Mathematical Analysis of Electrical Responses in Photoreceptor Cells Reveals Distinct Chemical Control of Visual Signal Transduction in Rods and Cones.

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Mathematical analysis of electrical responses in photoreceptor cells reveals distinct chemical control of visual signal transduction in rods and cones.

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Running header: Analysis of Light-Dependent Phototransduction in rod and cone

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Abstract

Retinal photoreceptor cells, rods and cones, convert photons of light into chemical and electrical signals as the first step of the visual transduction cascade. Although the chemical processes in the phototransduction system are very similar to each other in these photoreceptors, the light sensitivity and time resolution of the photoresponse in rods are functionally different than those in the photoresponses of cones. To systematically investigate how photoresponses are divergently regulated in rods and cones, we have developed a detailed mathematical model on the basis of the Hamer model. The current model successfully reconstructed light intensity-, ATP- and GTP-dependent changes in concentrations of phosphorylated visual pigments (VPs), activated transducins (Tr*s) and phosphodiesterases (PDEs), as well as cyclic nucleotide-gated currents (I_{CNG}) in rods and cones. In comparison to rods, the lower light sensitivity of cones was attributed not only to the lower affinity of activated VPs for Trs but also to the faster desensitization of the VPs. The assumption of an intermediate inactive state, MIIi, in the thermal decay of activated VPs was pivotal for inducing faster inactivation of VPs. In addition to the faster inactivation of VPs, calculating a faster rate of RGS9 intervention for PDE-induced Tr* inactivation in cones was indispensable for simulating the electrical waveforms of the light intensity-dependent I_{CNG} at higher temporal resolution in experimental systems in vivo.

Introduction

Retinal photoreceptor cells, the key players in the visual system, convert photons of light into chemical and electrical signals as the first step of the visual transduction cascade. When incident light stimulates a visual pigment (VP; a prototypical G protein-coupled receptor) on membranous disks at the outer segments of photoreceptors, the catalytic activity of a heterotrimeric G-protein, transducin (Tr), of exchanging GTPs for previously bound GDP increases. The GTP-bound α subunit of Tr dissociates from the $\beta\gamma$ subunits and subsequently activates phosphodiesterase (PDE) [1], which in turn hydrolyzes cyclic guanosine 3'-5' monophosphate (cGMP). The signals of visual transduction are highly amplifiable since a single stimulated VP (VP*) activates ~30 and ~140 molecules of Tr per second in cones and rods, respectively [2]. Activated VPs are simultaneously inactivated through two distinct mechanisms: phosphorylation by visual pigment-specific kinases (RKs) and thermal decay processes, thereby contributing to the termination of Tr activity.

Under scotopic conditions (dark), basal cGMP regulates the activities of cyclic nucleotide-gated (CNG) nonselective cation channels, allowing a steady inward current [3]. Photoreceptor cells are thus slightly depolarized (~40 mV, [4]) and spontaneously release a neurotransmitter, glutamate [5]. Upon stimulation of visual pigments the concentration of cGMP ([cGMP]) decreases, which deactivates the CNG channels and subsequently hyperpolarizes the photoreceptor to ~ -60 mV, preventing the release of neurotransmitters [4]. Postsynaptic responses to glutamate may be excitatory or inhibitory depending on the postsynaptic cell type involved [6]. Signals generated by switching "on" and "off" glutamate-mediated neurotransmission are comprehensively processed within the complex network of retinal cells (bipolar, horizontal, amacrine, and ganglion cells) and sent to optic nerve fibers.

There are two classic types of photoreceptors in mammalian eyes: rods and cones [7]. Although the chemical processes through the visual transduction cascades in these photoreceptors, as described above, are very similar to each other, several functional differences have been experimentally observed. Rods are highly sensitive to light, and visual transduction in rods may be triggered by a single photon. On the other hand, cones require significantly brighter light to activate the signaling cascade and generate electrical signals [8]. Another major difference is reflected in the time course of the photoresponses. The electrical waveforms of the flash light responses are activated and deactivated relatively slowly, exhibiting a strikingly prolonged response in rods than is observed in cones [8]. The differences in sensitivity and the temporal resolution of the light responses in rods and cones are attributed to different reaction rates throughout

the visual phototransduction system. However, the quantitative aspects of the molecular mechanisms underlying distinct phototransduction systems have not yet been conclusively clarified [9]. To quantitatively and systematically investigate how light intensity-dependent photoresponses are divergently regulated in rods and cones, a systems analysis of visual signal transduction systems for these photoreceptors is indispensable.

Former mathematical models of visual transduction cascades for rods and cones, the Hamer [10] and Dell'Orco [11] models, explain fundamental molecular reactions in the phototransduction system. Hamer et al. [10] and Dell'Orco et al. [11], however, seemed to place particular emphasis on photoresponses, especially single photon responses (SPRs). Their models thus generate macroscopic electrical events, including SPRs, but do not faithfully reproduce some of the microscopic biochemical responses, e.g., changes in concentrations and activity levels of VP*, Tr, or PDE, although these biochemical parameters were described thoroughly in the literature [2] [8] [1]. Furthermore, these previously developed mathematical models do not take into account [ATP] or [GTP]; therefore, ATP-dependent phosphorylation and the subsequent desensitization of VP* and GTP-dependent activation of Tr and cGMP production are not accurately estimated. These models remain powerful for demonstrating scientific aspects of the phototransduction system underlying photoresponses, especially SPRs; however, these models are not sufficient for practical application, e.g., in electroretinogram (ERG) simulation, where specific predictions of the molecular defects in microscopic biochemical reactions underlying pathophysiological electrical responses ([12] [13]) are required in medicine. In the present study, frameworks for all the molecular reactions of phototransduction cascades were mathematically elaborated on the basis of the Hamer model [10] to ensure that a wide variety of light intensity-, ATP-, and GTP-dependent microscopic biochemical reactions underlying macroscopic electrical responses are accurately reconstructed.

The proposed lower vertebrate rod and cone models successfully reproduced in vitro time courses of light intensity-, ATP- and GTP-dependent changes in the concentrations of phosphorylated VPs, Tr*s and PDEs and of cyclic nucleotide-gated currents (I_{CNG}) in rods and cones from frog and carp [14] [15]. Compared to that of rods, the lower light sensitivity of cones was attributed to the lower affinity of the activated VPs for Trs, as well as the faster desensitization (phosphorylation and inactivation) of VPs. The assumption of an intermediate inactive state, MIIi, during the thermal decay of activated VP was pivotal for inducing faster inactivation of VP. Furthermore, the combination of faster rates of VP desensitization and RGS9-mediated Tr* inactivation together were indispensable for simulating higher

temporal resolution of the electrical waveforms of the light intensity-dependent I_{CNG} in vivo experimental systems.

Methods

A dynamic mathematical model of the visual transduction system in rods and cones was constructed to analyze two distinct sets of experimental observations: fundamental microscopic “in vitro” biochemical reactions in phototransduction (Figures 2-4) and light intensity-dependent macroscopic photoresponses observed in “intact” retinal photoreceptor cells (Figure 5).

The fundamental component of the proposed “in vitro” model was developed on the basis of the Hamer model [10] and Dell’Orco model [11] by elaborating the frameworks for all the key molecular reactions of phototransduction cascades that account for the details of the underlying biochemistry as described in the subsections below. The molecular environment of the membranous disk where all chemical reactions for phototransduction take place in vivo (e.g., concentrations and spatial distributions of membrane-associated molecules, VP, RK, Tr, and PDE, present at the outer segments of photoreceptors), was assumed to be preserved and thus nearly identical to that isolated for biochemical experiments (see Figures 2-4). Taking into account the molecular environment of a single membranous disk, the in vivo concentrations of VP, RK, PDE, and were determined (Wet column in Table 1, see the Discussion section for more details). Note that different concentrations of freely diffusing factors, ATP, GTP, and cGMP, were applied depending on the corresponding biochemical experiments. The simulated results in Figures 2-4 were all reproduced using the parameters listed in Table 1 “Sim.” column and Table 3, in the “in vitro” model columns. The results are expressed as events per either VP* or VP_{tot}.

For the calculations of the light intensity-dependent macroscopic photoresponses observed in “intact” retinal photoreceptor cells (Figure 5), the effects of cytosolic factors, e.g., arrestin (Arr) and G protein signaling 9 (RGS9), were considered in addition to the “in vitro” system. The I_{CNG} presented in Figure 5 were calculated using the parameters listed in Table 3, in the “intact” model columns, and as described in detail in each subsection below.

The reaction scheme of the visual transduction cascade is shown in Figure 1, and the differential equations for the reaction steps in the model are described in the Supplementary materials under Equations. The abbreviations of signaling molecules are summarized in Table 2. The parameters used to define the present model, including the

concentrations and binding constants (K_d) of signaling factors and the rate constant (k), maximum activity ($V_{\max}$) and half-maximal effective concentration ($K_{1/2}$) of substances for activation of enzymes, among other parameters, are listed in Table 3. The initial values of some variables are also listed in Table 4. Model development and simulation-based analyses were both performed with simBio [16]. The time integration of the differential equations was conducted using the Euler method with a time step of 1 μ s.

A wide variety of light intensity-, ATP-, and GTP-dependent photoresponses in distinct types of photoreceptors in frogs and carp (extensively described by the biochemical experiments of Kawamura et al. [14] [2], see Figures 2-5) were successfully reconstructed and then quantitatively and systematically investigated in the current study.

Activation of visual pigments

Light stimulation activates a VP, which consists of an opsin combined with the chromophore 11-cis-retinal. The activation of a VP is initiated by light-induced isomerization of 11-cis-retinal to the all-trans form, causing conformational changes in the opsin. The intensity of the light stimulus (I_{light}), indicated in Figures 2-4, are given as a percentage (%), reflecting the % of visual pigments activated (bleached) by the light stimulus when considering the concentration of the total visual pigment as 100% (1).

$$I_{\text{light}} = \frac{[VP^*]}{[VP_{\text{total}}]} \quad (1)$$

Activated VP immediately after light stimulation (MII^*_0 at $t = 0$) is thus determined by (2).

$$[MII^*_0] = I_{\text{light}}[VP_{\text{total}}] \quad (2)$$

In the current simulation study, the stimulated VP, MII^* ($MII^*_0 - MII^*_3$, see Figure 1), is assumed to be capable of activating Tr, while being simultaneously inactivated through two distinct mechanisms: phosphorylation and thermal decay.

Phosphorylation of visual pigments

The phosphorylation of VPs is mediated by visual pigment-specific kinases (RKs).

In the Hamer model, phosphorylation reactions were simply calculated in 3 reaction steps: binding of the activated VP to an RK, phosphorylation of VP, and the dissociation of the RK from the VP. The Hamer model cannot be used to simulate the [ATP]-dependent phosphorylation or sequential inactivation of the VPs. Therefore, the binding of ATP to and dissociation of ADP from the MII*-RK complex were incorporated into the current model (see the reaction scheme in Figure 1B, reaction formulae R.1.1 - 1.8 below and the corresponding equations, Eqs. S1 - 26, S51 - 60, in the Supplementary materials under Equations) to ensure that the activities of Tr and PDE in the presence and absence of ATP, as estimated by experimental studies, are reconstructed and considered in this study (Figures 3 and 4)

where $0 \leq n \leq 3$ (n = number of phosphorylated VP sites) when simulating photoresponses in vitro (Figures 2 - 4) and $0 \leq n \leq 1$ when simulating I_{photo} recorded under in vivo conditions (Figure 5). The notation “pre” and “post” in above reaction distinguish RK-bound states of R* before and after phosphorylation, respectively.

Tachibanaki et al. [17] showed that the steady-state phosphorylation level in cones was ~ 3 , which is in good agreement with the number of phosphates necessary for the

complete suppression of the activated VPs in rods [18]. Although a maximum of 9 phosphorylation sites in the C terminus of MII have been suggested [19], only 3 sites were considered in the current model (for Figures 2-4, $0 \leq n \leq 2$ for R.1.1 - 1.8) based on biochemical experiment observations in vitro [18] [17]. For the calculation of the visual pigment phosphorylation shown in Figure 2, the total number of phosphate groups incorporated into activated visual pigments was determined by Eq. (3).

$$[Pi] = \sum_{n=1}^2 \{([MII_n^* \bullet RK_{pre}] + [MII_n^* \bullet RK_{pre} \bullet ATP] + [MII_n^i \bullet RK_{pre}] + [MII_n^i \bullet RK_{pre} \bullet ATP]) \times n\} + \sum_{n=1}^3 \{([MII_n^*] + [MII_n^* \bullet RK_{post}] + [MII_n^i] + [MII_n^i \bullet RK_{post}]) \times n\} \quad (3)$$

Rate constants for the phosphorylation reactions of the VPs, $k_{RK1} - k_{RK6}$, were estimated by model fitting to the experimental data exhibiting the time courses and the maximal rate of phosphorylation in rod and cone at given light flash intensities [17] (see Figure 2) The reaction rates for the phosphorylation of the activated VPs in the Hamer model were designed to vary as the phosphorylation reactions proceed, whereas those for the current model were set as constant parameters since the rate of phosphorylation up to $3Pi/VP^*_{total}$ does not seem to vary significantly (see Fig. 1 in Tachibanaki et al., [17]). The phosphorylation rate constants for MII^i , $k_{RK1}^i - k_{RK6}^i$, were assumed to be one-half those of MII^* ($k_{RK1} - k_{RK6}$) to reproduce continuing phosphorylation processes even after VP activity was completely terminated (based on the comparison of the time course of VP phosphorylation (see Figure 2A) with that of Tr activation in the presence of ATP (Figure 3A)) [17] [20].

For the simulation of the electrical waveforms of the light intensity-dependent photoresponses shown in Figure 5B, the phosphorylation reaction was calculated only for a single site ($n = 0$ for R.1.1 - 1.8), assuming a complete loss of VP activity quickly after arrestin (Arr) binds to MII^*_1 in the “intact” system. In this case, MII^*_0 was assumed to be only capable of activating Tr (see the Discussion section).

Thermal decay of visual pigments

Thermal decay of VPs is another pathway for the inactivation mechanism. The process is mediated by conformational changes in which MII^*_0 transitions to $MIII$ [21] directly or after undergoing a transition to an intermediate state, MII^i [22] (see the Discussion section), as well as by bleaching (dissociation of retinal from opsin) and the subsequent

recycling of VPs [23]. MIII may also undergo bleaching processes (see the reaction scheme in Figure 3, reaction formulae R.2.1 - 2.6 below, and corresponding equations, Eqs. S1 - 4, S14 - 17, S27 - 28, S61 - 62, in the Supplementary materials under Equations).

Rate constants for the inactivation reaction of the VPs, $k_2 - k_4$, were determined based on the reports of Kolesnikov et al. [24] and Kawamura et al. [2], while k_1 was estimated by model fitting. Note that the rates of $k_2 - k_4$ were assumed to be 6-fold faster, while k_1 was 120-fold faster in cones than in rods, since the lifetime of the MII intermediate was expected to be exceedingly shorter in cones. The reaction rates for inactivation were verified by reproducing Tr activation in the absence of ATP under the condition that no VP phosphorylation occurs, as shown in Figure 3.

Activation and inactivation of transducins

Transducin (Tr) is a prototypic heterotrimeric G protein comprising G_α and $G_{\beta\gamma}$ subunits. Stimulated visual pigments increase the catalytic activity of Tr by exchanging GTP for the GDP previously bound to G_α . G_α -GTP then dissociates from the $G_{\beta\gamma}$ subunits and subsequently binds to a PDE, increasing the degradation rate of cGMP (see the reaction scheme in Figure 3, reaction formulae R.3.1 - 3.5 below, and corresponding equations, Eqs. S29 - 42, S63 - 67, in the Supplementary materials under Equations). In the experiment, the number of $\text{GTP}\gamma\text{S}$, a nonhydrolyzable GTP analog, incorporated per Tr^* (G_α -GTP γS) was measured as an indicator of activated Tr.

where n indicates the number of phosphorylated sites ($n \geq 0$).

For the current model, the constraint of mass conservation was newly introduced to the concentration of Tr to prevent the continuous increase in G_{α} -GTP γ S in response to a stronger light stimulus (see Figure 3B). Since GTP γ S is not hydrolyzable, the initial rate of the change in G_{α} -GTP γ S (Figure 3A) purely reflects the rate of Tr activation because the VP* desensitization (inactivation and phosphorylation) reactions progress more slowly than those of Tr activation [25]. The rate constants for the reactions of Tr activation, $k_{G1,n} - k_{G7}$, were thus estimated by model fitting to the initial rate of Tr activation in response to light flash stimulation (0.0085% for rods and 0.25% for cones) as well as the light intensity-dependent activation of Tr in vitro [25] [20] (see Figure 3). Note that the affinity of G_{α} -GTP for MII*₀ ($k_{G1,0}/k_{G2}$) was approximately 6-fold higher in rods than in cones. The estimation was comparable to that of Chen et al. [26].

The G_{α} -GTP-binding rate to MII*₁ ($k_{G1,1}$) was estimated to be ~60% of that to MII*₀ ($k_{G1,0}$), while the rate ($k_{G1,2}$ and $k_{G1,3}$, see R.3.6) was assumed to further decrease through successive phosphorylation of the VPs (to MII*₂ and MII*₃, respectively), based on Gibson et al. [27] (see the Discussion section for more details).

$$k_{G1,n} = k_{G10} \exp(-\omega n) \quad \text{R.3.6}$$

The reaction rates for VP inactivation were verified by reproducing the experimental data obtained in the absence of ATP (Figure 3) under the condition that VP is not phosphorylated. The values of $k_{G1,n} - k_{G7}$ determined in the present study were comparable to these of the Hamer model [10].

Physiologically, activated Tr* (G_{α} -GTP) undergoes inactivation by the hydrolysis of its bound GTP to GDP on a minute time scale [20] (see the reaction scheme in Figure 1, reaction formulae R.4.1 - 4.3 below, and corresponding equations, Eqs. S42 - 45, S67 - 69, in the Supplementary materials under Equations). Calculating the elution of Tr* from the localized outer membrane complex where phototransduction takes place in experimental systems in vitro was indispensable for the reconstruction of the decay of PDE activity (Figure 4). Notably, inactivation does not occur with GTP γ S.

The GTPase activity of PDE-associated G_{α} dramatically increases as the PDE · G_{α} GTP complex binds to Regulator of RGS9, a GTPase-accelerating protein (see the reaction formulae R.7.1 - 7.2 below and corresponding equations, Eqs. S69 and 71, in the Supplementary materials under Equations). The RGS9 effects on free Tr^* (G_{α} -GTP) were assumed to be negligible since the binding affinity of RGS9 for free Tr^* was considerably lower than it was for PDE-bound Tr^* [28]

The rate constants for RGS9-dependent hydrolysis, k_{RGS1} - k_{RGS3} , were estimated by model fitting to the time courses of the electrical waveforms of the light intensity-dependent photoresponses, which are shown in Figure 5 recorded in a physiological condition [8] (see the Discussion section). Based on Tachibanaki et al. [20], an approximate 20-fold higher expression of RGS9 in cones than in rods was assumed for the simulation (see Table 3). The effect of RGS9 was excluded from the current model for the simulation of the in vitro experiments (Figures 2-4) under the assumption that RGS9 is translocated from the membranous disk in the outer segments to the inner segments of photoreceptors during sample preparation [29] (see the Discussion for more details).

Regulation of PDE activity

The catalytic activity of PDE in hydrolyzing cGMP at rest is elevated when the inhibitory γ subunit is removed from the enzyme upon binding activated Tr ($PDE^* \cdot G_{\alpha}$ -GTP, see R.5.1 - 6.2). The activity of PDE thus decreases as G_{α} -GTP is hydrolyzed by its GTPase activity. For the Hamer model, cGMP hydrolysis by PDE was simply described by a rate constant [10]. In contrast, in the current study, the chemical reaction of cGMP binding to PDE was calculated to indicate that cGMP hydrolysis (R.5.4) is also a $[cGMP]_i$ -dependent process (see the reaction scheme in Figure 1, reaction formulae R.5.1 - 6.4 below, and corresponding equations, Eqs. S42, S44-S45 and S67-S70, in the Supplementary materials under Equations). Notably, the PDE-independent basal degradation of cGMP was also assumed when the calculating steady-state $[cGMP]_i$ in the dark, taking into account the continuous cGMP generation by guanylate cyclase (GC) in vivo (see Figure 5).

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354

355 Based on Kawamura et al. [8], G_{α} -GTP was also assumed to be eluted from the localized
 356 outer membrane complex where phototransduction takes place in the in vitro system
 357 (see R.6.1 and the Discussion section). The eluted G_{α} -GTP was also presumed to be
 358 inactivated, as described in the previous section (see R.6.2).

359 The elution of G_{α} -GTP was estimated to be negligible in the “intact” system when
 360 simulating the electrical waveforms of the light intensity-dependent photoresponses in
 361 Figure 5.

362

365

366 The rate constants for the reactions of PDE activation (k_{P1} and k_{P2}), cGMP hydrolysis
 367 (k_{P3} , k_{P4} , and β_{sub}) and Tr^* (G_{α} -GTP) inactivation (k_{decay}) were estimated by model fitting
 368 to the time courses of cGMP hydrolysis per VP^* in response to a given light flash
 369 stimulation and to light intensity-dependent PDE activity [2] [8] [30] (see Figure 4).

370

371 Regulation of guanylate cyclase (GC) in the “intact” system

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374 Physiologically, the concentration of intracellular cGMP is determined by the balance
 375 between the rate of cGMP production and degradation. The rate of GTP-dependent
 376 cGMP production by GC was negatively regulated by Ca^{2+} (see the reaction scheme in
 377 Figure 1, reaction formula R.7.2 below, and corresponding differential equation, Eq. S72,
 378 in the Supplementary materials under Equations).

379

The half-maximal value of the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) for the inhibition of GC (K_c) and the Hill coefficient for Ca^{2+} -dependent inhibition were determined based on experimental reports [31] [32] [33] [34]. The rate constants for cGMP production, $k_{\text{GC1}} \sim k_{\text{GC3}}$, and for basal cGMP degradation, β_{dark} , were estimated by adjusting the resting cGMP level at $2\mu\text{M}$ [35] given that $[\text{Ca}^{2+}]_i$ in the dark is $\sim 500\text{ }\mu\text{M}$ [36] [37] [38]. The values of $k_{\text{GC1}} \cdot k_{\text{GC3}}$ and β_{dark} determined in the present study were comparable to those estimated by Kawamura et al. [8] and other experimental studies [39] [10] [40] [41] [15].

Regulation of CNG currents in the “intact” system

$[\text{cGMP}]_i$ regulates CNG currents (I_{CNG}) in the outer segments of photoreceptor cells (see the corresponding differential equation, Eq. S83, in the Appendix under Equations). The half-maximal value of $[\text{cGMP}]_i$ for the activation of I_{CNG} (K_m) and the Hill coefficient for its $[\text{cGMP}]_i$ -dependent activity (n_h) was determined based on experimental reports [42] [43] [44] [45]. The maximum conductance of I_{CNG} , G_{max} , in the dark was estimated by reproducing the steady-state current of ~ 4 and $\sim 12\text{ pA}$ in the rods and cones, respectively, given that membrane potential (V_m) of each photoreceptor cells under the scotopic conditions is $\sim 40\text{ mV}$ [46] [47].

Regulation of $[\text{Ca}^{2+}]_i$ in the “intact” system

$[\text{Ca}^{2+}]_i$ in the current model is regulated by Ca^{2+} influx through I_{CNG} , Ca^{2+} efflux via $\text{Na}^+/\text{Ca}^{2+}\text{-K}^+$ exchangers (NCKX), and Ca^{2+} binding to endogenous calcium buffer (see the reaction scheme in Figure 1C and the corresponding differential equations in the Appendix under Equations: for free Ca^{2+} ($[\text{Ca}^{2+}]_i$), Eq. S81, and for bound Ca^{2+} (Cab), Eq. S82).

The relative Ca^{2+} permeability of I_{CNG} (b) was set at 0.3 and 0.41 for rods and cones, respectively [48]. The minimum Ca^{2+} concentration within cells (α_0) was set at $0.01\text{ }\mu\text{M}$ based on experimental reports [10] [36] [38]. The conductance of the NCKX (γ_{Ca}), total content of endogenous Ca^{2+} buffer (ϵ_T) and the rate constants for Ca^{2+} binding to the endogenous buffer (k_{b1} and k_{b2}) in rods and cones were estimated by reproducing the resting $[\text{Ca}^{2+}]_i$ at 500 nM for both types of photoreceptor cells under scotopic conditions, considering that $[\text{Ca}^{2+}]_i$ may be reduced to $\sim 100\text{ nM}$ when I_{CNG} decrease to 0 pA in response to a strong light stimulus. The values of γ_{Ca} determined in the present study were comparable to those estimated by Hamer et al. [40].

Results

Phosphorylation of visual pigments

Activated VPs undergo processes of inactivation mediated by visual pigment-specific kinases (RKs). Time courses of visual pigment phosphorylation in membrane preparations of rods and cones in response to given light flash stimulation (Figure 2A, 1.3% for rods (*a*, circle) and 2.5% for cones (*b*, triangle)) were reported by Tachibanaki et al. [17]. VP phosphorylation in cones was evidently faster than it was in rods (half-maximum phosphorylation: ~12.5 sec in rods and ~250 ms in cones), whereas the rate of phosphorylation depended on the flash intensity (see below). The in vitro elements of the current visual transduction cascade model well reproduced the time course of phosphorylation of visual pigments in both rods and cones (see dotted lines). The simulation study clarified that the difference in the apparent rates of phosphorylation in these two types of photoreceptors was due to distinct amounts of receptor kinases (12 μ M in rods and 120 μ M cones) and reaction rates for each chemical process during the phosphorylation of the VPs in the rods and cones ($k_{RK1} - k_{RK6}$ and $k^i_{RK1} - k^i_{RK6}$, see Table 3), as predicted by Tachibanaki et al. [17].

Tachibanaki and colleagues have also reported distinct maximum rates of phosphorylation at different flash intensities (Figure 2B) in membrane preparations of rods (*a*, circle) and cones (*b*, circle) [17]. Fitting the experimental results by the Michaelis-Menten equation ($V/S = V_{max}/(S + K_m)$, where $S = VP^*/VP_{tot}$ (expressed as a percentage)) yielded an estimate of V_{max} (the maximum phosphorylation rates) and K_m (the half-maximal values of VP^*/VP_{tot} upon stimulation of the RKs) for the phosphorylation reactions of 0.0049 Pi/VP*/s (or 0.037 pmol Pi/s) and 0.61% in rods and 0.41 Pi/VP*/s (or 3.1 pmol Pi/s) and 10% in cones, respectively. V_{max} and K_m were determined by fitting the same equation to the corresponding simulation results for rods (Figure 2Ba, triangle) and cones (Figure 2Bb, triangle), and comparable values were obtained (0.0018 Pi/VP*/s and 0.36% in rods; 0.32 Pi/VP*/s and 4.8% in cones, respectively), indicating that the current model well reconstructed the flash intensity-dependent phosphorylation of VPs in rods and cones. These results showed that, with the chosen rate constants for the phosphorylation reaction of the VPs ($k_{RK1} - k_{RK6}$ and $k^i_{RK1} - k^i_{RK6}$) in both photoreceptors, the model provided reasonable descriptions of the experimental data.

Activation of Tr (G_{α} -GTP)

Stimulated visual pigment increases the catalytic activity of Tr in exchanging GTP for GDP. The time courses of Tr activation in the membrane preparations of rods and cones in response to given light stimulation (Figure 3A, 0.0085% for rods (*a*, circle) and 0.25% for cones (*b*, triangle)), in the presence (filled symbols) and absence of ATP (open symbols) were reported by Tachibanaki et al. [20]. For these experiments, the number of GTP γ S molecules, a nonhydrolyzable GTP analog, incorporated per VP* was measured as an indicator of activated Tr. The time course of GTP γ S binding to Tr in the absence of ATP, thus without VP phosphorylation, was fitted with a simple exponential function ($Y = A[1 - \exp(-kt)]$). The estimated initial rates (k) of Tr activation were 143 and 30 Tr*/VP*/s for rods and cones, respectively, suggesting that amplification of the incoming light signal at the level of Tr activation was approximately 5-fold more efficient in rods than it was in cones. Even in the absence of ATP, GTP γ S-binding reactions (A) led to eventual saturation (~ 497 GTP γ S/VP* in rods and ~ 8.5 GTP γ S/VP* in cones) due to termination of MII*₀ activity because of inactivation processes. In the presence of ATP (1 mM), the maximum amount of GTP γ S bound to Tr per VP* was diminished by $\sim 60\%$ in the membrane preparations of both types of photoreceptor cells owing to the faster desensitization of MII* by the additional phosphorylation processes. The in vitro elements of the current model well reconstructed time courses of Tr activation for both rods and cones in the presence and absence of ATP (Figure 3A, dashed lines (+ATP) and dotted lines (-ATP)) when corresponding light stimulation intensities (*a*, 0.0085% in rods, *b*, 0.25% in cones) were applied. As Tachibanaki et al. [25] suggested, the initial rates of Tr activation with ATP (~ 52.7 Tr*/VP*/s in rods and ~ 15.8 Tr*/VP*/s in cones), were almost the same to those in the absence of ATP for both types of photoreceptors (~ 52.7 Tr*/VP*/s in rods and ~ 15.8 Tr*/VP*/s in cones), indicating that the molecular reactions involved in VP* phosphorylation progressed relatively slowly compared to those of Tr activation.

Tachibanaki and colleagues have also reported light-induced Tr activation at different flash intensities (Figure 3B) in membrane preparations of rods (*a*, circle) and cones (*b*, circle) in the presence (filled symbols) and absence (open symbols) of ATP (100 μ M) [25]. The half-maximal flash intensity for the activation of Tr was approximately 100-fold higher in cones than it was in rods. It was also evident that ATP reduced the light sensitivity of Tr activation in both rods and cones, reflecting the facilitated desensitization of MII*₀ through phosphorylation processes. The model also well

simulated light intensity-dependent Tr activation in both rods and cones. Thus, with the rate constants for the Tr activation reactions ($k_{G1,0}$ - $k_{G1,3}$ and k_{G2} - k_{G6}) and the reaction rates for the VP phosphorylation and inactivation processes with and without ATP in both types of photoreceptors, the model provided reasonable descriptions of the experimental data.

Hydrolysis of cGMP by PDE

The catalytic activity of PDE in hydrolyzing cGMP increases when the gamma inhibitory subunit is removed from the enzyme upon binding Tr^* (G_α -GTP). The PDE activity thus decreases as G_α -GTP is hydrolyzed by its GTPase activity. Koshitani et al. [30] reported time courses of cGMP hydrolysis in membrane preparations of rods and cones in response to light stimulations (Figure 4A, 0.024% for rods (*a*, open circle) and 0.46% for cones (*b*, open circle)) in the presence and absence of ATP (solid and dashed lines, respectively) with GTP γ S (filled symbols) and GTP (open symbols). In rods, the number of cGMP hydrolyzed per activated VP* monotonically increased with GTP γ S increases, in either the presence or absence of ATP. On the other hand, cGMP hydrolyzed with ATP and GTP reached saturation at $\sim 1/3$ of that with GTP γ S at 50 sec after the light stimulus due to termination of PDE activity as GTP hydrolysis preceded. In contrast, cGMP hydrolysis measured with ATP and GTP γ S in cones was diminished by $\sim 99\%$ relative to that in rods and was further reduced to $\sim 1/6$ with GTP, 25 sec after the light stimulus. The lifetime of Tr was thus estimated to be ~ 2 -fold shorter in cones [30].

Peak PDE activities (first derivatives of the number of cGMPs hydrolyzed per activated VP* molecule) were also measured at various intensities of light stimulation (Figure 4Ba, circle for rods and triangle for cones) in the presence of ATP and either GTP (open symbols) or GTP γ S (filled symbols). The peak light intensity-dependent PDE activity with GTP (open symbols) and GTP γ S (filled symbols) was not drastically different in rods and cones, indicating that the rate of GTP hydrolysis is slower than that of GTP-dependent or GTP γ S-dependent activation of PDE. However, the peak PDE activity was significantly more sensitive to light when the phosphorylation of VPs was prohibited, that is, when ATP was absent (the comparison is shown in Figure 4Bb to *a*).

The in vitro elements of the current model well reconstructed the time courses of cGMP hydrolysis in membrane preparations of rods and cones in response to corresponding light stimulation in the presence and absence of ATP with either GTP or GTP γ S (*a*, 0.0085% in rods and *b*, 0.25% in cones; lines in Figure 4A). The model also

well simulated light intensity-dependent PDE activities (lines in Figure 4*B*) with the rate constants for the Tr activation reactions ($k_{G1,0}$ - $k_{G1,3}$ and k_{G2} - k_{G6}) as well as the reaction rates for VP phosphorylation and inactivation processes with and without ATP in both types of photoreceptors.

Generation of I_{CNG}

The $[cGMP]_i$ is determined by the balance between the rate of cGMP production and degradation under physiological conditions. When $[GTP]_i$ is elevated, $[cGMP]_i$ and I_{CNG} increase because GC generates cGMP at higher rates (see the reaction formulae R.8.1 - 8.2). Kawamura et al. [14] reported $[GTP]_i$ -dependent I_{CNG} recorded from a truncated outer segment of rods (Figure 5*A*, circle). Physiologically, the basal $[GTP]_i$ and $[cGMP]_i$ in the dark are 1 mM and $\sim 2 \mu M$, respectively, and the corresponding I_{CNG} are ~ -4 pA in rods and -12 pA in cones. Photoreceptor cells are thus slightly depolarized (~ -40 mV, [4]). Upon stimulation of the visual pigments, a decrease in the concentration of $[cGMP]_i$ deactivates the CNG channels, reducing I_{CNG} to ~ 0 pA in response to a strong light stimulus. Kawamura and Tachibanaki reported that light-dependent changes in I_{CNG} recorded from outer segments of rods (Figure 5*Ba*, solid lines) and cones (Figure 5*Bb*, solid lines) [8]. The intact elements of the current model simulated not only the $[GTP]_i$ -dependent I_{CNG} in rods in the dark (Figure 5*A*, solid line) but also light-dependent I_{CNG} responses in the outer segments of both types of photoreceptors (Figure 5*Ba*, rods and b, cones; dotted lines). Note that these cGMP-dependent currents were simulated under voltage clamp condition (see Eq. S83) since the proposed model does not include other membrane current to calculate changes in membrane potentials. It should be emphasized that the initial rising phases of I_{CNG} responses, especially in cone, in voltage clamp experiments seems to be faster than these recorded under unclamped condition, most likely due to unchanging driving force for I_{CNG} [49].

Discussion

Retinal photoreceptor cells, rods and cones, convert photons of light into chemical and electrical signals as the first step in the visual transduction cascade. The chemical processes of the phototransduction system are very similar to each other in these photoreceptors. The light sensitivity and time resolution of the photoresponse in rods, however, are functionally different from those in cones. To quantitatively and systematically investigate how light intensity-dependent photoresponses are divergently

regulated in rods and cones, a detailed mathematical models of the visual signal transduction system in these photoreceptors was developed on the basis of the Hamer model [40]. The current model successfully reconstructed a wide variety of light intensity-, ATP- and GTP-dependent changes in the concentrations and activities of phosphorylated VPs and activated Trs/PDEs, as well as CNG currents in rods and cones [14] [2] [8] [1] [30] [17] [25] [20] [45], when taking into account the localized molecular environment.

Compared to that in rods, the lower light sensitivity in cones was, at least in part, attributed to the lower affinity of the activated VP for Tr given that the concentration, as well as spatial distributions of VP and Tr at membranous disks in these photoreceptor cells, were very similar to each other. In experiments, the expression of cone Tr (G_α subunit) in rods decreased the light sensitivity of rods and the rate of Tr activation [26] [9]. This biochemical study indicated that the molecular nature of the Tr G_α subunit in cones, not VP, contributes to the lower light sensitivity and response kinetics of Tr.

Faster desensitization of activated VP through phosphorylation and thermal decay (inactivation) is another pivotal characteristic leading to lower light sensitivity in cones. While, properties of faster VP desensitization also contribute to the higher time resolution for phototransduction in cones. During the inactivation of MII^*_0 , the functional intermediate state of inactive MII, MII^i , was assumed before MII^* undergoing a complete transition to MIII in both types of photoreceptors [22]. Since the transition from MII^*_0 to MIII was suggested to be an event on the order of a few minutes in vitro [21] [50], considering that the state MII^i is indispensable to the termination of visual pigment activity: within ~ 10 sec in rods and ~ 1 sec in cones (see Figure 3Aa and b, respectively).

In cones, the current simulation experiments estimated that the rate of MII^* inactivation was ~ 120 -fold faster than that was in rods. The molecular nature of VP in cones (cone opsin), however, was indistinguishable from that in rods (rhodopsin), at least in terms of their light sensitivity in expression systems [26] [9]. The mechanisms underlying the faster inactivation of cone opsin may be due to other environmental factors and need to be further explored in future experimental studies.

On the other hand, to reproduce continuing phosphorylation of VP even after complete termination of its activity, the phosphorylation rate constants for MII^i ($k_{RK1} - k_{RK6}$) were assumed to be slower (1/2) than those for MII^* .

When complete abolition of VP activities, within ~ 10 sec after stimulus, PDE-dependent cGMP hydrolysis still progressed for ~ 30 sec in rods and cones (shown as + ATP/+GTP in Figure 4Aa and b, respectively) due to a longer lifetime of Tr^* in the in vitro

experimental systems. The assumption of faster inactivation of Tr^* by PDE was therefore indispensable to obtain a faster recovery of I_{CNG} , which was recorded in vivo for both types of photoreceptors (Figure 5). In the current study, the mechanism underlying the fast inactivation of Tr^* was attributed to the RGS9-mediated reaction, which was presumed to be intact under physiological conditions. Compared to that in rods, nearly 20 fold higher expression of RGS9 was assumed in cones ([20], see Table 3); therefore, the higher temporal resolution of the electrical waveforms of the light intensity-dependent I_{CNG} was achieved in these cells (see Figure 5). Note that RGS9-independent degradation of Tr^* was also assumed to be faster in cones due to the expression level of RGS9 [20]. Although RGS9 was simply assumed to be translocated from the membranous disk at the outer segments to the inner segments of photoreceptor cells during sample preparation in vitro experiments [29], different mechanisms may be involved in the distinct time courses of the termination of PDE activity and recovery of I_{CNG} in both rods and cones.

For the simulation experiments, the molecular environment of the membranous disk, where all the chemical reactions for phototransduction take place to generate I_{CNG} in vivo (Figure 5), was assumed to be identical to that in the in vitro system (see Figures 2 - 4 and Table 3). This assumption was based on the observations that VP, Tr, PDE, and RK are closely associated with membranous disks even after undergoing isolation procedures for the biochemical experiments [2]. If chemical reactions among freely diffusing factors are assumed, then the time course of phototransduction would be significantly slower due to the apparent reduction in the concentrations of key signaling molecules in the visual transduction cascade. The assumption made for the microdomain of the membranous disk seems to be verified since VP phosphorylation, Tr activation, and cGMP hydrolysis observed at different concentrations of phototransduction factors in different experimental studies were all well reconstructed by the current simulation study.

For the current model, most of the parameters were determined based on the experimental reports as fully described in the Methods or Results section, whereas some parameters were estimated by fitting experimental data. The rate constants for the reactions of Tr activation, $k_{\text{G1,1}} - k_{\text{G7}}$ (see Table 3), were estimated by model fitting to the initial rate of Tr activation in response to light flash stimulation as well as light intensity-dependent activation of Tr in vitro [25] [20] (see Figure 3). However, the simulation experiments revealed that the time course and the light intensity-dependent

activation of Tr, as shown in Figure 3, may be reproduced with various sets of $k_{G1,0}$ - k_{G7} values. Specifically, the higher light sensitivity of rods can be reproduced even when the affinity of the activated VP to Tr ratio is lower than it is in cones when the subsequent molecular reactions ($k_{G1,1}$ - k_{G7}) are faster. Since these parameters have not been conclusively determined by experimental studies, $k_{G1,1}$ - k_{G7} were set by referring to former simulation studies [11] [10] [40]. Furthermore, the G_α -GTP-binding rate in MII^*_0 - MII^*_3 ($k_{G1,0}$ - $k_{G1,3}$) was assumed to decrease with successive phosphorylation of the VPs [27]. The reduction rate was determined by the parameter denoted by ω in R.3.6 (see the Methods section under Activation and Inactivation of Transducins). The current simulation study clarified that the higher the values of ω , the slower the transducin activation (Figure 3) as well as PDE-mediated cGMP hydrolysis (Figure 4) in the presence of ATP. Although the value of ω for rods was estimated to be 0.6 by Gibson et al. [27], the value for cones was not given in the literature. In this study, ω was estimated by fitting the experimental data, as shown in Figures 3 and 4, and found to be 0.9 for cones.

The reproduction of a single photon response [51] has long been considered one of the most important characteristics to reproduce by a mathematical model. Prior theoretical analysis has concluded that the VP phosphorylation process requires multiple steps, and VP affinity for RK and Tr must exponentially decline as VP phosphorylation proceeds [15] [40] [10]. However, these theoretical models failed to reproduce the time courses of VP phosphorylation or Tr activation observed in the experiments, therefore, some unknown mechanisms must be involved to control these chemical processes. Importantly, the unknown mechanisms do not contradict the current model, since the model well reproduce both the microscopic and macroscopic experimental observations, although the current model failed to reproduce SPR experiments.

Finally, the uncertain rate for RGS9-mediated inactivation of Tr^* was estimated by fitting the recovery time course of the electrical waveforms of the light intensity-dependent I_{CNG} , as shown in Figure 5B, based on the concentration of RGS9 and the rate constants for GC-mediated cGMP production determined by Tachibanaki et al. [20] and Kawamura and Murakami [14], respectively. Since GC-mediated cGMP production is regulated by Ca^{2+} , the total content of endogenous Ca^{2+} buffer (e_T) and the rate constants for the reactions of Ca^{2+} binding to the endogenous buffers (k_{b1} and k_{b2}) also determine the recovery time course of the light intensity-dependent I_{CNG} . Although the parameters for the endogenous Ca^{2+} -buffering system estimated in the current study were

comparable to those in the Hamer model [40], these values need to be re-evaluated if the rate constants for RGS9-mediated inactivation of Tr^* was experimentally determined.

Meanwhile, other chemical reactions, listed below, were purely driven from assumptions due to limited details in literature. These model assumptions would derive new working hypothesis for future experimental studies to clarify corresponding uncertainties.

1. During the inactivation of MII^*_0 , intermediate state of inactive MII, MII_i , that has no capability to activate Tr, was introduced.
2. Number of VP phosphorylation for the in intact model was restricted to 1 by taking into consideration arrestin binding.
3. Elution of $\text{G}\alpha\text{-GTP}$, assumed for the in vitro model, was considered negligible for the intact model.
4. Reactions at membranous disk of the in vitro model needed to be evaluated after scaling substrate concentration.

As described above, the set of parameters used in the current model are not the only definitive values to reproduce experimental results. In order to analyze the significance of model parameters, sensitivity analysis were performed. 28 parameters related with VP phosphorylation, Tr activation and inactivation, PDE activation, GC activity, RGS activity were increased by 5% and the sum of squared differences between the resulting time courses of Pi/VP^* and I_{CNG} , and the original time courses were evaluated which are shown in Figure S1 A and B (in Supplement), respectively (results normalized). Figure S1 A shows that the phosphorylation process was strongly related to the $k_{\text{RK1}} - k_{\text{RK6}}$ parameters, however, especially for cone, they were also related with Tr activation and inactivation, and PDE activation processes. Similarly, Figure S1 B shows that the I_{CNG} were strongly related with both phosphorylation, Tr activation and cGMP production processes. To see the detail of the relation between these parameters and the rising phase and the falling phase of I_{CNG} , sensitivity analysis on the maximum and the minimum slope of I_{CNG} were performed (see Figure S2 in Supplement). The rising phase was closely related with phosphorylation, Tr activation and PDE activation parameters (Figure S2 A), while the falling phase was related with phosphorylation, Tr activation and cGMP production and RGS activities (Figure S2 B).

Limitations

Although the current models well demonstrated the phototransduction in response to

light stimulation that lasted for only a short period, corresponding to experimental conditions, the cellular responses during light/dark adaptation may not be accurately predicted since the reactions for VP re-formation from opsin and 11-cis-retinal were not included in the systems. For future studies, it will be worthwhile to include the molecular reactions regarding the retinal cycle to predict the adaptation phenomena of the nonlinear behaviors of photoreceptors in response to light stimuli, as they refer to previous conditions.

It also should be emphasized that arrestin is another key molecule to determine VP inactivation through binding to phosphorylated VP. Therefore, incorporating arrestin reactions to the current model is fundamental to understand the entire biological process of VP inactivation. However, experimental data available in literature, as far as we were aware, were not sufficient enough to derive the binding rates especially when changes depending on number of phosphorylation of VP. Accordingly, for the current model, macroscopic data fitting was performed for arrestin-dependent VP inactivation, whereas it needs to be updated when sufficient experimental evidences are available in the future.

Finally, few parameters, ATP and GTP concentrations and K_c (the calcium concentration of half maximal cGMP production rate) for the current lower vertebrate model were obtained from experimental studies performed on mammals. Although ATP and GTP concentrations do not require temperature conversion or any additional scaling processes, K_c value need to be replaced when available in literature.

Table Legends

Table 1. Parameters of the membrane-associated molecules applied for simulation in comparison to these estimated in the experimental studies. Wet column represents in vitro substrate concentrations calculated after taking into consideration the experimental test tube volume, while Sim. refers to physiological in vivo concentrations. The Sim. values were applied for the simulation experiments assuming that the in vivo membrane structures were preserved in the in vitro experimental environments (see Discussion section for more details).

			VP (μM)	RK (μM)	PDE (μM)	Tr (μM)	ATP (μM)	GTP (μM)
Phosphorylation	Wet	Rod	0.5	0.002	0.0018	0.047	100	500
		Cone	0.5	0.02	0.0018	0.047	100	500
	Sim.	Rod	3000	12	11	280	100	500
		Cone	3000	120	11	280	100	500
Tr activation (time-dependent)	Wet	Rod	0.6	0.0024	0.0022	0.056	1000	100
		Cone	0.3	0.012	0.011	0.028	1000	100
	Sim.	Rod	3000	12	11	280	1000	100
		Cone	3000	120	11	280	1000	100
Tr activation (light-dependent)	Wet	Rod	3	0.012	0.011	0.28	100	5
		Cone	0.3	0.012	0.0011	0.028	100	5
	Sim.	Rod	3000	12	11	280	100	5
		Cone	3000	120	11	280	100	5
PDE activation	Wet	Rod	0.75	0.003	0.0028	0.07	250	100
		Cone	0.75	0.03	0.0028	0.07	250	100
	Sim.	Rod	3000	12	11	280	250	100
		Cone	3000	120	11	280	250	100
Photocurrent	Wet	Rod	3000	12	11	280	1000	1000
		Cone	3000	120	11	280	1000	1000
	Sim.	Rod	3000	12	11	280	1000	1000
		Cone	3000	120	11	280	1000	1000

k_{RK1}	Rate constant of binding MII_n^* to RK ($\mu M^{-1}s^{-1}$)
k_{RK2}	Rate constant of dissociation of MII_n^* from $MII_n^* \cdot RK_{pre}$ (s^{-1})
k_{RK3}	Rate constant of binding $MII_n^* \cdot RK_{pre}$ to ATP ($\mu M^{-1}s^{-1}$)
k_{RK4}	Rate constant of dissociation of $MII_n^* \cdot RK_{pre}$ from $MII_n^* \cdot RK_{pre} \cdot ATP$ (s^{-1})
k_{RK5}	Rate constant of dissociation of ADP from $MII_n^* \cdot RK_{pre}$ (s^{-1})
k_{RK6}	Rate constant of dissociation of MII_{n+1}^* from $MII_n^* \cdot RK_{post}$ following phosphorylation (s^{-1})
k_{RK1}^i	Rate constant of binding MII_n^i to RK ($\mu M^{-1}s^{-1}$)
k_{RK2}^i	Rate constant of dissociation of MII_n^i from $MII_n^i \cdot RK_{pre}$ (s^{-1})
k_{RK3}^i	Rate constant of binding $MII_n^i \cdot RK_{pre}$ to ATP ($\mu M^{-1}s^{-1}$)
k_{RK4}^i	Rate constant of dissociation of $MII_n^i \cdot RK_{pre}$ from $MII_n^i \cdot RK_{pre} \cdot ATP$ (s^{-1})
k_{RK5}^i	Rate constant of dissociation of ADP from $MII_n^i \cdot RK_{pre}$ (s^{-1})
k_{RK6}^i	Rate constant of dissociation of MII_{n+1}^i from $MII_n^i \cdot RK_{post}$ following phosphorylation (s^{-1})
k_1	Rate constant of inactivation of MII_n^* (s^{-1})
k_2	Rate constant of inactivation of MII_n^* and MII_n^i (s^{-1})
k_3	Rate constant of MIII (s^{-1})
k_4	Rate constant of dissociation of retinal and opsin from MII_n (s^{-1})
$k_{G1,0}$	Rate constant of binding Tr to MII_0^* ($\mu M^{-1}s^{-1}$)
$k_{G1,1}$	Rate constant of binding Tr to MII_1^* ($\mu M^{-1}s^{-1}$)
$k_{G1,2}$	Rate constant of binding Tr to MII_2^* ($\mu M^{-1}s^{-1}$)
$k_{G1,3}$	Rate constant of binding Tr to MII_3^* ($\mu M^{-1}s^{-1}$)
k_{G2}	Rate constant of dissociation of MII_n^* from $MII_n^* \cdot G \cdot GDP$ (s^{-1})
k_{G3}	Rate constant of dissociation of GDP from $MII_n^* \cdot G \cdot GDP$ (s^{-1})
$k_{G4}[GDP]$	Rate constant of binding GDP to $MII_n^* \cdot G$ (s^{-1})
k_{G5}	Rate constant of binding GTP to $MII_n^* \cdot G$ (s^{-1})
k_{G6}	Rate constant of dissociation of MII_n^* from $MII_n^* \cdot G \cdot GTP$ (s^{-1})
k_{G7}	Rate constant of dissociation of $G\alpha \cdot GTP$ from $G \cdot GTP$ (s^{-1})
k_{P1}	Rate constant of binding PDE to $G\alpha \cdot GTP$ ($\mu M^{-1}s^{-1}$)
k_{P2}	Rate constant of activation of $PDE \cdot G\alpha \cdot GTP$ (s^{-1})
k_{P3}	Rate constant of binding cGMP to $PDE^* \cdot G\alpha \cdot GTP$ ($\mu M^{-1}s^{-1}$)
k_{P4}	Rate constant of dissociation of cGMP from $cG \cdot PDE^* \cdot G\alpha \cdot GTP$ (s^{-1})
$k_{elution}$	Rate constant of elution of $G\alpha \cdot GTP$ (s^{-1})
k_{RGS1}	Rate constant of binding RGS9 to $PDE^* \cdot G\alpha \cdot GTP$ ($\mu M^{-1}s^{-1}$)

k_{RGS2}	Rate constant of dissociation of RGS9 from $\text{RGS9} \cdot \text{PDE}^* \cdot \text{G}\alpha \cdot \text{GTP}$ (s^{-1})
k_{RGS3}	Rate constant of dissociation of RGS9 from $\text{RGS9} \cdot \text{PDE}^* \cdot \text{G}\alpha \cdot \text{GTP}$ and GTP hydrolysis (s^{-1})
k_{decay}	Rate constant of inactivation of $\text{G}\alpha \cdot \text{GTP}$ (s^{-1})
k_{GC1}	Rate constant of binding GC to GTP (s^{-1})
k_{GC2}	Rate constant of dissociation of cGMP and GC from $\text{GC} \cdot \text{GTP}$ (s^{-1})
k_{GC3}	Rate constant of dissociation of GMP from $\text{GC} \cdot \text{GTP}$ (s^{-1})
K_c	Calcium ion at which synthesis of cGMP is half of maximum rate of cGMP (μM)
m	Hill coefficient for the action of Calcium ion on cyclase rate (-)
β_{dark}	Dark rate of cGMP hydrolysis (s^{-1})
β_{sub}	Rate constant of cGMP hydrolysis by $\text{cG} \cdot \text{PDE}^* \cdot \text{G}\alpha \cdot \text{GTP}$ (s^{-1})
b	Ratio of calcium ion to Photocurrent
γ_{Ca}	Rate constant of calcium ion extrusion by the NCKX (s^{-1})
c_0	Minimum intracellular calcium ion (μM)
e_T	Concentration calcium ion buffers total (μM)
k_{b1}	Rate constant of binding calcium ion to buffers ($\mu\text{M}^{-1}\text{s}^{-1}$)
k_{b2}	Rate constant of dissociation of calcium ion from buffers (s^{-1})
K_m	Half maximum of cGMP concentration (μM)
n_h	Hill coefficient for opening CNG channels (-)
F	Faraday constant (C/mol)
Vol	Cytoplasmic volume (L)
G_{max}	Conductance of photocurrent (pA/mV)
E_p	Reversal potential (mV)
V_m	Membrane potential (mV)
VP_{total}	Concentration of visual pigment total (μM)
MII^*	Concentration of activated metarhodopsin II (μM)
MII^i	Concentration of intermediate state of activated metarhodopsin II and metarhodopsin III (μM)
RK_{free}	Concentration of rhodopsin kinase total (μM)
Tr_{free}	Concentration of transducin total (μM)
PDE_{free}	Concentration of phosphodiesterase (μM)
$\text{RGS9}_{\text{free}}$	Concentration of RGS9 free (μM)
cGMP	Concentration of cGMP (μM)
ATP	Concentration of ATP (μM)
GTP	Concentration of GTP (μM)
GC_{free}	Concentration of Guanylate Cyclase total (μM)

Ca^{2+}	Concentration of calcium ion (μM)
Ca_b	Concentration of intracellular calcium bound to buffers (μM)
I_{photo}	Photocurrent (pA)

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740 Table 3. Parameters in the current models

Parameter	Unit	in vitro model		intact model		References	Species
		Rod	Cone	Rod	Cone		
k_{RK1}	$\mu\text{M}^{-1}\text{s}^{-1}$	0.462	9.6	0.462	9.6	[17]	carp
k_{RK2}	s^{-1}	6.0	80	6.0	80	[17]	carp
k_{RK3}	$\mu\text{M}^{-1}\text{s}^{-1}$	0.16	0.26	0.16	0.26	[17]	carp
k_{RK4}	s^{-1}	1.5	7.0	1.5	7.0	[17]	carp
k_{RK5}	s^{-1}	1.083	37.5	1.083	37.5	[17]	carp
k_{RK6}	s^{-1}	100	1500	100	1500	[17]	carp
k_{RK1}^i	$\mu\text{M}^{-1}\text{s}^{-1}$	0.231	4.8	0.231	4.8	[17] [20]	carp
k_{RK2}^i	s^{-1}	3.0	40	3.0	40	[17] [20]	carp
k_{RK3}^i	$\mu\text{M}^{-1}\text{s}^{-1}$	0.08	0.13	0.08	0.13	[17] [20]	carp
k_{RK4}^i	s^{-1}	0.75	3.5	0.75	3.5	[17] [20]	carp
k_{RK5}^i	s^{-1}	0.542	18.75	0.542	18.75	[17] [20]	carp
k_{RK6}^i	s^{-1}	50	750	50	750	[17] [20]	carp
k_1	s^{-1}	1.0	120	1.0	120	[2]	carp
k_2	s^{-1}	0.0056	0.0336	0.0056	0.0336	[2] [24]	carp,frog
k_3	s^{-1}	0.001	0.006	0.001	0.006	[2] [24]	carp,frog
k_4	s^{-1}	0.0053	0.0318	0.0053	0.0318	[2] [24]	carp,frog
$k_{G1,0}$	$\mu\text{M}^{-1}\text{s}^{-1}$	3.57	35.7	3.57	35.7	[10] [20]	vertebrate, carp
$k_{G1,1}$	$\mu\text{M}^{-1}\text{s}^{-1}$	1.96	14.5	0	0	[20]	carp
$k_{G1,2}$	$\mu\text{M}^{-1}\text{s}^{-1}$	1.08	5.9	0	0	[20]	carp
$k_{G1,3}$	$\mu\text{M}^{-1}\text{s}^{-1}$	0.59	2.4	0	0	[20]	carp
k_{G2}	s^{-1}	50	2250.34	50	2250.34	[11] [20]	vertebrate, carp
k_{G3}	s^{-1}	1000	370.4	1000	370.4	[10] [20]	vertebrate, carp
$k_{G4}[\text{GDP}]$	s^{-1}	600	2.0	600	2.0	[10] [20]	vertebrate, carp
k_{G5}	s^{-1}	3.0	1	3.0	1	[10] [20]	vertebrate, carp
k_{G6}	s^{-1}	2000	2000	2000	2000	[10]	vertebrate
k_{G7}	s^{-1}	200	200	200	200	[10]	vertebrate, carp
k_{P1}	$\mu\text{M}^{-1}\text{s}^{-1}$	250	300	250	300	[8] [30]	carp

k_{p2}	s^{-1}	1000	1000	1000	1000	[8] [30]	carp
k_{p3}	$\mu M^{-1}s^{-1}$	100	100	100	100	[8] [30]	carp
k_{p4}	s^{-1}	1.0	1.0	1.0	1.0	[8] [30]	carp
$k_{elution}$	s^{-1}	80000	80000	0.0	0.0	[8] [30]	carp
k_{RGS1}	$\mu M^{-1}s^{-1}$	-	-	0.85	0.7	[8]	carp
k_{RGS2}	s^{-1}	-	-	0.05	0.05	[8]	carp
k_{RGS3}	s^{-1}	-	-	5.0	4.0	[8]	carp
k_{decay}	s^{-1}	0.045	0.64	0.045	0.64	[10]	carp
k_{GC1}	s^{-1}	0.1	0.006	0.1	0.006	[14] [45]	frog, carp
k_{GC2}	s^{-1}	100	108.45	100	108.45	[14] [45]	frog, carp
k_{GC3}	s^{-1}	8.5	8.967	8.5	8.967	[14] [45]	frog, carp
K_c	μM	-	-	0.25	0.25	[13]	bovine
m	-	-	-	2.0	2.0	[10]	vertebrate
β_{dark}	s^{-1}	-	-	3.5	3.5	[9]	carp
β_{sub}	s^{-1}	2000	2400	2000	2400	[2]	carp
b	$\mu Ms^{-1}pA^{-1}$	-	-	0.303	0.413	[8] [14]	carp, salamander, bass
γ_{Ca}	s^{-1}	-	-	106.7	582.4	[8]	carp
c_0	μM	-	-	0.01	0.01	[11]	vertebrate
e_T	μM	-	-	400	400	[11]	vertebrate
k_{b1}	$\mu M^{-1}s^{-1}$	-	-	4.0	1.0	[8]	carp
k_{b2}	s^{-1}	-	-	16	4.0	[8]	carp
K_m	μM	-	-	20	60	[8] [45]	carp
n_h	-	-	-	2.0	4.0	[8] [45]	carp
F	C/mol	-	-	96486.7	96486.7		
vol	L	-	-	1.2e-13	9.0e-14	[8]	carp
G_{max}	pA/mV	-	-	10.1	243000.3	[8]	carp
K_p	mV $^{-1}$	-	-	0.03	0.015	[8]	carp
E_p	mV	-	-	10	10	[52]	lizard
V_m	mV	-	-	-30	-30	[52]	lizard
VP_{total}	μM	3000	3000	3000	3000	[2]	carp
RK_{free}	μM	12	120	12	120	[2]	carp
Tr_{free}	μM	280	280	280	280	[2]	carp
PDE_{free}	μM	11	11	11	11	[2]	carp
$RGS9_{free}$	μM	-	-	3.3	75	[2]	carp

cGMP	μM	-	-	2.0	2.0	[53]	triturus
ATP	μM	Depen dence on experi ment	Depende nce on experim ent	1000	1000	[54] [55] [56] [57] [58] [59]	rabbit, frog, bovine
GTP	μM	Depen dence on experi ment	Depende nce on experim ent	1000	1000	[54] [58]	rabbit, frog
GC_{free}	μM	4.2	72	4.2	72	[45]	carp
Ca^{2+}	μM	-	-	0.5	0.5	[10]	vertebrate
Cab	μM	-	-	44.4	44.4	[8]	carp
I_{photo}	pA	-	-	-4	-12	[2]	carp

742 Table 4. Initial set of time-dependent variables

	Rod	Cone
VP_{free}	3000	3000
RK_{free}	12	120
PDE_{free}	11	11
T_{free}	280	280
$RGS9_{\text{free}}$	3.3	75
GC_{free}	8.3	75.9
ATP	1000	1000
GTP_{free}	1000	1000
cGMP	2.0	2.0
$GC \cdot GTP$	4.11	3.90
Ca^{2+}	0.5	0.5
Cab	44.4	44.4
I_{photo}	-4.0	-12.0

Figure Legends

Figure 1 Reaction scheme of visual phototransduction in rods and cones

A, Visual phototransduction, including activation and inactivation of VP, Tr, and PDE, in rods and cones (see Table 2 for abbreviations). B, Details of the phosphorylation reactions. Phosphorylation of VP at 3 sites in vitro (Figures 2-4), where only 1 site in vivo (Figure 5) was assumed. Phosphorylation reactions and Tr* elution indicated in light gray were not included, whereas RGS9-dependent inactivation of Tr* and GC-dependent cGMP synthesis, depicted in dark gray, were added for simulating I_{CNG} in Figure 5.

Figure 2 Phosphorylation of visual pigments in rods and cones.

A, The time courses for the phosphorylation of VPs (the number of phosphate groups incorporated into an activated visual pigment molecule) measured in the membrane preparations of purified frog rod (a, circle) and carp cone (b, triangle) in response to light flash at 1.3% and 2.5%, respectively, in the experiments [17]. The corresponding simulation results (dotted lines in a and b) are also shown in the figures. B, Maximum rates of phosphorylation reaction per activated visual pigment at different flash intensities in rods (a, circle) and cones (b, circle), determined 10 sec and 0.6 sec after light stimuli, respectively (data modified from Tachibanaki et al. [17]). Experimental results were fitted by the Michaelis-Menten equation ($V/S = V_{max}/(S + K_m)$, solid lines). Simulated responses for both the carp rod (a, dotted line triangle) and cone (b, dotted line triangle) were superimposed onto the experimental results.

Figure 3 Transducin activation in rods and cones

A, The time courses for Tr activation (the number of GTP γ S molecule, a nonhydrolyzable GTP analog, incorporated per VP*) in the membrane preparations of purified carp rods and cones in response to light stimulation (0.0085% for rods (a, circle) and 0.25% for cones (b, circle)) in the presence (filled symbols) and absence (open symbols) of ATP (1 mM) as determined by biochemical experiments [20]. Corresponding simulation results (dotted lines (-ATP) and dashed lines (+ATP) in a and b were superimposed onto the experimental results. B, Light-induced GTP γ S binding as a

function of flash intensity in rods (a, circle, after 40 sec stimulation) and cones (b, circle, after 20 sec stimulation) in the presence (filled symbols) and absence (open symbols) of ATP (0.1 mM) normalized to maximum values. Simulated results for rods (a, triangle) and cones (b, triangle) reproduced under corresponding experimental conditions in the presence (filled symbols) and absence (open symbols) of ATP are also shown in the figure. Values for the rate constants for VP phosphorylation, $k_{RK1} - k_{RK6}$ and $ki_{RK1} - ki_{RK6}$, were set to 0 for simulation of experimental results obtained without ATP.

Figure 4 PDE-mediated cGMP hydrolysis in rods and cones

A, The time courses for PDE activities (the number of cGMP molecules hydrolyzed per VP*) in the membrane of carp rods and cones in response to light stimulations (a, 0.024% for rods (open circle); b, 0.46% for cones (open circle)) in the presence of ATP (0.25 mM), cGMP (2500 μ M) and either GTP (open closed circle) or GTP γ S (filled triangle) determined by biochemical experiments [25] [19]). Corresponding simulation results (solid lines (with GTP γ S) and dotted lines (with GTP) in a and b) were superimposed onto the experimental data. The continuous presence of saturating levels of cGMP was assumed for the simulation study, and thus β_{dark} was set to 0. B, Light-induced peak PDE activity as a function of flash intensity in rods (circle) and cones (triangle) either in the presence of ATP (a, 0.25 mM) or without ATP (b) and either GTP (open symbols) or GTP γ S (filled symbols) are normalized to maximum values. Simulated PDE activity of the rods and cones reproduced under the corresponding experimental conditions (with either GTP (dotted lines) or GTP γ S (solid lines)) were superimposed onto the experimental results. The value for the rate constant, k_{decay} , for cGMP hydrolysis was set to 0 for the simulation of the experimental results obtained with GTP γ S.

Figure 5 I_{CNG} in rods and cones

A, $[GTP]_i$ -dependent I_{CNG} recorded from a truncated outer segment of frog rods (circle), normalized to I_{CNG} at 8 mM $[GTP]_i$ [14]. Corresponding simulation results at steady-state were superimposed onto the experimental data (solid line). B, Time courses of light-induced I_{CNG} as a function of flash intensity recorded from the outer segments of the carp rods (a, light stimuli; 5.1E-6, 1.6E-5, 5.1E-5, 1.6E-4, and 5.1E-4%) and cones (b, light stimuli; 1.6E-3, 5.2E-3, 1.6E-2, and 0.16%) as solid lines [14]. Simulated I_{CNG} for rods (a) and cones (b) reproduced under the corresponding experimental conditions was superimposed onto the experimental results (dotted lines in a and b).

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Figures

Figure 1

Reaction scheme of visual phototransduction in rods and cones A, Visual phototransduction, including activation and inactivation of VP, Tr, and PDE, in rods and cones (see Table 2 for abbreviations). B, Details of the phosphorylation reactions. Phosphorylation of VP at 3 sites in vitro (Figures 2-4), where only 1 site

in vivo (Figure 5) was assumed. Phosphorylation reactions and Tr* elution indicated in light gray were not included, whereas RGS9-dependent inactivation of Tr* and GC-dependent cGMP synthesis, depicted in dark gray, were added for simulating ICNG in Figure 5.

Figure 2

Phosphorylation of visual pigments in rods and cones. A, The time courses for the phosphorylation of VPs (the number of phosphate groups incorporated into an activated visual pigment molecule) measured in the membrane preparations of purified frog rod (a, circle) and carp cone (b, triangle) in response to light flash at 1.3% and 2.5%, respectively, in the experiments [17]. The corresponding simulation results (dotted lines in a and b) are also shown in the figures. B, Maximum rates of phosphorylation reaction per activated visual pigment at different flash intensities in rods (a, circle) and cones (b, circle), determined 10 sec and 0.6 sec after light stimuli, respectively (data modified from Tachibanaki et al. [17]). Experimental results were fitted by the Michaelis-Menten equation ($V/S = V_{max}/(S + K_m)$, solid lines). Simulated responses for both the carp rod (a, dotted line triangle) and cone (b, dotted line triangle) were superimposed onto the experimental results.

Figure 3

Transducin activation in rods and cones A, The time courses for Tr activation (the number of GTPyS molecule, a nonhydrolyzable GTP analog, incorporated per VP*) in the membrane preparations of purified carp rods and cones in response to light stimulation (0.0085% for rods (a, circle) and 0.25% for cones (b, circle)) in the presence (filled symbols) and absence (open symbols) of ATP (1 mM) as determined by biochemical experiments [20]. Corresponding simulation results (dotted lines (-ATP) and dashed lines (+ATP) in a and b were superimposed onto the experimental results. B, Light-induced GTPyS binding as a function of flash intensity in rods (a, circle, after 40 sec stimulation) and cones (b, circle, after 20 sec stimulation) in the presence (filled symbols) and absence (open symbols) of ATP (0.1 mM) normalized to maximum values. Simulated results for rods (a, triangle) and cones (b, triangle) reproduced under corresponding experimental conditions in the presence (filled symbols) and absence (open symbols) of ATP are also shown in the figure. Values for the rate constants for VP phosphorylation, $k_{RK1} - k_{RK6}$ and $k_{iRK1} - k_{iRK6}$, were set to 0 for simulation of experimental results obtained without ATP.

Figure 4

PDE-mediated cGMP hydrolysis in rods and cones A, The time courses for PDE activities (the number of cGMP molecules hydrolyzed per VP*) in the membrane of carp rods and cones in response to light stimulations (a, 0.024% for rods (open circle); b, 0.46% for cones (open circle)) in the presence of ATP (0.25 mM), cGMP (2500 μ M) and either GTP (open closed circle) or GTP γ S (filled triangle) determined by biochemical experiments [25] [19]). Corresponding simulation results (solid lines (with GTP γ S) and dotted lines (with GTP) in a and b) were superimposed onto the experimental data. The continuous presence of saturating levels of cGMP was assumed for the simulation study, and thus β_{dark} was set to 0. B, Light-induced peak PDE activity as a function of flash intensity in rods (circle) and cones (triangle) either in the presence of ATP (a, 0.25 mM) or without ATP (b) and either GTP (open symbols) or GTP γ S (filled symbols) are normalized to maximum values. Simulated PDE activity of the rods and cones reproduced under the corresponding experimental conditions (with either GTP (dotted lines) or GTP γ S (solid lines)) were superimposed onto the experimental results. The value for the rate constant, k_{decay} , for cGMP hydrolysis was set to 0 for the simulation of the experimental results obtained with GTP γ S.

Figure 5

ICNG in rods and cones A, [GTP]i-dependent ICNG recorded from a truncated outer segment of frog rods (circle), normalized to ICNG at 8 mM [GTP]i [14]. Corresponding simulation results at steady-state were superimposed onto the experimental data (solid line). B, Time courses of light-induced ICNG as a function of flash intensity recorded from the outer segments of the carp rods (a, light stimuli; 5.1E-6, 1.6E-5, 5.1E-5, 1.6E-4, and 5.1E-4%) and cones (b, light stimuli; 1.6E-3, 5.2E-3, 1.6E-2, and 0.16%) as solid lines [14]. Simulated ICNG for rods (a) and cones (b) reproduced under the corresponding experimental conditions was superimposed onto the experimental results (dotted lines in a and b).

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