu., Kiselev, A.V. and Lobanov, N.A. (1979) FEBS Lett. 100, 219–224.
[57] Ovchinnikov, Yu.A. (1980) in: Sov. Sci. Rev. (Biol.) (Skulachev, V.P. ed) vol. 1, pp. 1–42, Harwood Academic, New York.
[58] Ovchinnikov, Yu.A. (1981) in: Chemiosmotic Proton Circuits in Biological Membranes (Skulachev, V.P. and Hinkle, P.C. eds) pp. 311–320, Addison-Wesley, London, Amsterdam; Don Mills, Sydney, Tokyo.
[59] Henderson, R. and Unwin, P.N.T. (1975) Nature 257, 29–32.
[60] Unwin, P.N.T. and Henderson, R. (1975) J. Mol. Biol. 94, 425–440.

[61] Henderson, R. (1977) Annu. Rev. Biophys. Bioeng. 6, 87–109.
[62] Chalazonitis, N. and Arvanitaki, A. (1956) Arch. Sci. Physiol. 10, 291–319.
[63] Brown, A.M., Baur, P.S. jr and Tuley, F.H. jr (1975) Science 188, 157–160.
[64] Baur, P.S. jr., Brown, A.M., Rogers, T.D. and Brower, M.E. (1977) J. Neurobiol. 8, 19–42.
[65] Krauhs, J.M., Sordahl, L.A. and Brown, A.M. (1977) Biochim. Biophys. Acta 471, 25–31.
[66] Brown, A.M. and Brown, H.M. (1973) J. Gen. Physiol. 62, 239–254.
[67] Brown, A.M., Brodwick, M.S. and Eaton, D.C. (1977) J. Neurobiol. 8, 1–18.
[68] Cohen, R.J. and Atkinson, M.M. (1978) Biochem. Biophys. Res. Commun. 83, 616–621.
[69] Cohen, R.J. (1974) Nature 251, 144–146.
[70] Cassel, D. and Selinger, Z. (1978) Proc. Natl. Acad. Sci. USA 75, 4155–4159.
[71] Pfeuffer, T. (1979) FEBS Lett. 101, 85–89.
[72] Citri, Y. and Schramm, M. (1980) Nature 287, 297–300.
[73] Szuts, E.Z. (1980) J. Gen. Physiol. 76, 253–286.
[74] Fleischman, D. and Denisevich, M. (1979) Biochemistry 18, 5060–5066.
[75] Lipton, S.A., Rasmussen, H. and Dowling, J.E. (1977) J. Gen. Physiol. 70, 771–791.
[76] Abdulaev, N.G., Feigina, M.Yu., Kiselev, A.V., Ovchinnikov, Yu.A., Drachev, L.A., Kaulen, A.D., Khitrina, L.V. and Skulachev, V.P. (1978) FEBS Lett. 90, 190–194.
[77] Botchkin, L.M., Zak, P.P. and Ostrovsky, M.A. (1982) in preparation.
[78] Barany, M. and Barany, K. (1980) Annu. Rev. Physiol. 42, 275–292.