Hypothesis

An extended Ca^{2+}-hypothesis of visual transduction with a role for cyclic GMP

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A model is described having the following features: Light induces Ca^{2+} release from vertebrate rod outer segments discs via pores composed of multimeric rhodopsin. Cytoplasmic Ca^{2+} reversibly blocks Na^{+} channels of the surface membrane, with the time course of development and amplitude of the response to light being influenced by restrictions on intradiscal Ca^{2+} diffusion. The falling phase of response reflects a decline in cytoplasmic [Ca^{2+}] due to a Ca^{2+}-binding protein controlled by cyclic GMP so that its binding capacity is increased by the reduction in cytoplasmic [cyclic GMP] which follows rhodopsin bleaching.

Visual transduction *Rod outer segment* *Ca^{2+}-hypothesis* *Cyclic GMP*

1. INTRODUCTION

The idea that Ca^{2+} might act as the intracellular transmitter for visual signalling in the vertebrate photoreceptor (with reference particularly to the rod photoreceptor) has been actively discussed since it was put forward a decade ago [1]. While initially received with enthusiasm, no doubt in large measure because of the analogy which could be drawn with other Ca^{2+}-controlled biological systems, the Ca^{2+}-hypothesis has made little progress in its acceptability by workers in the field and recently its standing has declined markedly. Three factors have contributed to this:

(1) There has been a failure under certain experimental conditions to observe Ca^{2+} movements that would appear to be required by the hypothesis;
(2) Certain features of the light-evoked surface membrane response appear to indicate a transduction mechanism with different kinetics from that offered by the hypothesis;
(3) An alternative model of intracellular transmission involving a different transmitter substance (cyclic GMP) has appeared.

It is proposed here to show how much of the criticism directed at the Ca^{2+}-hypothesis can be answered and to produce an extension to the hypothesis which takes into account some observations made on cyclic GMP.

2. ESSENTIAL FEATURES OF THE Ca^{2+}-HYPOTHESIS

A model for visual signal transduction, whereby bleaching of a rhodopsin molecule situated in the disc membrane in the interior of a rod outer segment affects the ionic permeability of the surface membrane, is illustrated in section A of fig. 1. According to this, in the dark-adapted state (i.e., when there are no photically activated rhodopsin molecules present) Ca^{2+} is present in a high concentration (~20 nM) in the narrow intradiscal space (constituting about 0.1 of the rod outer segment volume), while the concentration in the cytoplasmic space (about 0.5 of the volume) is at a

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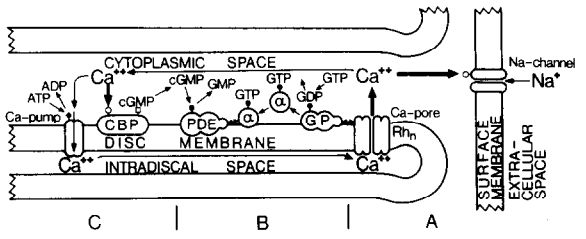

Fig. 1. A model of visual signal transduction in the rod outer segment of the veretebrate retina. Action of Ca^{2+} as intracellular transmitter is shown in the section on the right (A). Photo-activation of cyclic GMP-phosphodiesterase is shown in the middle section (B). Cyclic GMP-controlled Ca^{2+} binding and ATP-driven Ca^{2+}-pump activity are shown in the section on the left (C). Short double-headed arrows indicate protein–protein interaction. Other active sites on proteins are indicated as follows: (○) site of reversible Ca^{2+} binding; (□) site of reversible cyclic GMP binding; (●) site of GDP and GTP binding and of GTP hydrolysis; (■) site of cyclic GMP hydrolysis; (♦) site of ATP hydrolysis.

much lower level (~1 μM). The first of these figures for Ca^{2+} concentration is not to be equated with Ca^{2+} activity, since, with the paired membranes of a disc separated by only 3 nm, one would expect interaction of Ca^{2+} with the membrane surfaces to be important. Despite this, Ca^{2+} activity within the disc is still expected to be >100-times that in the cytoplasm, thus providing a large driving force for an outward movement of Ca^{2+} from the disc. Also, the reduction of its activity need not significantly restrict the amount of Ca^{2+} that can leave the disc, since its removal may occur largely by exchange for other cations; viz., Na^+ and K^+.

Following absorption of a photon and conversion of a rhodopsin molecule to its active form (probably metarhodopsin II), a passage is formed across the disc membrane, through which Ca^{2+} move from the intradiscal to the cytoplasmic space (shown in the figure by the upward directed heavy arrow). This passage is designated a 'Ca^{2+}-pore', although there are indications that it allows the movement of a wide range of ions. The term 'pore' is used to indicate a water-filled, relatively non-selective passage, as distinct from a more selective 'channel'. The special position of Ca^{2+} in connection with this pore is that it is expected to be the only ion present and capable of moving through the pore that is not in equilibrium across the disc membrane in the dark-adapted state. The number of Ca^{2+} moving through the pore during the first second after absorption of a photon (corresponding to the time-to-peak of the surface membrane response) is estimated to be ~5000 (a figure which is arrived at on the basis that a single-photon response of a frog rod outer segment would entail an increase of ~5% in cytoplasmic Ca^{2+} concentration extending over ~5% of its volume). The pore is considered to open with a mean delay of ~10 ms following photon absorption, as inferred from a component of light-evoked conductance change observed in suspensions of rod outer segments and attributed to a non-selective ionic permeability increase of the disc membrane [2]. In the figure, the pore-forming structure is shown as composed of *n* rhodopsin molecules, based on observation of the saturation of the light-evoked conductance change [3] and on protein separation studies [4], which together suggest that 12 rhodopsin molecules of at least 2 forms are involved in the pore structure. That a complex arrangement of this sort is required might be expected on the grounds that, apart from its terminal portions, the rhodopsin polypeptide chain is composed very largely of non-polar amino acids [5], so that several such chains would be needed to furnish a sufficient number of polar side-groups to establish an hydrophilic region penetrating the membrane.

Having passed through the pore, Ca^{2+} will diffuse in the cytoplasmic space to raise the concentration adjacent to the surface membrane (shown by the heavy arrow directed to the right). In this membrane there are present Na^+-channels which, in the dark-adapted state, permit a nett inward movement of Na^+ under the influence of the electrochemical potential difference for Na^+ between the cytoplasmic and extracellular spaces. These channels are considered to be capable of being blocked by reversible combination of a Ca^{2+} with a specific binding site on the cytoplasmic side of the channel-forming structure or possibly within the channel.

3. CRITICISM OF THE HYPOTHESIS

Objections to the fundamental idea of the Ca^{2+}-hypothesis (that is apart from details of pore- and channel-forming structures) have come from a number of directions. It has been argued that there

is a lack of experimental evidence for an accumulation of diffusible Ca^{2+} in the discs or for a light-evoked movement of Ca^{2+} across the disc membrane [6]. A possible way of discounting such negative findings is to suppose that they are a consequence of damage to the rod outer segment in the preparation of material for examination. It is perhaps significant that those studies which have provided evidence for Ca^{2+} movements in support of the hypothesis have depended on the use of undamaged rod outer segments; i.e., with the surface membrane intact or even with the entire photoreceptor cell intact in the retina [7–9]. This requirement for the surface membrane to be intact applies also to observation of the disc-membrane conductance increase [10]. An implication of these findings is that some as yet unidentified diffusible substance present in the cytoplasm is essential for the functioning of the disc membrane.

Other objections have been based on the argument that the light-evoked response of the surface membrane observed in the intact rod outer segment (closure of Na^+-channels) does not correspond in its time course or in certain characteristics of its amplitude to predictions from the hypothesis. It may be noted in this connection that, even before Ca^{2+} was proposed as an intracellular transmitter, the possibility was considered that the delay in transmission of a visual signal could be accounted for by diffusion [11]. It was shown, using certain criteria for a threshold response of the retina, that over a 10^4–10^5-fold range of stimulating light intensities the same mathematical function as applies for diffusion can be used to describe this delay. The analysis however ignores the absolute time scale of the delay in visual transmission. Considering the response of the rod outer segment surface membrane, one finds that the time course of its rising phase is such that the postulated transmitter substance, assuming it to be released uniformly through the interior of the outer segment and to act at the surface membrane, would have to have a diffusion coefficient between 0.1–0.01 that of a simple water-soluble particle, such as the Ca^{2+}.

In view of the more delayed development of the surface membrane response compared with that predicted for Ca^{2+} diffusion (especially in the case of the large diameter amphibian rod outer segment), it has been widely accepted, as an alternative delay mechanism, that the time course for the closure of Na^+-channels following rhodopsin activation is determined by a succession of 4 first-order reactions with similar rate constants [12]. In [13] however, it has been argued that the time for development of the surface membrane response can be ascribed to the diffusion of Ca^{2+} on the grounds that Ca^{2+} will exist largely in an adsorbed state due to electrical interaction with fixed negative changes on the surface of the disc membrane, so that only a small fraction of the Ca^{2+} are free to diffuse at any instant. This is expected to be specially important in the intradiscal space, owing to the narrowness of the gap between the membranes of a disc. With intradiscal diffusion being the major factor influencing the development of response, it will also serve to account for the observation that the response is not significantly affected by whether the region of the disc illuminated is near the surface of the outer segment or near the rod axis, at the maximum distance from the surface membrane [14].

Yet a further objection to the Ca^{2+}-hypothesis has been based on the observation that unitary responses, recorded as transient membrane currents generated in a single rod outer segment by single-photon absorptions, display a strikingly small variation in amplitude as well as in time course [15, 16]. Statistical analysis of such unitary responses shows their amplitude to be approximately normally distributed with a standard deviation equal to $\sim 1/3$ of the mean. If one were to suppose that the amplitude of response is proportional to the amount of Ca^{2+} released into the cytoplasmic space, it would appear that this quantity cannot be determined by the time that the pore remains open. The reason is that for any simple mechanism for initiating pore closure, as for example by the dissociation of one particle (or molecular group) from another, first-order kinetics would apply. This means that in a set of pore operations the time during which the pore remains open will be distributed according to a decaying exponential function with the most probable duration of pore-open time being zero. The observed distribution of amplitudes is quite different.

With closure of the pore being thus excluded as a primary factor limiting the amount of Ca released from the disc to generate the response, one may assume that the pore remains open for an average time extending beyond the time-to-peak of the

response. Other factors may then be sought that will limit the amount of Ca^{2+} released by operation of a single pore. The combined effect of slow intradiscal diffusion of Ca^{2+} and the sub-division of the disc by incisures into lobes of fairly uniform size might function in this capacity. In any case, as a disc lobe (~1/20 of a frog disc or ½ of a mammalian disc) would contain ~40 000 Ca^{2+}, a single pore would be expected to deplete its Ca^{2+} content to the extent of at least 10% during the time of development of the response.

4. LIGHT-INDUCED CHANGES IN CYCLIC GMP

Recently, there has been a sudden shift of interest away from the Ca^{2+}-hypothesis toward a rival hypothesis which aims to account for visual transduction by a change in concentration of cytoplasmic cyclic GMP. The starting point of the cyclic GMP-hypothesis is the finding that in the dark-adapted state cyclic GMP is present in the cytoplasmic space at ~100 μM and that bleaching of rhodopsin causes a fall in its concentration [17–21]. The way in which this occurs is illustrated in section B of fig. 1. Bleaching of rhodopsin, probably to the stage of metarhodopsin II, causes a reaction site to appear on the protein allowing it to combine with another protein of the disc membrane variously termed 'G-protein', 'GTP-binding protein' or 'transducin' (GP in the figure). This protein adheres loosely to the cytoplasmic side of the disc membrane. It is a trimer with a molecule of GDP attached to the highest M_r subunits (α). As a consequence of the reaction with activated rhodopsin, the GDP is exchanged from GTP present in the cytoplasm. The G-protein then dissociates from rhodopsin and separates into its component subunits which leave the membrane to diffuse in the cytoplasmic space. By this process a single molecule of activated rhodopsin will react successively with many molecules of G-protein.

The α-subunit of G-protein with attached GTP has the capability of reacting with another protein on the cytoplasmic surface of the disc membrane or possibly on the surface of a different disc from that on which the G-protein was initially situated. This second protein is likewise a trimer and the reaction of it with the α-subunit of G-protein plus GTP causes its activation as a cyclic GMP-specific phosphodiesterase (PDE), probably through a change in the relation between its subunits. The active state of the enzyme is terminated by hydrolysis of the GTP on the α-subunit to GDP. Through the complete process, operating at low levels of rhodopsin bleaching, a single bleached rhodopsin molecule will activate several hundred phosphodiesterase molecules to give a rate of hydrolysis of cyclic GMP which can reach 400000 molecules/s. The time course over which this capacity to hydrolyse cyclic GMP developes is considered to be determined by diffusion of the active rhodopsin photoproduct and G-protein in the disc membrane. Experimental evidence for the reaction scheme has come from biochemical work involving the separation of disc membrane proteins as well as measurement of guanine nucleotide levels [22–24]. In addition indirect methods have been employed for detecting cyclic GMP hydrolysis by the H^+ released [25] and for reactions of the G-protein by light-scattering changes of suspensions of rod outer segments [26].

The idea that cyclic GMP would have an action on the surface membrane, such that cyclic GMP would be required to maintain Na^+-channels in the open condition (possibly by promoting phosphorylation of a channel-forming protein), makes this substance a candidate for intracellular transmitter. In this case, it would be a decrease in cytoplasmic concentration that transmits the visual signal. The consideration that cyclic GMP would have the proposed action is based mainly on inference, associating observed light-induced changes in cyclic GMP concentration with light-induced changes of the surface membrane. There is however one type of experiment which has been interpreted as giving a direct indication of cyclic GMP acting on the surface membrane [27]. This involves the injection of cyclic GMP directly into a rod cell in the retina via an intracellular micropipette which is also used for recording the electrical response of the cell. The result of cyclic GMP injection is a change in membrane potential (depolarization) consistent with an opening of Na^+-channels, together with an increase in latency of the light-evoked response. It is further observed that the cyclic GMP effect on membrane electrical properties is suppressed following an intense light stimulus which would be expected to cause activation of the phosphodiesterase of the rod outer segment. While these results are

consistent with the proposed action of cyclic GMP to open Na^+-channels of the rod outer segment surface membrane, it should be noted that the technique employed suffers from the fact that there is extensive electrical coupling between rod cells in the retina. Because of this, massive changes in electrical properties would have had to be produced in the cell into which the micropipette was introduced to give the observed effects. It is possible under these circumstances that the effects have been influenced by changes in electrical properties of the cell other than those involving the Na^+-channels of the outer segment.

A criticism of the cyclic GMP-hypothesis is that in the intact rod outer segment, unlike the case for fragmented material, the decline in cyclic GMP content is found to take place with a delay of ~1 s from the time of illumination and therefore is too late to be involved in generation of the surface membrane response [28, 29].

5. ACTION OF CYCLIC GMP IN AN EXTENDED Ca^{2+}-HYPOTHESIS

The present proposal for a role of cyclic GMP within the Ca^{2+}-hypothesis is shown in section C of fig. 1. The essential feature of the scheme is suggested by the observation that cyclic GMP causes a rapid release of absorbed Ca^{2+} from disc membranes prepared by mechanical disruption of rod outer segments [30]. A graded action is produced over 50–150 μM cyclic GMP, which covers the region of concentrations in which light-induced changes of cyclic GMP have been observed in intact rods. No effect is produced by cyclic AMP, GMP, GDP or GTP. The effect does not require nor is it influenced by the presence of ATP or GTP. The effect is also independent of whether the rhodopsin in the disc membrane has been bleached or not. The magnitude of the effect indicates that ~1/4 of adsorbed, tightly bound Ca (with a dissociation constant of ~1 μM) can be released by cyclic GMP. The capacity for this tightly bound Ca^{2+} in the absence of cyclic GMP is measured to be ~0.1 of the rhodopsin content (on a molar basis). Accordingly, referred to volume of cytoplasmic space the Ca^{2+} capable of release by cyclic GMP would amount to ~100 μM. Considering that the concentration of free Ca^{2+} in the cytoplasmic space is estimated to be only 1 μM, one is aware that even a small fractional change in cyclic GMP concentration would have an appreciable effect on the free Ca^{2+} content. The high-affinity binding is distinguished in Ca^{2+}-equilibration studies on bovine rod outer segment disc membrane from a low-affinity component of binding with a capacity some 100-times greater [31]. It is the latter which is considered to be involved in slowing the diffusion of Ca^{2+} by adsorption, particularly in the intradiscal space.

The involvement of cyclic GMP-dependent binding of Ca^{2+} in visual excitation is envisaged to be as follows. Light-evoked hydrolysis of cyclic GMP, taking place via the G-protein activation of phosphodiesterase, leads to a fall in cyclic GMP concentration of the cytoplasm. This in turn affects Ca^{2+}-binding sites on the disc membrane which are considered to reside in a specific Ca^{2+}-binding protein (CBP). The dissociation of cyclic GMP from this protein permits an uptake of Ca^{2+}, as the reverse of the process whereby addition of cyclic GMP causes a release of Ca^{2+}. It is presupposed that the association of cyclic GMP with the protein is reversible. As an extension of the Ca^{2+}-hypothesis, it is proposed that the cytoplasmic Ca^{2+} concentration, after being increased from its dark-adapted level by the release of Ca^{2+} from the intradiscal space and after having exerted its effect in closing Na^+-channels of the surface membrane, will then be reduced through the uptake of ions by the Ca^{2+}-binding protein. (The last of these movements is indicated by the downward directed heavy arrow in the figure.) Between the opening of the Ca^{2+}-pore in the disc membrane and the change in Ca^{2+} binding, there is expected to be an interval of ~1 s, caused by the delay in the decline of cyclic GMP concentration and this could be the main factor determining the time-to-peak of the surface membrane response.

An evident difficulty with this scheme is that the uptake of Ca^{2+} by the Ca^{2+}-binding protein cannot be expected to balance the preceding release of Ca^{2+} from the intradiscal space, owing to the independence of these two processes controlling cytoplasmic Ca^{2+}. Thus, on completion of a response the Ca^{2+} concentration and hence the fraction of Na^+-channels in the open state will not have returned to the dark-adapted condition. To some extent fluctuations of the Ca^{2+} concentration would be limited by the circumstance that the

dissociation constant for tightly bound Ca^{2+} is in the region of the prevailing concentration. Further stability, with increased likelihood of reversibility of response, would be achieved if the Ca^{2+} concentration were to affect the dark-adapted level of cyclic GMP, so that a decrease in Ca^{2+} concentration would cause an increase in cyclic GMP concentration. This, together with the action of cyclic GMP on Ca^{2+} binding, would then constitute a negative feedback loop. The required effect of Ca^{2+} on cyclic GMP level has been observed [32]. It is possible that this occurs via a regulatory action of Ca^{2+} on guanylate cyclase, the enzyme responsible for synthesis of cyclic GMP.

Several phenomena involving changes in the time course of the surface membrane response can be explained by an action of cyclic GMP on the falling phase. Exposure of the retina to a low concentration of IBMX (when the drug is expected to act specifically as an inhibitor of the phosphodiesterase) causes a prolongation of the surface membrane response for low levels of photic stimulation [33]. Similarly, in mutant mice, heterozygous for the retinal dystrophy gene (the C3H strain), for which there is evidence of a reduction in the light-regulated phosphodiesterase, the response has a prolonged falling phase compared with that in normal mice [34]. This form of behaviour is consistent with the light-induced reduction in cyclic GMP being responsible for the falling phase of the response in accordance with the present model. A change of response in the opposite direction (an accelerated falling phase with curtailment of the time-to-peak) is produced by background illumination [35]. This effect appears to reach a steady state in ~10 s from the onset of background illumination. It suggests a priming of the process controlling the falling phase of the response by the continued presence of activated rhodopsin molecules. One way in which this might occur is if more than one G-protein molecule were required for activation of a molecule of phosphodiesterase. Another way is if more than one molecule of cyclic GMP were required to affect the Ca^{2+}-binding protein.

6. MODIFICATION OF RESPONSE BY Ca^{2+}

The short-term effects of variation in Ca^{2+} concentration, produced either by changing the Ca^{2+} concentration of the extracellular solution or by introducing Ca^{2+} or Ca^{2+}-buffering reagents into the cytoplasm of the outer segment, are quite different from those described above. Provided the response is in the linear region (i.e., there is a linear relation between the intensity of light stimulus and the amplitude of response) its time course is not affected appreciably by changes of Ca^{2+} which produce large changes in amplitude of response [36–39]. Increased Ca^{2+} causes a reduction in response amplitude which can be accounted for most simply by blockage of a proportion of the Na^{+}-channels so that fewer of them are available to be closed by the light stimulus. A decrease in Ca^{2+} has the reverse effect. The response is augmented up to several times its amplitude under normal Ca^{2+}. This is accounted for by a larger number of Na^{+}-channels being in the open state before the stimulus, which closes a fixed fraction of channels that are open. These effects are clearly directly explicable in terms of the Ca^{2+}-hypothesis, with going beyond the proposal for a control site on the Na^{+}-channel which combines reversibly with Ca^{2+}.

Exposure of rod outer segments to a low concentration of Ca^{2+} (<1 μM in the extracellular medium) for a prolonged period (>5 min) results in a different form of behaviour. The response to a weak light stimulus, after having been initially increased by exposure to low Ca^{2+}, now becomes drastically reduced in amplitude. This is consistent with the Ca^{2+}-hypothesis on the assumption that the maintenance of cytoplasmic Ca^{2+} at a low level (below that prevailing in the dark-adapted state) for an extended period causes a reduction in the Ca^{2+} content of the discs, so that the amount of Ca^{2+} released by the opening of a pore is correspondingly reduced. Under these conditions a new phenomenon appears – that of superlinearity of the response to weak stimuli. The response amplitude, instead of increasing in proportion to the stimulating light intensity, increases more steeply so that there appears to be a threshold for eliciting a detectable response. This behaviour can be accounted for by the existence of Ca^{2+}-binding sites which take up the small amounts of Ca^{2+} released from the discs in response to weak, effectively subthreshold stimuli, but these sites become saturated with stronger stimuli. Presumably, the ability to detect this form of behaviour is facilitated by the magnification of response for a

given amount of Ca^{2+} released, due to the number of Na^+-channels available to be closed being increased at the prevailing low cytoplasmic Ca^{2+} level. On the other hand, the low Ca^{2+} level would have an opposing effect through increasing the cyclic GMP concentration, which, on the present scheme, would reduce the number of exposed Ca^{2+}-binding sites.

7. PROCESS INVOLVED IN RESTORATION OF INTRADISCAL Ca^{2+}

The surface membrane response reflecting a rise and fall of cytoplasmic Ca^{2+} concentration is, for weak light flashes, completed in ~3 s. For stronger stimuli, giving a response of maximum amplitude (corresponding to closure of all the Na^+-channels of the surface membrane), the response is prolonged, but its falling phase is probably still determined by cyclic GMP-controlled binding of Ca^{2+}. Restoration of the Ca^{2+} content of the intradiscal space to its dark-adapted level is envisaged to be a much slower process, being dependent on a Ca^{2+}-pump for the active transfer of Ca^{2+} inward across the disc membrane and probably requiring 5–10 min for its completion. As a preliminary step, this will necessitate closure of the pore in the disc membrane. There is uncertainty about the timing of this event. It may conceivably correspond to phosphorylation of metarhodopsin II, which has been observed to take place 1–2 min following flash illumination and which has been proposed also as the reaction terminating the susceptibility of the rhodopsin photoproduct to combine with G-protein [40]. Another possibility is that pore closure may closely follow the decay of metarhodopsin II. In intact frog rod outer segments the time constant for this has been found to depend on the fraction of rhopsin bleached, with bleaches of $<10\%$ yielding a time constant of ≤ 10 s [41]. Evidence is lacking for a component of light adaptation at low levels of bleaching which could be attributed to the Ca^{2+}-pore remaining open and hence the relevant portion of the disc being incapable of accumulating Ca^{2+}. However, if pore closure were as rapid as the decay of metarhodopsin II, it would be difficult to differentiate its effect on responsiveness from that of changes involving cyclic GMP.

With the pore closed, operation of the Ca^{2+}-pump will cause a gradual accumulation of Ca^{2+} in the intradiscal space, returning the system to its dark-adapted condition. This pump is envisaged to be no different from that found in many other biological systems in which Ca^{2+} has a physiological role, with energy for the transport of Ca^{2+} against an electrochemical potential difference being provided by hydrolysis of ATP to ADP. By analogy with the situation in vertebrate striated muscle, in which the major protein constituent of the sarcoplasmic reticulum of M_r 100000 has both a Ca^{2+}-binding and a Ca^{2+}-pump function [42], one is tempted to speculate that those two functions of the rod disc membrane may likewise reside in a single protein-constituted system. In that case the two functional units represented in section C of fig. 1 should be combined.

8. CONCLUSION: MECHANISM OF Ca^{2+} BINDING

In this model of visual transduction the major innovative feature is the role of the Ca^{2+}-binding protein controlled by the cyclic GMP level of the cytoplasm. It does not seem possible at present to provide a molecular description of the functioning of this protein. Evidently, the regulatory action of cyclic GMP does not take place through activation of a protein kinase, as in the usually observed mechanism of action of cyclic AMP, since the effect of cyclic GMP is exerted in the absence of a phosphate donor. A similar effect of cyclic GMP on Ca^{2+} binding in the absence of a phosphate donor has been observed in a preparation of microsomes from heart muscle sarcoplasmic reticulum [43]. Furthermore, in the case of the rod outer segment the possibility of Ca^{2+} binding being controlled by either phosphorylation or dephosphorylation appears to be excluded by studies that have been made on light-induced changes of protein phosphorylation, in which there appears to be no membrane-bound protein having the necessary properties [44]. One is left to suppose that combination of cyclic GMP with the protein induces a configuration change which results in the disappearance of one or more Ca^{2+}-binding sites.

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