ACS Chemical Neuroscience

Review

pubs.acs.org/chemneuro

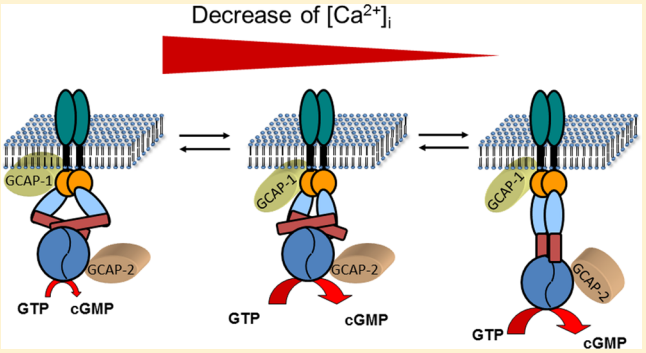
A Calcium-Relay Mechanism in Vertebrate Phototransduction

Karl-Wilhelm Koch*,† and Daniele Dell'Orco‡

†Department of Neurosciences, Biochemistry Group, University of Oldenburg, Carl-von-Ossietzky-Strasse 9-11, D-26129 Oldenburg, Germany

‡Department of Life Sciences and Reproduction, Section of Biological Chemistry and Center for BioMedical Computing (CBMC), University of Verona, Strada le Grazie 8, I-37134 Verona, Italy

ABSTRACT: Calcium-signaling in cells requires a fine-tuned system of calcium-transport proteins involving ion channels, exchangers, and ion-pumps but also calcium-sensor proteins and their targets. Thus, control of physiological responses very often depends on incremental changes of the cytoplasmic calcium concentration, which are sensed by calcium-binding proteins and are further transmitted to specific target proteins. This Review will focus on calcium-signaling in vertebrate photoreceptor cells, where recent physiological and biochemical data indicate that a subset of neuronal calcium sensor proteins named guanylate cyclase-activating proteins (GCAPs) operate in a calcium-relay system, namely, to make gradual responses to small changes in calcium. We will further integrate this mechanism in an existing computational model of phototransduction showing that it is consistent and compatible with the dynamics that are characteristic for the precise operation of the phototransduction pathways.



KEYWORDS: *Calcium-signaling, cGMP, phototransduction*

Critical biochemical reactions in rod and cone photoreceptor cells are under the control of negative feedback loops that determine the cell's light sensitivity and its precise operation under different light regimes.[1−4] One key factor that is involved in various feedback loops is Ca^{2+}. Its concentration is balanced by two transport systems: In the dark state of the cell, the second messenger guanosine 3′,5′-cyclic monophosphate (cGMP) binds to a cyclic nucleotide-gated (CNG) channel in the plasma membrane, thereby opening the channel,[5] which allows the influx of Na^+ and Ca^{2+}. The second transport system in the plasma membrane is a Na^+/Ca^{2+}, K^+-exchanger that is extruding Ca^{2+}. Illumination of a photoreceptor cell triggers the hydrolysis of cGMP, leading to the closure of CNG-channels, but the continuous operation of the exchanger is shuffling Ca^{2+} out of the cell resulting in a concomitant decrease of the cytoplasmic Ca^{2+}-concentration.[1,2] Ca^{2+}-sensor proteins of the EF-hand superfamily of Ca^{2+}-binding proteins are expressed in photoreceptor cells and detect these changes in Ca^{2+}. Among them are guanylate cyclase-activating proteins (GCAPs) that constitute a subfamily of the neuronal Ca^{2+}-sensor proteins.[4,6−8] GCAPs control the activity of membrane bound guanylate cyclases in a Ca^{2+}-dependent manner and are therefore essential key factors in shaping the rod and cone photoresponse under different light regimes.[9,10] Two to eight different GCAP isoforms exist in species from fish to human. The apparently redundant expression of different GCAP forms in one cell type (rod or cone cell) was puzzling at the time, when these forms were first described. For example, mammalian GCAP1 and GCAP2 were found in rod and cone cells exhibiting almost identical biochemical properties.[11−14] However, a few years later, electrophysiological recordings on transgenic mice lacking GCAP1, GCAP2, or both indicated that these neuronal calcium sensors have different roles in shaping the light response of a rod cell.[15−17] Soon after this report, detailed biochemical studies showed that the Ca^{2+}-sensitive activation profiles of bovine GCAP1 and 2 differ, which provided a molecular basis for interpreting the physiological data.[18] The operation of different GCAP forms in rod or cone cells can be described in a Ca^{2+}-relay model,[19] for which we discuss in the following sections the experimental evidence and its relation to cone−rod diseases caused by GCAP1 mutations.

CONTRIBUTION OF GCAP1 AND GCAP2 TO THE FLASH SENSITIVITY OF PHOTORECEPTOR CELLS

Rods from transgenic mice lacking GCAP1 and 2 ($GCAPs^{-/-}$) show a single flash response with a larger amplitude, a delayed time-to-peak, and a larger integration time when compared with wild-type mice (Figure 1).[15] These characteristic features demonstrate the importance of guanylate cyclase regulation by GCAPs for the normal shape of the rod photoresponse. Expression of GCAP2 on a $GCAP^{-/-}$ genetic background resulted in variable flash responses, but in general GCAP2

Received: January 23, 2013
Accepted: March 10, 2013
Published: March 11, 2013

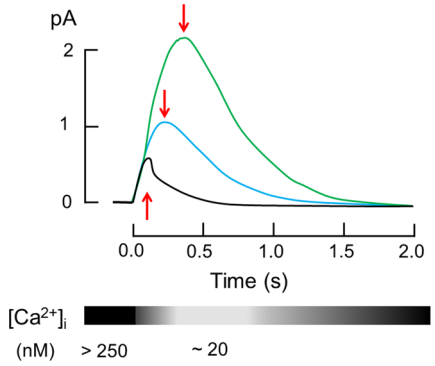


Figure 1. Idealized single photon responses of mice rods. Responses of rods from wild-type mice (black trace), from GCAPs$^{-/-}$ mice (green trace), and from mice expressing GCAP2 on a GCAPs$^{-/-}$ genetic background (blue trace) were superimposed. Red arrows indicate the time at which maximum amplitude is reached (time-to-peak). Traces represent typical idealized responses and were drawn according to published results in ref 15. The lower part of the figure shows the change of intracellular Ca^{2+}-concentration during a light response.

expression could not rescue the initial rapid recovery phase typically observed in wildtype rods (see black trace in Figure 1).[15] Consistent with these observations are single photon responses that were recorded from mice rods lacking GCAP2 (GCAP2$^{-/-}$), which have a slower recovery to the baseline. Interestingly, the initial rapid recovery phase seems unchanged, whereas the later part of the recovery phase is delayed (see Figure 7 in ref 20). A recent complementary study[21] on mice lacking GCAP1 (GCAP1$^{-/-}$) yields a qualitatively similar shape of the flash response as shown in Figure 1, that is, larger response amplitudes and delayed time-to-peak (the original recordings shown in Figure 6A from ref 21 are reproduced here in Figure 2A for comparison). However, Makino et al. report also in their study[21] that the single photon responses from WT and GCAP1$^{-/-}$ rods show a remarkable heterogeneity (their Figure 6C−E is reproduced here in Figure 2B−D) in amplitudes and kinetics and some responses do not exhibit a full recovery to the baseline within 2 s (Figure 5 in ref 21). It remains therefore an open question for further investigations, whether compensatory mechanisms change the transcription and translation pattern of GCAPs.[21]

Nevertheless, in comparison to WT rods, the time-to-peak is shifted by ~100 ms in GCAP1$^{-/-}$ rods[21] and only about 12 ms in GCAP2$^{-/-}$ rods[20] indicating that GCAP1 and GCAP2 respond on a different time scale or on different free cytoplasmic Ca^{2+}-concentration during the photoresponse.

The cytoplasmic Ca^{2+}-concentration in mice is expected to drop from its initial dark value around 250 nM to 20 nM within a few hundred milliseconds.[22] Different steady-state background light intensities probably adjust steady-state Ca^{2+}-concentrations at intermediate levels. Although this has not been experimentally verified for mice rod or cone cells, one can anticipate it from data obtained on amphibian rods, where different intracellular Ca^{2+} concentrations were measured at different illumination conditions.[23] While the precise value of the changing range of Ca^{2+} also depends on the species (dark values in the literature span a range from 250 to 800 nM, see, for instance, refs 22−29), dynamic changes in Ca^{2+} are believed to strongly correlate with the illuminations state of the photoreceptor cell.

Collectively, these results point to a differential operation mode of GCAP1 and GCAP2 in rods and probably cones as well. In the next paragraph, we will review the biochemical studies that provide strong support of such a step-by-step activation modus of rod guanylate cyclases.

■ ACTIVATION PROFILES OF GCAP1 AND GCAP2 DIFFER IN KEY PROPERTIES

Although GCAP1 and GCAP2 fold into similar three-dimensional structures that are also present as general folding motives in other neuronal calcium sensor proteins,[30,31] they nevertheless display remarkable differences in their regulatory properties. This was explicitly stated for the first time in two biochemical in vitro studies by Hwang and Koch[32] and Hwang et al.,[18] in which the authors used bovine recombinant GCAP1 and GCAP2 reconstituted with either native guanylate cyclase 1 (ROS-GC1 or GC-E) in bovine rod outer segment membranes or tested GCAP function with ROS-GC1 expressed in HEK293 cells. The main findings of the studies were:

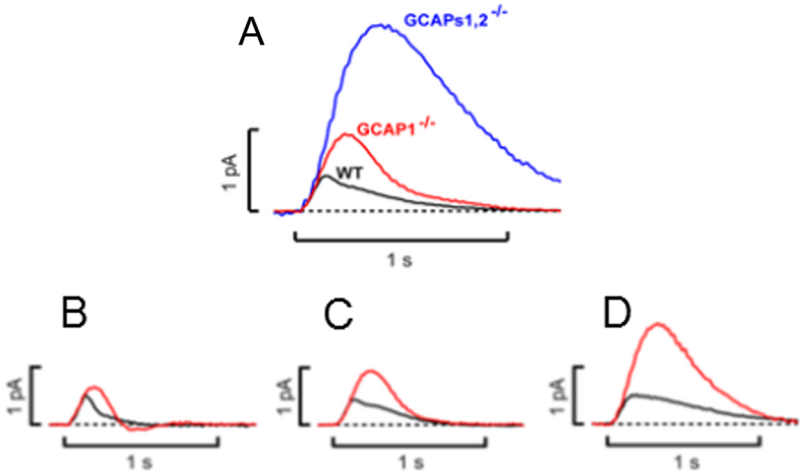


Figure 2. Heterogeneity of light responses from WT and transgenic mice. Original recordings were adapted from Figure 6 of ref 21 (Makino et al., PLoS One 7, e47637) in accordance with the Creative Commons Attribution License. (A) Comparison of averaged dim flash recordings from WT, GCAP1$^{-/-}$, and GCAPs1,2$^{-/-}$ as indicated. (B−D) Variability of integration times of single photon responses as observed by Makino et al.[21]

(a) GCAP1 is activating ROS-GC1 at higher free Ca^{2+}-concentration than GCAP2 (half-maximal activation expressed as IC_{50}-value was at 700−1000 nM and at 100−200 nM, respectively).
(b) GCAPs operate in a way that they increase the catalytic efficiency (k_{cat}/K_m) of the target ROS-GC1.
(c) Myristoylation has a stronger impact on the activation profiles of GCAP1 than on the one of GCAP2.

While these studies were focused on ROS-GC1, subsequent work showed that GCAP1 and GCAP2 also activate bovine ROS-GC2 (alternatively named GC-F) with different Ca^{2+}-sensitivities for each GCAP form (IC_{50} was 306 nM and 46 nM Ca^{2+} for GCAP1 and GCAP2, respectively).[33] ROS-GC2 is a second sensory membrane guanylate cyclase in ROS that is expressed in a lower molar ratio to ROS-GC1. The exact ratio, however, seems to differ among species. For example, the ratios of ROS-GC1 to ROS-GC2 are 25:1 and 4:1 in bovine[33] and mice[34] outer segments, respectively. Biochemical studies on mice further supported the emerging picture that both guanylate cyclases are regulated in a differential manner by both GCAPs.[34] A summarizing sketch of these results is shown in Figure 3: the Ca^{2+}-dependent activation profiles of photoreceptor guanylate cyclases in the presence of either GCAP1 or GCAP2 represent a Ca^{2+}-relay mode of GCAP action.

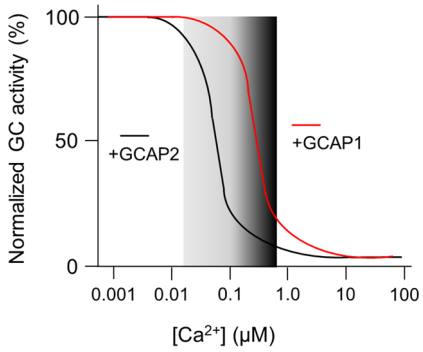


Figure 3. Ca^{2+}-dependent activation profile of the photoreceptor guanylate cyclase is regulated by GCAP1 and GCAP2. The GC activity is normalized to 100%. The curves are typical for mammalian GCAPs and summarize the principal finding of many references (see main text). The gray bar symbolizes the physiological range of cytoplasmic Ca^{2+} that changes from a high (dark gray) to a low value (light gray).

To achieve a better comparison of electrophysiological data obtained from transgenic mice (see above) with data from biochemical measurements, Peshenko et al. performed a study using retinal homogenates from transgenic mice lacking the two ROS-GCs and GCAPs in different combinations.[34] Further, Ca^{2+}-dependent guanylate cyclase activities in retina homogenates of $GCAP1^{-/-}$ or $GCAP2^{-/-}$ displayed a characteristic shift of the Ca^{2+}-dependent activation curves. While these studies confirm a differential regulatory mode of GCAP1 and GCAP2 action, the investigation by Peshenko et al. also revealed differences between mouse and bovine rod cells. For example, the ratio of the two GCs differed and their catalytic constants were in general higher in mice (see above).

In agreement with a differential action of GCAPs is the finding that GCAP1 and GCAP2 interact with different regions on ROS-GC1.[35−39] Site-directed mutagenesis, the use of synthetic peptides representing the interaction domains, and cross-linking studies in combination with mass spectrometry revealed for GCAP1 a total of four interaction regions, where two are located in the juxtamembrane domain and two were found in the kinase homology domain.[35,36] While these target regions are upstream from the cyclase catalytic domain, the interaction site of GCAP2 is located downstream on the C-terminal extension of the catalytic domain.[37−39]

In summary, the biochemical data obtained from mice, bovine, and purified recombinant samples is consistent with a model in which GCAP1 is turned on at higher free calcium concentration than GCAP2. Taking this observation into a physiological context means that both GCAPs are operating in a different time frame of the photoresponse, but also under different illumination conditions characterized by a changing pattern or oscillation of the intracellular Ca^{2+}-concentration.

WHAT MECHANISMS CONTROL THE TUNING OF CA^{2+}-SENSITIVITY?

Peshenko and Dizhoor demonstrated that GCAPs are Ca^{2+}/Mg^{2+} sensors and that Mg^{2+} is a critical factor affecting the Ca^{2+}-sensitivity.[40] Decreasing the Mg^{2+}-concentration in the incubation buffer can shift the Ca^{2+}-sensitivity of ROS-GC regulation to lower Ca^{2+}-concentration while keeping a difference between the IC_{50} values of GCAP1 and GCAP2. Moreover, recent results indicate that both the apparent affinity for Ca^{2+} of GCAP1 and GCAP2 and the dynamics of the conformational changes upon metal binding are significantly influenced by the presence of 1 mM free Mg^{2+}.[41] Another important factor involved in sensitivity regulation is the covalent fatty acylation, mainly a myristoylation (as mentioned already above) in GCAP1, which has almost no effect in GCAP2. Presence of the myristoyl group in GCAP1 is essential to shift the Ca^{2+}-sensitivity into the physiological range and controls also the affinity of the GCAP1-ROS-GC1 interaction.[18,42,43] Without an attached myristoyl group Ca^{2+}-saturated GCAP1 remains in an active conformation and can significantly activate ROS-GC1 to about 20% of its maximal activity.[32,43] The myristoyl group is located in a hydrophobic pocket[31] and, in contrast to recoverin, it is not extruded by a switch mechanism upon changes in Ca^{2+}-concentration. Interestingly, substitution of critical hydrophobic amino acids (Leu and Val) in the pocket by more bulky Phe residues restores almost the maximal activation of ROS-GC1 by GCAP1 and increases its affinity for ROS-GC1, but filling (or compensating) the hydrophobic pocket with bulky hydrophobic substituents does not change much the Ca^{2+}sensitivity of ROS-GC1 regulation.[43] A third parameter controlling the Ca^{2+}-sensitivity could be the intermolecular tuning of the Ca^{2+}-sensor with the corresponding target as this has been experimentally observed for calmodulin,[44] but this is currently a controversial issue in the case of GCAPs. The observation that the control of GC by Ca^{2+} via the GCAPs is cooperative,[45] while the binding of Ca^{2+} to individual GCAPs has been shown to occur sequentially and without cooperativity,[41,46] suggests that the interaction with the target may influence the Ca^{2+}-affinity of the sensor. This issue however awaits further clarification in future studies.

WHAT ABOUT CONES?

Electroretinogram (ERG) recordings are sum potentials of retinal cells responding to light stimulation. ERG recordings on $GCAPs^{-/-}$ mice indicated that deletion of GCAPs delayed the

dx.doi.org/10.1021/cn400027z | ACS Chem. Neurosci. 2013, 4, 909−917

recovery of cone light responses, which were partially restored in mice expressing transgenic GCAP1 on a GCAP null background.[16,17] In a more recent study, single-cell recordings from cones of GCAPs$^{-/-}$ mice gave a detailed picture of the physiological function of GCAPs in cone photoreceptors.[47] Characteristic features of light responses were a 2-fold longer time-to-peak and a 3-fold longer integration time, indicating that GCAPs are required for an efficient shutoff of the response on a short time scale, which is typical for cone response kinetics. However, as the authors of the study noted, the impact of GCAPs on modulation of the light response was smaller in comparison to rods despite the fact that cones adapt to a larger range of light intensities and have a wider span of dynamic changes in intracellular Ca^{2+}. But mice are nocturnal animals and preferentially process olfactory information for orientation. Cones of mice might therefore express proteins with reduced capabilities. Thus, it will be worth testing whether animals with a cone system similar to humans have a more elaborated operation of GCAPs. Zebrafish, for example, possess four types of cone cells expressing a total set of nine cone opsins, thereby making the zebrafish retina responsive to visible and UV light.[48] Due to the advantages of facilitated genetic manipulation and rapid larval development, work on zebrafish vision has increased tremendously in the past decade. Although we lack so far information from single-cell recordings of zebrafish cones with deletion of GCAPs, biochemical studies indicate a complex regulatory system for cGMP synthesis in zebrafish photoreceptor cells. Rod and cone cells express a total of three sensory guanylate cyclases and six different GCAP isoforms.[49−51] Transcription of one guanylate cyclase (zGC3) and four GCAPs (zGCAP3, zGCAP4, zGCAP5, and zGCAP7) has been exclusively found in cones,[49−51] and expression on the protein level has been demonstrated for zGCAP3.[52] Two isoforms, zGCAP1 and zGCAP2, are expressed in rods and UV cones. During development, the cellular transcription pattern in the retina seems to change and may adapt to specific challenges of the aqueous habitat.[50,51] Using purified samples of recombinant zGCAPs in biochemical reconstitution experiments showed a pattern of different activation profiles,[53,54] which was similar to the ones observed with mammalian GCAPs (Figure 3). One can group the six zebrafish GCAP isoforms in two categories of different Ca^{2+}- sensitivities, zGCAP4 and 7 switching between different GC activation profiles at higher Ca^{2+} compared to zGCAP1, 2, and 3. In addition, zGCAPs exhibit different properties with respect to Ca^{2+}-binding and Ca^{2+}-induced conformational changes pointing also to a step-by-step action of GCAPs in zebrafish cones.[53]

THE CA^{2+}-RELAY MECHANISM IN A PHYSIOLOGICAL CONTEXT

The cytoplasmic Ca^{2+}-concentration decreases during the single flash response of a rod cell from the dark state value to a level well below 100 nM. Within the first 50 ms, the cytoplasmic Ca^{2+} has not reached its final value. This kind of intermediate state triggers activation of mammalian GCAP1 (or zGCAP4 and 7 in the zebrafish retina; see Figures 3 and 4 for comparison), which loses its bound Ca^{2+} and is transformed to its Mg^{2+}-bound activator state (Figure 5). Several lines of experimental evidence show that GCAP1 undergoes a conformational change during this transformation, which is further transmitted to the catalytic center of the target GC (e.g., refs 11, 13, 31, 32, and 36). Thus, GCAP1 steps into action and causes a first pulse of rapid cGMP synthesis, which contributes

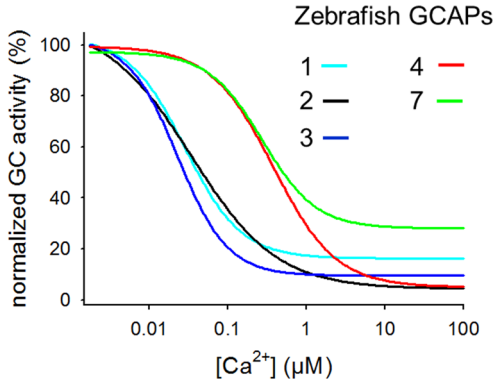


Figure 4. Activity profiles of cone specific GCAPs from zebrafish. The GC activity is normalized as in Figure 3. The curves were obtained by measuring the GC activity in the presence of zGCAPs as indicated. The figure is based on the original data published in ref 53, where a bovine ROS membrane preparation containing ROS-GCs was used for assaying zebrafish GCAPs.

to effectively shut off the photoresponse. A characteristic fast response kinetics (Figure 1) is due to this step of cGMP synthesis, which is not seen in transgenic mice lacking GCAP1 or both GCAPs (Figures 1 and 2).[15,21] A further decrease of cytoplasmic Ca^{2+} on a time scale of 0.5−1 s triggers the dissociation of Ca^{2+} from GCAP2 (or zGCAP1, 2, or 3 in the zebrafish retina), which now undergoes a conformational change leading to the activator state (Figure 5). The maximal activities of both GCAP-ROS-GC complexes are partially additive,[20] but it remains unclear whether both GCAPs act synergistically on either ROS-GC1 or ROS-GC2 or whether they act on different GCs, which may even include a switch from a GCAP1 to a GCAP2 modus. In order to investigate the mechanistic implications of the Ca^{2+}-relay model of guanylate cyclase regulation by GCAPs within its physiological context, a recently developed comprehensive kinetic model of phototransduction can be used. The model is optimized for amphibian rods and describes the synergic action of the biochemical events occurring in a rod photoreceptor cell upon light stimulation ranging over 5 orders of magnitude. The output consists of the simulated time course of the photocurrents, which can be compared with experimental electrophysiological recordings.[55] The modular nature of the model allows the insertion of new mechanisms and the quantitative effects on the overall network can thus be directly assessed.[56,57] In such a framework, the addition of the Ca^{2+}-relay model can be realized by assuming that the rate of cGMP synthesis by ROS-GC1 is regulated by both GCAP1 and GCAP2 as follows:

$$\frac{d[cGMP]_{synth}}{dt} = \frac{a_{max}}{1 + \left(\frac{[Ca^{2+}_{free}](t)}{IC_{50}^{GCAP1}}\right)^{m_1}} + \frac{a_{max}}{1 + \left(\frac{[Ca^{2+}_{free}](t)}{IC_{50}^{GCAP2}}\right)^{m_2}}$$

where a_{max} = 60 μM/s[45] is the maximal activation of GC1, assumed to be the same for both GCAP1 and GCAP2, $[Ca^{2+}_{free}]$ is the free intracellular calcium depending on the illumination level, and IC_{50} (139 nM and 59 nM[34]) and m (1.7 and 2.4 [32]) indicate, respectively, the free Ca^{2+}-concentration for half-maximal ROS-GC1 activation contributed by the specific GCAP and the Hill coefficient. We neglected any specific contribution of cGMP synthesis by ROS-GC2, because we lack at the moment essential data on putative switch mechanisms between a ROS-GC1 and ROS-GC2 operation

dx.doi.org/10.1021/cn400027z | ACS Chem. Neurosci. 2013, 4, 909−917

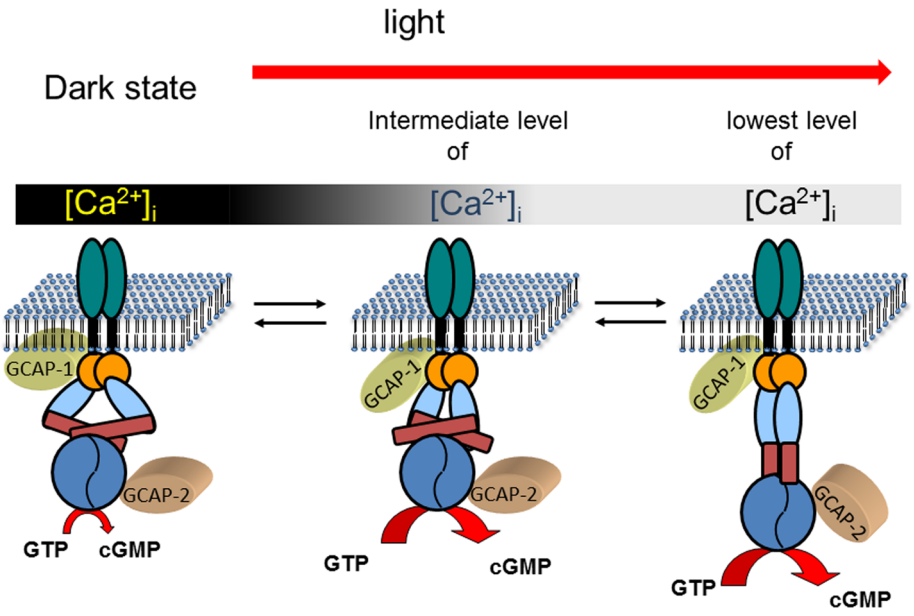


Figure 5. Ca^{2+}-relay model of sequential GCAP action. Photoreceptor guanylate cyclases are dimers with one transmembrane domain in each monomer. In addition, each monomer consists of one extracelllar domain (in rods, this domain is in the lumen of the disks), one juxtamembrane domain (orange), and one kinase homology domain (light blue). Further, the dimerization domain (dark red rectangle) is important for the formation of an active enzyme controlling the correct positioning of the two catalytic domains (blue). In the dark state of the cell, GCAPs are fully or partially saturated with Ca^{2+} which keeps the guanylate cyclase activity at a very low level that is sufficient to keep a fraction of the CNG-channels in the plasma membrane open. GCAPs form with the target guanylate cyclase a complex in the presence and absence of Ca^{2+}, which enables a rapid response to changing Ca^{2+}-concentration after illumination. When the intracellular Ca^{2+}-concentration falls to an intermediate level, Ca^{2+} dissociates form GCAP1. This process triggers a conformational change in GCAP1, leading to the activation of guanylate cyclase. When Ca^{2+} reaches its final lower intracellular level, GCAP2 turns into an activator. The different conformations of the guanylate cyclase are hypothetical and need to be verified in future experiments. It is further suggested that GCAPs stabilize the transition states of the cGMP catalytic step, but experimental proof is also lacking so far. The stepwise and reversible action of GCAPs would allow the cell to react on small incremental changes in Ca^{2+} with a fine-tuned response system.

modules (see above). However, the implementation of the Ca^{2+}-relay mechanism based on ROS-GC1 regulation is fully compatible with the typical kinetics of photoresponses, as shown in Figure 6. The simulation of the photocurrent generated by six flashes of increasing intensity leading from 1.5 up to 11.5×10^4 photoisomerizations, that is, from very dim light to saturating conditions, is in line with the typical electrophysiological recordings. The significantly slower time-scale compared to mouse photoresponse is due to the fact that the model was developed for amphibian photoreceptors.

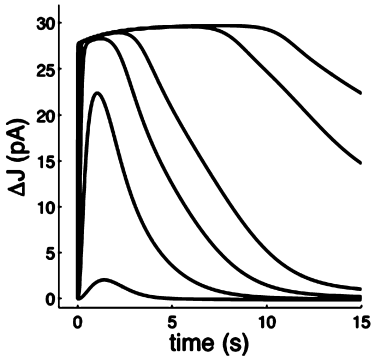


Figure 6. Simulated photocurrents of an amphibian rod stimulated by flashes of light of increasing intensity. The duration of each flash is 24 ms, and each stimulus leads to 1.54, 46, 280, 985, 30 400, and 115 000 photoisomerizations of rhodopsin. The mathematical model used for the simulation is the one reported in ref 57 in which the Ca^{2+}-relay mechanism has been implemented as described here.

The computational implementation of the Ca^{2+}-relay model allows assessing each term defining the rate of cGMP synthesis, thus deriving the specific contribution of GCAP1 and GCAP2 regulation of ROS-GC1 over time. Figure 7 shows that, at dim light intensities, when the Ca^{2+}-concentration decreases mildly compared to the dark state, the most important contribution to the cGMP synthesis (black line) derives almost entirely from the GCAP1-mediated stimulation of ROS-GC1 (blue line), while the contribution of GCAP2 (red line) is modest. GCAP1 is thus the first calcium sensor stepping into action for a fast activation of ROS-GC1 (and maybe ROS-GC2 as well). However, already at intermediate illumination levels, the contribution of GCAP2 becomes more prominent, and at saturating conditions (Figure 7E and F) it quantitatively equals that of GCAP1, although the activation profile is slower and, due to the shorter time the cell spends at very low calcium, less broad over the whole photoresponse time range. Overall, the simulations show full consistence with the concept of Ca^{2+}-relay mechanism shaping the photoresponse of vertebrates.

DISTURBANCE OF THE CA^{2+}-RELAY MODUS IN RETINAL DISEASES

Cone and cone−rod dystrophies are hereditary diseases that correlate with mutations in retinal proteins. One group of patients carries mutations in the GUCA1A gene coding for human GCAP1 (for a summary, see refs 58 and 59. So far, only one very rare mutation was identified in GCAP2.[60] Nine

dx.doi.org/10.1021/cn400027z | ACS Chem. Neurosci. 2013, 4, 909−917

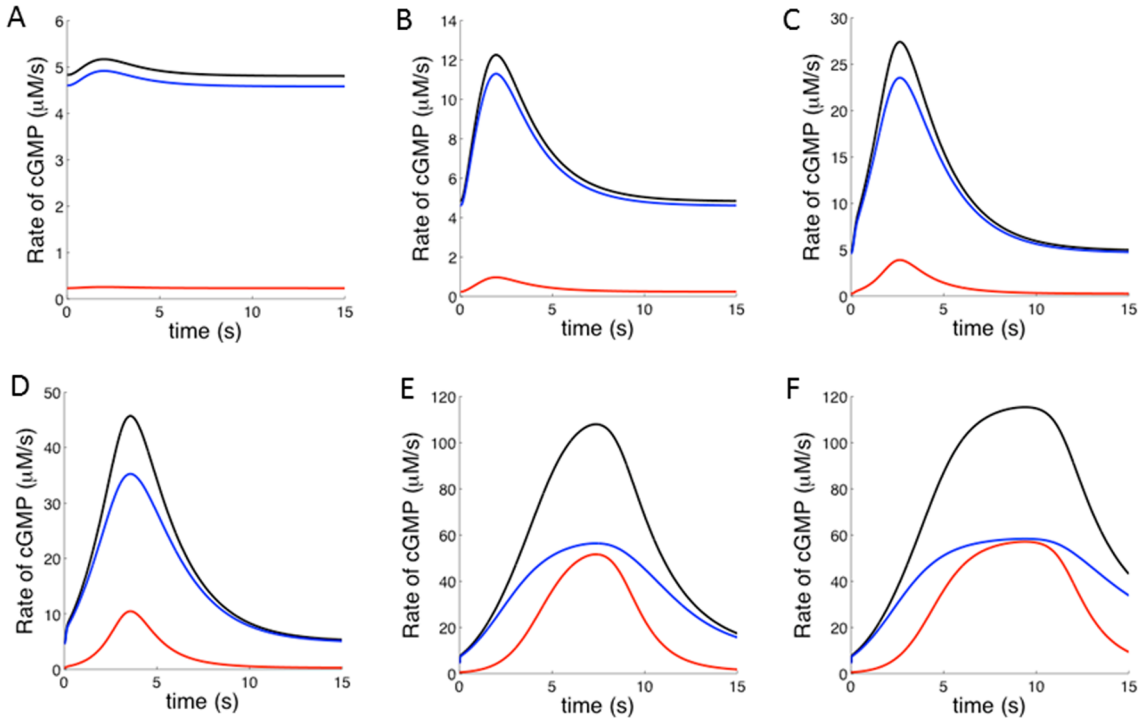


Figure 7. Simulated time course of the synthesis of cGMP by GC1 under the Ca^{2+}-mediated regulation by GCAP1 and GCAP2 according to the Ca^{2+}-relay mechanism. Panels A–F refer to the same flashes of increasing intensity reported in Figure 6 (i.e., panel A refers to 1.54 photoisomerization, panel B to 46 photoisomerizations, and so forth). Blue lines report on the contribution of GCAP1 to the rate of synthesis, while red lines to that of GCAP2. The two contributions add up to form the overall rate (black line).

mutations found in GCAP1 have been investigated in more detail including in particular the Ca^{2+}-sensitive regulatory properties. Eight mutations are found in or near the EF-hands 3 and 4, indicating an impairment of Ca^{2+}-binding and/or Ca^{2+}-dependent properties. When recombinant forms of these GCAP1 mutants were investigated in reconstitution experiments with membranes containing photoreceptor guanylate cyclase, the Ca^{2+}-dependent activation profiles of ROS-GC1 were shifted to higher free Ca^{2+}-concentration.[61−68] Thus, under normal physiological conditions these GCAP forms are permanently active leading to continuous high synthesis rates of cGMP. Some GCAP1 mutants would even require Ca^{2+}-concentrations well above 100 μM to be turned off. These findings demonstrate that an imbalance of the finely tuned Ca^{2+}-cGMP homeostasis can lead to severely impaired cellular functions. Since GCAP1 and GCAP2 respond sequentially to Ca^{2+} in a narrow range, the shifted response curve of GCAP1 is setting the Ca^{2+}-relay operation modus out of control.

OTHER CA^{2+}-DEPENDENT FEEDBACK LOOPS

Two other Ca^{2+}-sensor proteins, calmodulin and recoverin, are also mainly involved in regulation of phototransduction processes. Ca^{2+}-calmodulin binds directly to the CNG-channel and thereby decreases the affinity of the channel for its ligand cGMP[69−71] and Ca^{2+}-loaded recoverin inhibits rhodopsin kinase in the dark state of the cell.[72,73] When Ca^{2+}-levels drop after illumination, calmodulin dissociates from binding sites in the CNG channel, making the channel susceptible for lower cGMP concentration, which facilitates the reopening of the channel at lower cGMP levels that are present in dark adapted cells.

Experiments on photoreceptor cells of recoverin knockout mice revealed that recoverin is an essential component of the light-dependent modulation of the lifetime of light-activated rhodopsin.[74] A more recent biochemical study also showed that the Ca^{2+}-sensitive inhibition of rhodopsin kinase by recoverin can be increased by the synergetic effect of calmodulin placing the inhibitory action in a range of 0.05−1 μM free Ca^{2+}-concentration.[75] This mechanism of shifting the Ca^{2+}-sensitive regulation of the target enzyme rhodopsin kinase is reminiscent of the Ca^{2+}-relay model described above for the GCAPs and its physiological relevance is an open question for future studies.

CONCLUSION AND PROSPECTS

GCAPs in the vertebrate retina are calcium-sensor proteins that operate in a relay mode and make thereby the cell responsive to incremental changes in intracellular calcium. Biochemical and physiological data as well as results from a systems biology approach support this Ca^{2+}-relay model, but also impose further questions. For example, flash responses delivered in the presence of background light showed that the flash sensitivity of rods and cones of GCAP−/−mice displayed a deviation from normal Weber-Fechner relation, indicating a partial loss of adaptation mechanisms.[15,47] These results lead to unresolved questions such as how does a step-by-step action of GCAPs operate under constant illumination or under different background light intensities? Furthermore, is there a shift in the Ca^{2+}-sensitivities of GCAPs under prolonged illumination? Which other mechanisms beside the Ca^{2+}-dependent regulation of ROS-GCs control light adaptation of photoreceptor cells? Further, is the larger diversity of GCAPs in teleost fish the molecular basis for the dynamic range extension in cone adaptation of the fish retina? The heterogeneity and variability of flash responses obtained with transgenic mice[21] impose also further questions about compensatory mechanisms (see above)

dx.doi.org/10.1021/cn400027z | ACS Chem. Neurosci. 2013, 4, 909−917

and certainly reflect our incomplete understanding of GCAP function.

GCAP1 and GCAP2 are also localized in the synaptic layer of photoreceptor cells,[13,14] and it has recently been reported that GCAP2 interacts with the protein ribeye that is part of the synaptic ribbon structure.[76,77] Further, overexpression of GCAP2 leads to morphological changes of the ribbon synapse.[78] Does GCAP2 operate as a Ca^{2+}-sensor for more than one target in synaptic terminals including ribeye and ROS-GC1? The latter has also been localized in the outer plexiform layer of vertebrate retinae and could be under control of changing Ca^{2+}-levels. Finally, a Ca^{2+}-relay mode of operation could involve other Ca^{2+}-sensors as well regulating, for example, synaptic vesicle release or light-dependent transport and translocation processes.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +49-(0)441-798-3640. Fax: +49-(0)441-798-3250. E-mail: karl.w.koch@uni-oldenburg.de.

Author Contributions

K.W.K. conceived the study, D.D.O. performed the computational simulation, both authors wrote and approved the paper.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

GCAP, guanylate cyclase-activating protein; ROS, rod outer segment; GC, guanylate cyclase; CNG, cyclic nucleotide-gated

REFERENCES

(1) Pugh, E. N. Jr., and Lamb, T. D. (2000) Phototransduction in vertebrate rods and cones: molecular mechanisms of amplification, recovery and light adaptation. In *Handbook of Biological Physics* (Stavenga, D. G., DeGrip, W. J., and Pugh, E. N., Jr., Eds.), Vol. 3, pp 183−255, Elsevier Science BV: Amsterdam, The Netherlands.

(2) Luo, D. G., Xue, T., and Yau, K.-W. (2008) How vision begins: an odyssey. *Proc. Natl. Acad. Sci. U.S.A. 105*, 9855−9862.

(3) Stephen, R., Filipek, S., Palczewski, K., and Sousa, M. C. (2008) Ca^{2+}-dependent regulation of phototransduction. *Photochem. Photobiol. 84*, 903−910.

(4) Koch, K.-W., Duda, T., and Sharma, R. K. (2010) Ca^{2+}-modulated vision-linked ROS-GC guanylate cyclase transduction machinery. *Mol. Cell. Biochem. 334*, 105−115.

(5) Kaupp, U. B., and Seifert, R. (2002) Cyclic nucleotide-gated ion channels. *Physiol. Rev. 82*, 769−824.

(6) Palczewski, K., Polans, A. S., Baehr, W., and Ames, J. B. (2000) Ca^{2+}-binding protiens in the retina: structure, function, and the etiology of human visual diseases. *BioEssays 22*, 337−350.

(7) Dizhoor, A. M., Olshevskaya, E. V., and Peshenko, I. V. (2010) Mg2+/Ca2+ cation binding cycle of guanylyl cyclase activating proteins (GCAPs): role in regulation of photoreceptor guanylyl cyclase. *Mol. Cell. Biochem. 334*, 117−124.

(8) Burgoyne, R. D. (2007) Neuronal calcium sensor proteins: generating diversity in neuronal Ca^{2+} signaling. *Nat. Rev. Neurosci. 8*, 182−193.

(9) Burns, M. E., Mendez, A., Chen, J., and Baylor, D. A. (2002) Dynamics of cyclic GMP synthesis in retinal rods. *Neuron 36*, 81−91.

(10) Gross, O. P., Pugh, E. N., Jr, and Burns, M. (2012) Calcium feedback to cGMP synthesis strongly attenuates single-photon responses driven by long rhodopsin lifetimes. *Neuron 76*, 370−382.

(11) Palczewski, K., Subbaraya, I., Gorczyca, W. A., Helekar, B. S., Ruiz, C., Ohguro, H., Huang, J., Zhao, X., Crabb, J. W., Johnson, R. S., Walsh, K. A., Gray-Keller, M. P., Detwiler, P. B., and Baher, W. (1994) Molecular cloning and characterization of retinal photoreceptor guanylyl cyclase-activating protein. *Neuron 13*, 395−404.

(12) Gorczyca, W. A., Polans, A. S., Surgucheva, I. G., Subbaraya, I., Baehr, W., and Palczewski, K. (1995) Guanylyl cyclase activating protein. A calcium-sensitive regulator of phototransduction. *J. Biol. Chem. 270*, 22029−22036.

(13) Dizhoor, A. M., Olshevskaya, E. V., Henzel, W. J., Wong, S. C., Stults, J. T., ankoudinova, I., and Hurley, J. B. (1995) Cloning, sequencing, and expression of a 24-kDa Ca(2+)-binding protein activating photoreceptor guanylyl cyclase. *J. Biol. Chem. 270*, 25200−25206.

(14) Frins, S., Bonigk, W., Muller, F., Kellner, R., and Koch, K.-W. (1996) Functional characterization of a guanylyl cyclase-activating protein from vertebrate rods. Cloning, heterologous expression, and localization. *J. Biol. Chem. 271*, 8022−8027.

(15) Mendez, A., Burns, M. E., Sokal, I., Dizhoor, A. M., Baehr, W., Palczewski, K., Baylor, D., and Chen, J. (2001) Role of guanylate cyclase-activating proteins (GCAPs) in setting the flash sensitivity of rod photoreceptors. *Proc. Natl. Acad. Sci. U.S.A. 98*, 9948−9953.

(16) Howes, K. A., Pennesi, M. E., Sokal, I., Church-Kopish, J., Schmidt, B., Margolis, D., Frederick, J. M., Rieke, F., Palczewski, K., Wu, S. M., Detwiler, P. B., and Baehr, W. (2002) GCAP1 rescues rod photoreceptor response in GCAP1/GCAP2 knockout mice. *EMBO J. 21*, 1545−1554.

(17) Pennesi, M. E., Howes, K. A., Baehr, W., and Wu, S. M. (2003) Guanylate cyclase-activating protein (GCAP) 1 rescues cone recovery kinetics in GCAP1/GCAP2 knockout mice. *Proc. Natl. Acad. Sci. U.S.A 100*, 6783−6788.

(18) Hwang, J. Y., Lange, C., Helten, A., Höppner-Heitmann, D., Duda, T., Sharma, R. K., and Koch, K.-W. (2003) Regulatory modes of rod outer segment membrane guanylate cyclase differ in catalytic efficiency and Ca^{2+}-sensitivity. *Eur. J. Biochem. 270*, 3814−3821.

(19) Koch, K.-W. (2006) GCAPs, the classical neuronal calcium sensors in the retina − a Ca^{2+}-relay model of guanylate cyclase activation. *Calcium Binding Proteins 1*, 3−6.

(20) Makino, C. L., Peshenko, I. V., Wen, X. H., Olshevskaya, E. V., Barrett, R., and Dizhoor, A. M. (2008) A role for GCAP2 in regulating photoresponse. *J. Biol. Chem. 283*, 29135−29143.

(21) Makino, C. L., Wen, X. H., Olshevskaya, E. V., Peshenko, I. V., Savchenko, A. B., and Dizhoor, A. M. (2012) Enzymatic relay mechanism stimulates cyclic GMP synthesis in rod photoresponse: biochemical and physiological study in guanyly cyclase activating protein 1 knockout mice. *PLoS One 7*, e47637.

(22) Woodruff, M. L., Sampath, A. P., Matthews, H. R., Krasnoperova, N. V., Lem, J., and Fain, G. L. (2002) Measurement of cytoplasmic calcium concentration in the rods of wild-type and transducin knock-out mice. *J. Physiol. 543*, 843−854.

(23) Gray-Keller, M. P., and Detwiler, P. B. (1996) Ca^{2+} dependence of dark- and light-adapted flash responses in rod photoreceptors. *Neuron 17*, 323−331.

(24) Korenbrot, J. I., and Miller, D. L. (1989) Cytoplasmic free calcium concentraton in dark-adapted retinal rod outer segments. *Vision Res. 29*, 939−948.

(25) McCarthy, S. T., Younger, J. P., and Owen, W. G. (1996) Dynamic, spatially nonuniform calcium regulation in frog rods exposed to light. *J. Neurophysiol. 76*, 1991−2004.

(26) Sampath, A. P., Matthews, H. R., Cornwall, M. C., Bandarchi, J., and Fain, G. L. (1999) Light-dependnet changes in outer segment free Ca^{2+} concentration in salamander cone photoreceptors. *J. Gen. Physiol. 113*, 267−277.

(27) Nakatani, K., Chen, C., Yau, K.-W, and Koutalos, Y. (2002) Calcium and phototransduction. *Adv. Exp. Med. Biol. 514*, 1−20.

(28) Matthews, H. R., and Fain, G. L. (2002) Time course and magnitude of the calcium release induced by bright light in salamander rods. *J. Physiol. 542.3*, 829−841.

(29) Matthews, H. R., and Fain, G. L. (2003) The effect of light on outer segment calcium in salamander rods. *J. Physiol. 552.3*, 763−776.

(30) Ames, J. B., Dizhoor, A. M., Ikura, M., Palczewski, K., and Stryer, L. (1999) Three-dimensional structure of guanylyl cyclase activating

protein-2, a calcium-sensitive modulator of photoreceptor guanylyl cyclases. *J. Biol. Chem. 274*, 19329−19337.
(31) Stephen, R., Bereta, G., Golczak, M., Palczewski, K., and Sousa, M. C. (2007) Stabilizing function for myristoyl group revealed by the crystal structure of a neuronal calcium sensor, guanylate cyclase-activating protein 1. *Structure 15*, 1392−1402.
(32) Hwang, J.-Y., and Koch, K.-W. (2002) Calcium- and myristoyl-dependent properties of guanylate cyclase-activating protein-1 and protein-2. *Biochemistry 41*, 13021−13028.
(33) Helten, A., Säftel, W., and Koch, K.-W. (2007) Expression level and activity profile of membrane bound guanylate cyclase type 2 in rod outer segments. *J. Neurochem. 103*, 1439−1446.
(34) Peshenko, I. V., Olshevskaya, E. V., Savchenko, A. B., Karan, S., Palczewski, K., Baehr, W., and Dizhoor, W. (2011) Enzymatic properties and regulation of the native isozymes of retinal membrane guanylyl cyclase (RetGC) from mouse photoreceptors. *Biochemistry 50*, 5590−5600.
(35) Lange, C., Duda, T., Beyermann, M., Sharma, R. K., and Koch, K.-W. (1999) Regions in vertebrate photoreceptor guanylyl cyclase ROS-GC1 involved in Ca^{2+}-dependent regulation by guanylyl cyclase-activating protein GCAP-1. *FEBS Lett. 460*, 27−31.
(36) Krylov, D. M., and Hurley, J. B. (2001) Identification of proximate regions in a complex of retinal guanylyl cyclase 1 and guanylyl cyclase-activating protein-1 by a novel mass spectrometry-based method. *J. Biol. Chem. 276*, 30648−30654.
(37) Sokal, I., Haeseleer, F., Arendt, A., Adman, E. T., Hargrave, P. A., and Palczewski, K. (1999) Identification of a guanylyl cyclase-activating protein-binding site within the catalytic domain of retinal guanylyl cyclase 1. *Biochemistry 38*, 1387−1393.
(38) Duda, T., Fik-Rymarkiewicz, E., Venkataraman, V., Krishnan, R., Koch, K.-W., and Sharma, R. K. (2005) The calcium-sensor guanylate cyclase activating protein type 2 specific site in rod outer segment membrane guanylate cyclase type 1. *Biochemistry 44*, 7336−7345.
(39) Pettelkau, J., Schröder, T., Ihling, C. H., Olausson, B. E. S., Kölbel, K., Lange, C., and Sinz, A. (2012) Structural insights into retinal guanylycyclase-GCAP2 interaction determined by cross-linking and mass spectrometry. *Biochemistry 51*, 4932−4949.
(40) Peshenko, I. V., and Dizhoor, A. M. (2004) Guanylyl cyclase-activating proteins (GCAPs) are Ca2+/Mg2+ sensors: implications for photoreceptor guanylyl cyclase (RetGC) regulation in mammalian photoreceptors. *J. Biol. Chem. 279*, 16903−16906.
(41) Dell'Orco, D., Sulmann, S., Linse, S., and Koch, K.-W. (2012) Dynamics of conformational Ca2+-switches in signaling networks detected by a planar plasmonic device. *Anal. Chem. 84*, 2982−2989.
(42) Peshenko, I. V., Olshevskaya, E. V., and Dizhoor, A. M. (2012) Interaction of GCAP1 with retinal guanylyl cyclase and calcium: sensitivity to fatty acylation. *Front. Mol. Neurosci. 5*, 19.
(43) Peshenko, I. V., Olshevskaya, E. V., Lim, S., Ames, J. B., and Dizhorr, A. M. (2012) Calcium-myristoyl tug is a new mechanisms for intramolecular tuning of calcium sensitivity and tarbet enzyme interaction for guanylyl cyclase-activating protein 1. *J. Biol. Chem. 287*, 13972−13984.
(44) Peersen, O. B., Madsen, T. S., and Falke, J. J. (1997) Intermolecular tuning of calmodulin by target peptides and proteins: differential effects on Ca2+ binding and implications for kinase activation. *Protein Sci. 6*, 794−807.
(45) Koch, K.-W., and Stryer, L. (1988) Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. *Nature 334*, 64−66.
(46) Dell'Orco, D., Behnen, P., Linse, S., and Koch, K.-W. (2010) Calcium binding, structural stability and guanylate cyclase activation in GCAP1 variants associated with human cone dystrophy. *Cell. Mol. Life. Sci. 67*, 973−984.
(47) Sakurai, K., Chen, J., and Kefalov, V. J. (2011) Role of guanylyl cyclase modulation in mouse cone phototransduction. *J. Neurosci. 31*, 7991−8000.
(48) Chinen, A., Hamaoka, T., Yamada, Y., and Kawamura, S. (2003) Gene duplication and spectral diversification of cone visual pigments of zebrafish. *Genetics 163*, 663−675.
(49) Imanishi, Y., Yang, L., Sokal, I., Filipek, S., Palczewski, K., and Baehr, W. (2004) Diversity of guanylate cyclase-activating proteins (GCAPS) in teleost fish: characterization of three novel GCAPs (GCAP4, GCAP5, GCAP7) from zebrafish (Danio rerio) and prediction of eight GCAPs (GCAP1−8) in pufferfish (Fugu rubripes). *J. Mol. Evol. 59*, 2204−2217.
(50) Rätscho, N., Scholten, A., and Koch, K.-W. (2009) Expression profiles of three novel sensory guanylate cyclases and guanylate cyclase-activating proteins in the zebrafish retina. *Biochim. Biopyhs. Acta 1793*, 1110−1114.
(51) Rätscho, N., Scholten, A., and Koch, K.-W. (2010) Diversity of guanylate cyclases in teleost fishes. *Mol. Cell. Biochem. 334*, 207−214.
(52) Fries, R., Scholten, A., Säftel, W., and Koch, K.-W. (2012) Operation profile of zebrafish guanylate cyclase-activating protein 3. *J. Neurochem. 121*, 54−65.
(53) Scholten, A., and Koch, K.-W. (2011) Differential calcium signaling by cone specific guanylate cyclase-activating proteins from the zebrafish retina. *PLoS One 6*, e23117.
(54) Behnen, P., Scholten, A., Rätscho, N., and Koch, K.-W. (2009) The cone-specific calcium sensor guanylate cyclase activating protein 4 from the zebrafish retina. *J. Biol. Inorg. Chem. 14*, 89−99.
(55) Dell'Orco, D., Schmidt, H., Mariani, S., and Fanelli, F. (2009) Network-level analysis of light adaptation in rod cells under normal and altered conditions. *Mol. Biosyst. 5*, 1232−1246.
(56) Dell'Orco, D., and Koch, K.-W. (2010) Systems biochemistry approaches to vertebrate phototransduction: towards a molecular understanding of disease. *Biochem. Soc. Trans. 38*, 1275−1280.
(57) Dell'Orco, D., and Koch, K.-W. (2011) A dynamic scaffolding mechanism for rhodopsin and transducin interaction in vertebrate vision. *Biochem. J. 440*, 263−271.
(58) Hunt, D. M., Buch, P., and Michaelides, M. (2010) Guanylate cyclases and associated activator proteins in retinal diseases. *Mol. Cell. Biochem. 334*, 157−168.
(59) Behnen, P., Dell'Orco, D., and Koch, K.-W. (2010) Involvement of the calcium sensor GCAP1 in hereditary cone dystrophies. *Biol. Chem. 391*, 631−637.
(60) Sato, M., Nakazawa, M., Usui, T., Tanimoto, N., Abe, H., and Ohguro, H. (2005) Mutations in the gene coding for guanylate cyclase-activating protein 2 (GUCA1B gene) in patients with autosomal dominat retinal dystrophies. *Graefes Arch. Clin. Exp. Ophthalmol. 243*, 235−242.
(61) Kitiratschky, V. B., Behnen, P., Kellner, U., Heckenlively, J. R., Zrenner, E., Jägle, H., Kohl, S., Wissinger, B., and Koch, K.-W. (2009) Mutations in the GUCA1A gene involved in hereditary cone dystrophies impair calcium-mediated regulation of guanylate cyclase. *Hum. Mutat. 30*, E782−E796.
(62) Sokal, I., Li, N., Surgucheva, I., Warren, M. J., Payne, A. M., Bhattacharya, S. S., Baehr, W., and Palczewski, K. (1998) GCAP1 (Y99C) mutant is constitutively active in autosomal dominant cone dystrophy. *Mol. Cell 2*, 129−133.
(63) Olshevskaya, E. V., Calvert, P. D., Woodruff, M. L., Peshenko, I. V., Savchenko, A. B., Makino, C. L., Ho, Y. S., Fain, G. L., and Dizhoor, A. M. (2004) The Y99C mutation in guanylyl cyclase-activating protein 1 increases intracellular Ca2+ and causes photoreceptor degeneration in transgenic mice. *J. Neurosci. 24*, 6078−6085.
(64) Jiang, L., Wheaton, D., Bereta, G., Zhang, K., Palczewski, K., Birch, D. G., and Baehr, W. (2008) A novel GCAP1(N104K) mutation in EF-hand 3 (EF3) linked to autosomal dominant cone dystrophy. *Vision Res. 48*, 2425−2432.
(65) Nishiguchi, K. M., Sokal, I., Yang, L., Roychowdhury, N., Palczewski, K., Berson, E. L., Dryja, T. P., and Baehr, W. (2004) A novel mutation (I143NT) in guanylate cyclase-activating protein 1 (GCAP1) associated with autosomal dominant cone degeneration. *Invest. Ophthalmol. Vis. Sci. 45*, 3863−3870.
(66) Sokal, I., Dupps, W. J., Grassi, M. A., Brown, J., Jr., Affatigato, L. M., Roychowdhury, N., Yang, L., Filipek, S., Palczewski, K., Stone, E. M., and Baehr, W. (2005) A novel GCAP1 missense mutation (L151F) in a large family with autosomal dominant cone-rod dystrophy (adCORD). *Invest. Ophthalmol. Vis. Sci. 46*, 1124−1132.

(67) Jiang, L., Katz, B. J., Yang, Z., Zhao, Y., Faulkner, N., Hu, J., Baird, J., Baehr, W., Creel, D. J., and Zhang, K. (2005) Autosomal dominant cone dystrophy caused by a novel mutation in the GCAP1 gene (GUCA1A). *Mol. Vis. 11*, 143−151.
(68) Wilkie, S. E., Li, Y., Deery, E. C., Newbold, R. J., Garibaldir, D., Bateman, J. B., Zhang, H., Lin, W., Zack, D. J., Bhattacharya, S. S., Warren, M. J., Hunt, D. M., and Zhang, K. (2001) Identification and functional consequences of a new mutation (E155G) in the gene for GCAP1 that causes autosomal dominant cone dystrophy. *Am. J. Hum. Genet. 69*, 471−480.
(69) Hsu, Y.-T., and Molday, R. S. (1993) Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. *Nature 361*, 76−79.
(70) Weitz, D., Zoche, M., Müller, F., Beyermann, M., Körschen, H. G., Kaupp, U. B., and Koch, K.-W. (1998) Calmodulin controls the rod photoreceptor CNG channel through an unconventional binding site in the N-terminus of the beta-subunit. *EMBO J. 17*, 2273−2284.
(71) Grunwald, M. E., Yu, W.-P., Yu, H.-H., and Yau, K.-W. (1998) Identification of a domain on the β-subunit of the rod cGMP-gated cation channel that mediates inhibition by calcium-calmodulin. *J. Biol. Chem. 273*, 9148−9157.
(72) Kawamura, S., Hisatomi, O., Kayada, S., Tokunaga, F., and Kuo, C.-H. (1993) Recoverin has S-modulin activity in frog rods. *J. Biol. Chem. 268*, 14579−14582.
(73) Gorodovikova, E. N., Senin, I. I., and Philippov, P. P. (1994) Calcium-sensitive control of rhodopsin phosphorylation in the reconstituted system consisting of photoreceptor membranes, rhodopsin kinase and recoverin. *FEBS Lett. 353*, 171−172.
(74) Chen, C.-K., Woodruff, M. L., Chen, F. S., Chen, D., and Fain, G. L. (2010) Background light produces a recoverin-dependent modulation of activated-rhodopsin lifetime in mouse rods. *J. Neurosci. 30*, 1213−1220.
(75) Grigoriev, I. I., Senin, I. I., Tikhomirova, N. K., Komolov, K. E., Permyakov, S. E., Zernii, E. Y., Koch, K.-W., and Philippov, P. P. (2012) Synergetic effect of recoverin and calmodulin on regulation of rhodopsin kinase. *Front. Mol. Neurosci. 5*, 1−13.
(76) Venkatesan, J. K., Natarajan, S., Schwarz, K., Mayer, S. I., Alpadi, K., Magupalli, V. G., Sung, C.-H., and Schmitz, F. (2010) Nicotinamide adenine dinucleotide-dependent binding of the neuronal Ca^{2+} sensor protein GCAP2 to photoreceptor synaptic ribbons. *J. Neurosci. 30*, 6559−6576.
(77) Schmitz, F., Natarajan, S., Venkatesan, J. K., Wahl, S., Schwarz, K., and Grabner, C. P. (2012) EF hand-mediated Ca^{2+}- and cGMP signaling in photoreceptor synaptic terminals. *Front. Mol. Neurosci. 5*, 26.
(78) López-del Hoyo, N., Fazioli, L., López-Begines, S., Fernández-Sánchez, L., Cuenca, N., Llorens, J., de la Villa, P.d, and Mendez, A. (2012) Overexpression of guanylate cyclase activating protein 2 in rod photoreceptors in vivo leads to morphological changes at the synaptic ribbon. *PLoS One 7*, e42994.