The Molecular Design of Visual Transduction

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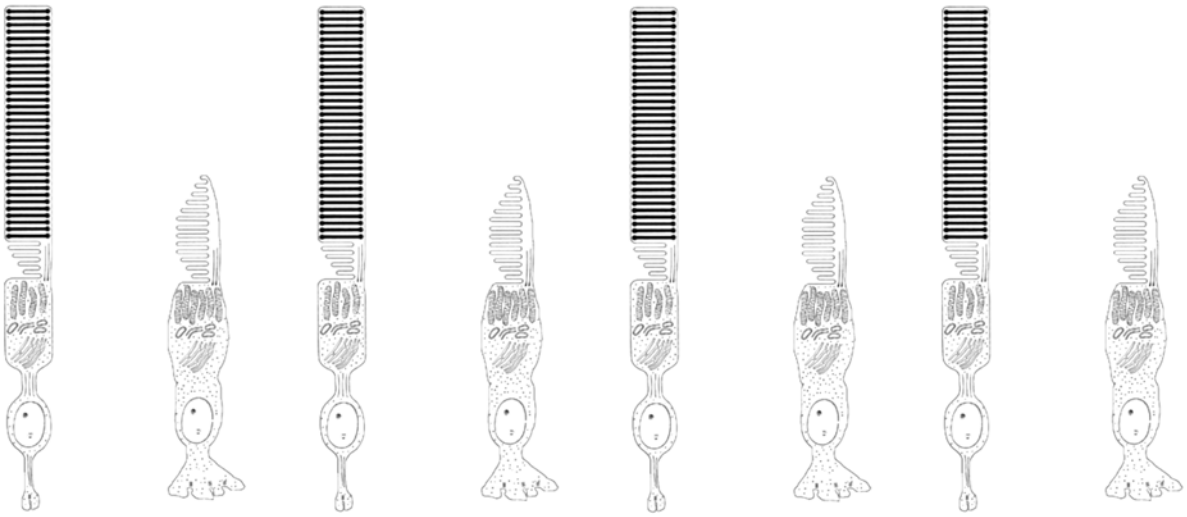

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Our daily lives are so resplendent with activities that include or require vision, that it is easy to overlook the complexity of the underlying processes involved. Here we describe the first step in vision…detection of light by our photoreceptors. This input stage sets limits on what we can and cannot see. The goal of this resource page is to impart a better understanding of the molecular design behind visual transduction.

Structure and Function of Rods and Cones

The photoreceptors are located in the deepest layer of the retina (**Fig.1**), farthest from the incoming light. There are two kinds, rods and cones, so named for their overall shapes. Rods operate in very low light, such as at night. Cones operate under brighter conditions and provide the basic units for color vision. The recently discovered intrinsically photoreceptive ganglion cells will be excluded from this review because while they respond to light, they have not been shown to contribute to image-forming vision.

Rods and cones are specialized unipolar neurons. All vertebrate visual receptors follow a simple blueprint. They can be divided into two portions, termed inner and outer segments, according to their radial position within the retina (**Fig.1B**). The inner segment consists of the cell body and contains the cellular organelles found in other neurons, including a synaptic terminal. The outer segment is an elaborate, modified cilium that contains the biochemical machinery needed for visual transduction. The components of the phototransduction enzyme cascade are packed into stacks of flattened, membranous vesicles ("disks") that in rods are enclosed by the plasma membrane of the outer segment (**Fig.1C**). Cones have a similar structure, but the disk membranes are an extension of the plasma membrane, arranged into a series of infoldings (**Fig.1B**).

Most neurons maintain a resting membrane potential of about -60 to -70 mV and when excited, they open cation channels and allow Na^+ to flow in. The resulting depolarization opens voltage-gated Ca^{2+} channels at the synapse. Ca^{2+} flows in and promotes fusion of synaptic vesicles which release neurotransmitter. Rods and cones work "backwards". At rest, that is in darkness, rods and cones are depolarized to -35 to -45 mV because channels in the outer segment membrane that pass Na^+ are already open. An efflux of K^+ through voltage-gated channels at the inner segment balances the influx of cations at the outer segment, completing an electrical circuit, referred to as the "dark" or circulating current (**Fig.1D**). Since photoreceptors are already depolarized in darkness, Ca^{2+} channels are open and the influx of Ca^{2+} ions supports a steady release of the neurotransmitter glutamate from rod and cone terminals onto second order neurons (**Fig.1D, left**). When rods and cones receive light, the channels in their outer segments close and the membrane hyperpolarizes towards the equilibrium potential for K^+. At the synapse, voltage-sensitive Ca^{2+} channels close and neurotransmitter release subsides (**Fig.1D, right**).

The Phototransduction Cascade

How does photon capture lead to closure of ion channels in the outer segment? The following outline will focus on rods since their phototransduction pathway is better

understood, but cones operate in a qualitatively similar way.

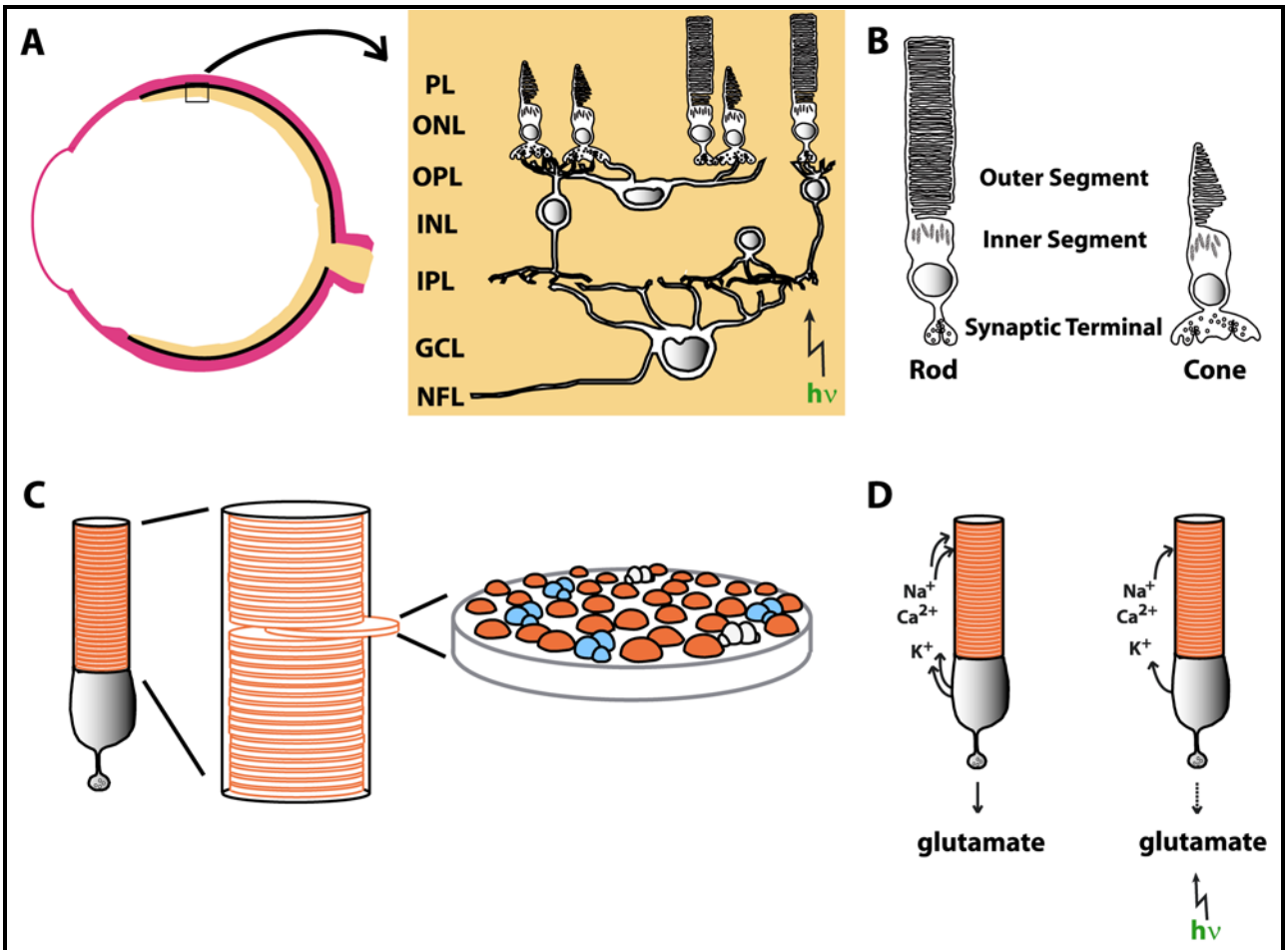

Figure 1. Cellular plan of vertebrate photoreceptors. **A.** Encapsulation of a thin layer of brain tissue--the neural retina (tan)--within the vertebrate eye (left). The retina (right) is laminated with neurons that detect light, process the information, and convey it to higher visual centers in the brain. Light must pass through all layers of the retina to reach the photoreceptors. **B.** Morphology of rods and cones. **C.** Outer segment disk membranes containing the biochemical components of the light-detecting pathway. **D.** The "dark" current. Na^+ and a lesser amount of Ca^{2+} enter through cyclic nucleotide-gated channels in the outer segment membrane while K^+ departs through voltage-gated channels in the inner segment. In darkness (left), the rod is depolarized and releases the neurotransmitter glutamate continuously. In response to light (right), channels in the outer segment membrane close, the rod hyperpolarizes, and glutamate release decreases. PL, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fiber layer.

In order for light to be seen, it must first be absorbed by the visual pigment rhodopsin. Rhodopsin consists of two parts. One part is a protein called opsin, and the other is 11-*cis* retinal. Opsin was sequenced independently by Ovchinnikov in the former Soviet Union and by Hargrave in the United States. Opsin is a single polypeptide

chain with 7 helical segments that span the membrane (**Fig.2A**). The cloning of opsin by Nathans, then a grad student, paved the way for the discovery of other hepta-helical receptors that turned out to be the largest class of receptors in the human genome. Earlier in this century, opsin became the first hepta-helical receptor to have its X-ray crystal structure solved, thanks to Okada and Palczewski (**Fig.2B**).

The other part of rhodopsin, 11-*cis* retinal (**Fig.2C**), binds to residue number 296, a lysine, on the seventh helix of opsin (**Fig.2A**). A Nobel prize was awarded to George Wald for identifying 11-*cis* retinal as the chromophore and for working out the photochemistry. 11-*cis* Retinal is a derivative of vitamin A, which cannot be made *de novo* by the body and must be taken in constantly in the diet. Hence vitamin A deficiency causes night blindness; depletion of 11-*cis* retinal leads to fewer functional rhodopsins and lower sensitivity of the rods -- the cells that do the heavy lifting during low light situations.

The structure of 11-*cis* retinal makes it well suited to serve as the light-absorbing molecule in rod and cone pigments. Whenever a string of carbons are connected with alternating double and single bonds (**Fig.2C**), the bonds take on a special character, not exactly a single bond but not exactly a double bond either. Furthermore, the bonding electrons do not stay with a particular carbon pair, rather they de-localize over the entire conjugated chain. It does not take much energy to raise the electrons to the excited state. By itself, 11-*cis* retinal would absorb near UV light. But opsin perturbs the distribution of the electrons so that the excited state can be achieved with less energy, i.e., with longer wavelength light. Cone opsins have similar hepta-helical structures, but with different amino acid residues surrounding the bound 11-*cis* retinal; thus they tune the chromophore's absorption to different wavelengths.

Photon absorption isomerizes the retinal from the bent 11-*cis* form to the all-*trans* conformation (**Fig.2C**), and it does so with blazing speed--it only takes a few hundred femtoseconds (10^{-13} sec). This discovery by Mathies, Shank and colleagues caused quite a stir because no one knew that nuclear rearrangements could occur on this time scale. Slower rearrangements in the surrounding opsin reposition its cytoplasmic loops, converting the protein to its active R* state. All subsequent steps occur in darkness. Thus, the chromophore converts the energy of a photon into a conformational change in protein structure. This structural change is but the first step in the phototransduction cascade, because most of the rhodopsin resides in disk membranes within the rod, physically separated from the plasma membrane where the ion channels are located. How does rhodopsin communicate with channels in the plasma membrane?

Fung, Hurley and Stryer showed that rhodopsin is a G protein-coupled receptor and in rods and cones, named the corresponding G protein transducin (**Fig.3A**). Its X-ray crystal structure was solved by Sigler and Hamm and co-workers. G proteins are heterotrimeric, consisting of α, β, and γ subunits. In its inactive state, transducin's α subunit has a GDP bound to it, but R* binds transducin and allows the GDP to dissociate (**Fig.3B**). GDP is the glue that holds the transducin heterotrimer together, so without GDP, the $\beta\gamma$ subunits come off. R* and the α subunit remain conjoined until a passing GTP binds the α subunit, allowing it to detach from R*. R* repeats this action over and over until its activity is shut off.

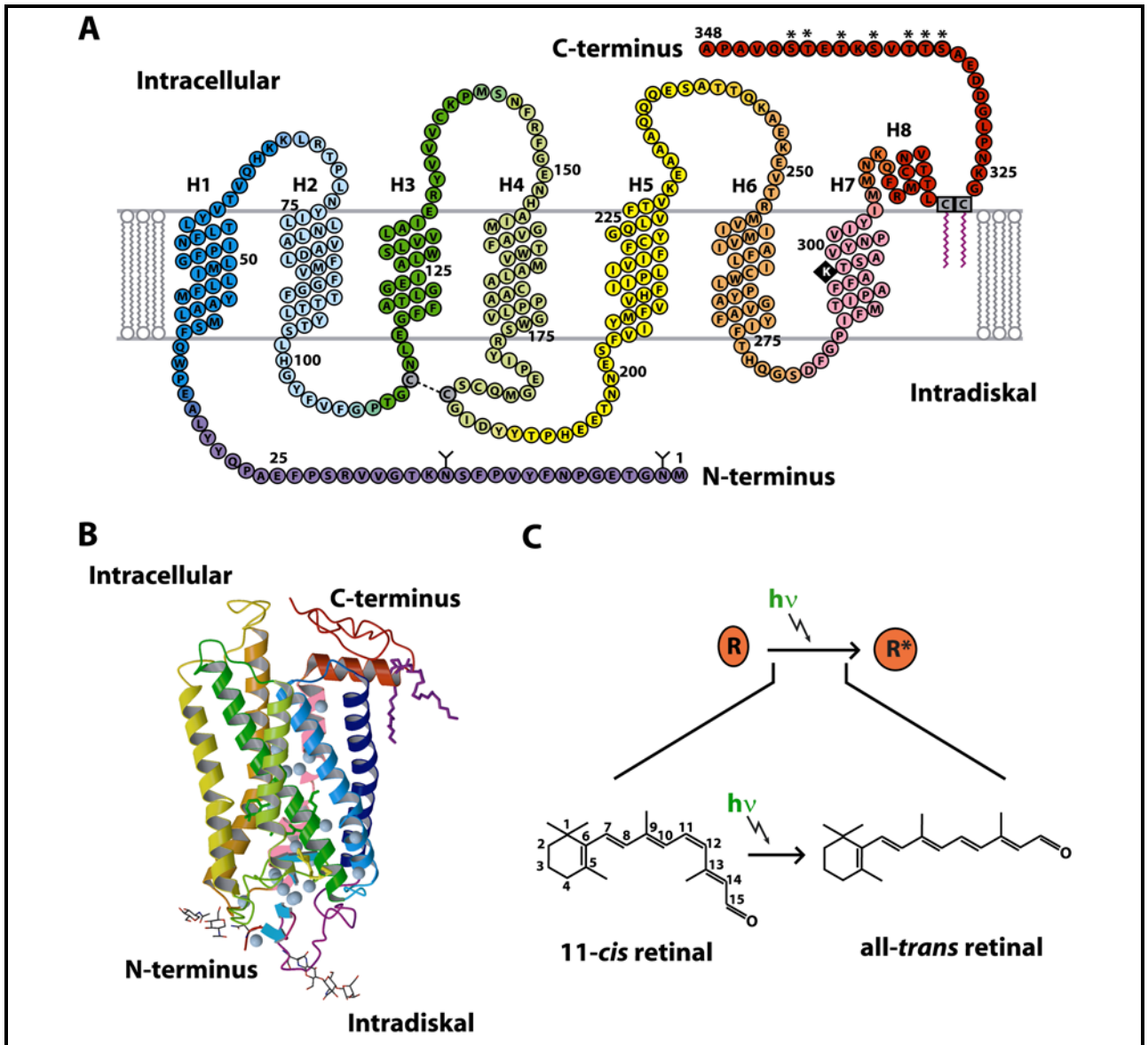

Figure 2. Structure of rhodopsin. **A.** Amino acid sequence of bovine opsin. The glycosylated N-terminus (violet residues) is located within the outer segment disk (intradiskal/extracellular) while the C-terminus (red residues) is exposed to the cytoplasm, with the seven α helices (H1-H7) spanning the disk membrane. An eighth helix (H8) sits on the membrane surface. There are seven phosphorylation sites (*) and two palmitoylated cysteines (gray squares) on the C-terminus. Two conserved cysteines (gray circles) on the first and second extracellular loops form a disulfide linkage. The 11-*cis* retinal chromophore binds as a protonated Schiff base to Lysine^{296} (black diamond). **B.** X-ray crystal structure of bovine rhodopsin. The color scheme for the helices (H1-H8) correspond to that in **A**. The blue spheres represent water molecules, and the arrows represent beta strand structure. Courtesy of T. Okada, Gakushuin University. **C.** The light-absorbing chromophore of rhodopsin. Photoisomerization of the 11-*cis* retinal in opsin's chromophore-binding pocket to the all-*trans* conformation (right) transforms rhodopsin to the activated state (R*).

Transducin serves as an intermediary between the activated receptor and an effector. In this case, the effector is a phosphodiesterase (PDE) that enzymatically cleaves the phosphodiester bond of cGMP to form 5'-GMP (**Fig.4**). PDE is a heterotetramer that consists of a dimer of two catalytic subunits, α and β, each with an active site inhibited by a PDEγ subunit. The activated transducin α subunit-GTP binds to PDEγ and relieves the inhibition on a catalytic subunit.

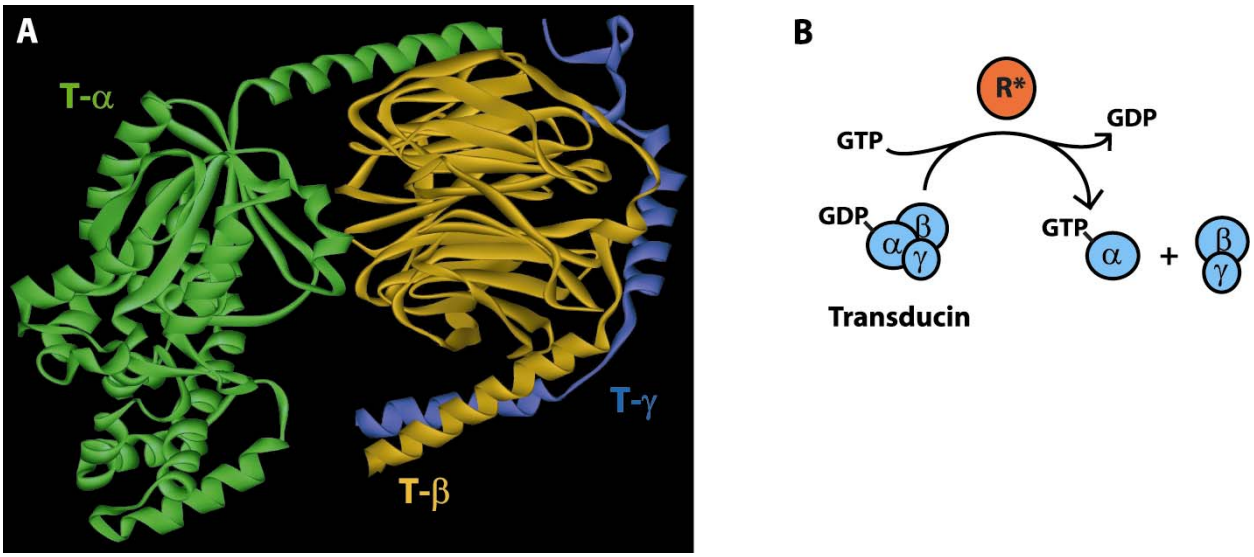

Figure 3. Structure of transducin. **A.** Heterotrimeric composition of transducin (PDB ID: 1GOT). Courtesy of K. Martemyanov, University of Minnesota. **B.** Activation of transducin. R* binds the transducin heterotrimer and catalyzes the exchange of a bound GDP for a GTP, leading to the release of the α-GTP subunit from transducin.

So far, all of the steps have taken place on the disk membrane (**Fig.4, left**), separate from the ion channels in the plasma membrane of the outer segment. A small, soluble molecule, cGMP, links the two compartments. When activated, PDE hydrolyzes cGMP to 5'-GMP, the cGMP concentration inside the rod decreases, and cyclic nucleotide-gated ion channels respond by closing (**Fig.4, right**). Na^+ entry into the outer segment is blocked, and as a result the rod hyperpolarizes. The discovery by Fesenko and colleagues that these channels were gated directly by cGMP was a revelation in biology, because until that time people thought that cyclic nucleotides elicited their effects through the phosphorylation of proteins only. Cyclic nucleotide-gated channels are now widely studied in other neurons, as well as in kidney, heart and other organs.

Seeing Single Photons

Rods do more than just respond to light; Baylor, Lamb and Yau demonstrated by single cell recording that they possess quantal sensitivity. In order to signal individual photons, three important factors need to be addressed. First, background noise needs to be minimal. Second, the quantal response needs to be large. Third, the response must be reproducible.

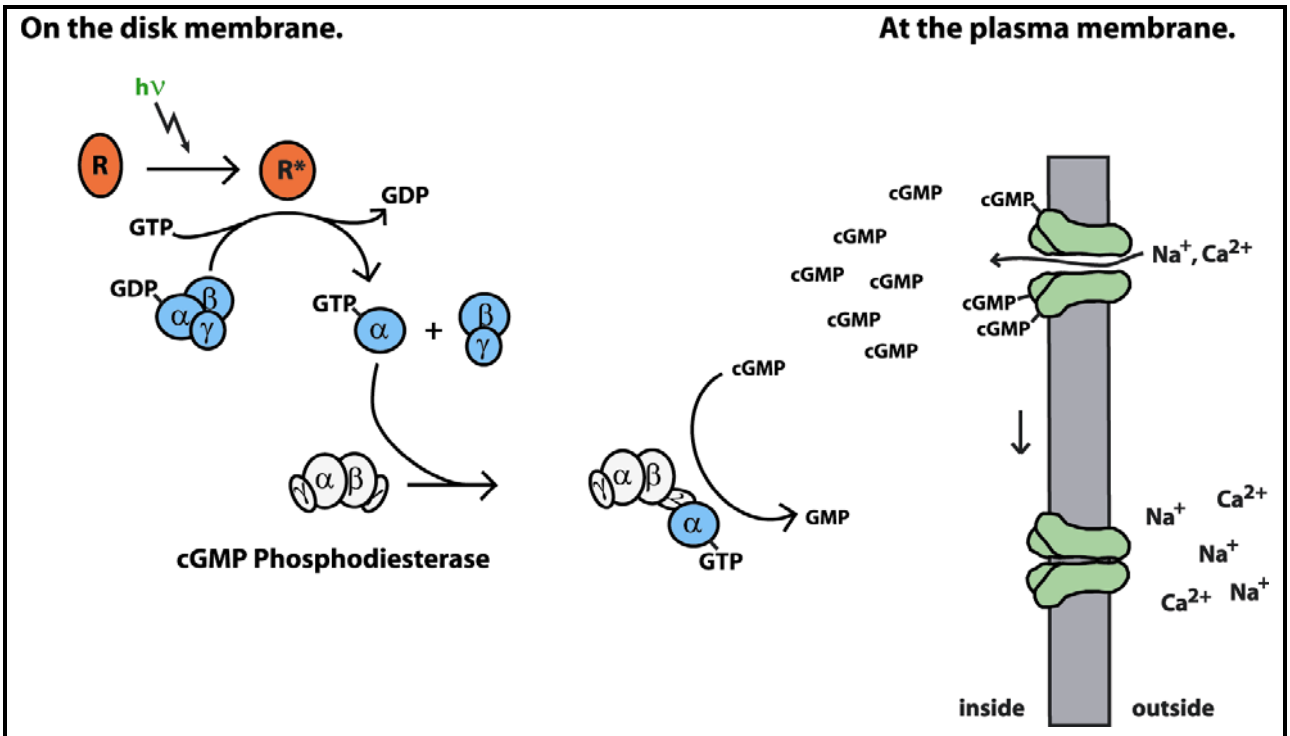

Figure 4. Bridging the gap between photon absorption at the disk membrane and channel closure at the plasma membrane. On the disk membrane (**left**), absorption of a photon by rhodopsin (R) leads to photoisomerization and formation of the active state of the pigment (R*). R* catalyzes the transfer of a bound GDP for a GTP on transducin, causing the βγ subunits to come off, and leaving the transducin α-GTP free to activate phosphodiesterase (PDE). A transducin α-GTP can bind one PDE γ subunit and remove the inhibition from one of the PDE catalytic subunits (α or β). Activated PDE hydrolyzes cGMP, the intracellular messenger that is required for cGMP-gated channels in the outer segment plasma membrane to remain open (**right**). Reduction in the intracellular levels of cGMP closes channels, blocking entry of Na^+ and Ca^{2+} and thereby decreases the circulating current.

First: the quiet baseline. By analogy, let's equate the detection of a single photon by a rod with a person trying to hear the sound of a single pin dropped onto the floor. It is far easier to hear the sound of the pin hitting the floor when it is quiet, and impossible when there is a lot of background noise. Consider the sources of noise in the visual transduction cascade. For any temperature above absolute zero, molecules are in constant motion, fidgeting and banging into each other. Sometimes enough energy accumulates within a rhodopsin molecule to set it off, just as if it had absorbed a photon. At body temperature, the rhodopsin in your eye has a half-life for thermal isomerization exceeding 400 years (**Fig.5A**). That is 23 log units slower than light activation since photoisomerization takes a few hundred femtoseconds. Of course, for many rhodopsins thermal isomerization occurs much sooner than 400 years. Moreover, rods contain about a hundred million copies of rhodopsin, so there will be a thermal isomerization about once every minute.

How does this affect the rod's response? **Figure 5B** is a recording of a single rod's response to dim flashes. Poisson statistics are obeyed; sometimes the rod does not respond, while at other times it detects a photon, and sometimes it detects two. Once in a while, due to thermal activation, the rod gives a "response" even though no photon was absorbed (**Fig.5B, asterisk**). Spontaneous events do not interfere with vision as long as their rate of occurrence is overwhelmed by the rate of responses arising from photon absorptions. But as the light becomes exceedingly dim, our absolute visual sensitivity is limited by this rate of thermal activation. So while rhodopsin is incredibly stable, it has not been grossly over-engineered.

The next component in the phototransduction cascade, transducin, is the most stable G protein known. Random activation, that is spontaneous exchange of GDP for GTP, takes hours. Transducin is not nearly as stable as rhodopsin, but there are ten-fold fewer of them in the outer segment than there are rhodopsins. Also as we shall see, the effect generated by a single transducin is a hundred times smaller than that of a single R*.

PDE is even noisier than transducin, but there are ten-fold fewer PDE than transducins. Nevertheless, at any given instant in time, there are hundreds of active PDE in the absence of light. Most of the time, PDE shuts off quickly without hydrolyzing too much cGMP. But once in a while, the PDE stays on long enough to produce a single photon response-like event. Luckily that only happens once every 10 or 20 minutes, which is less than the rate of thermal activation of rhodopsin. The activity of PDE actually serves an important function. In darkness, cGMP is constantly synthesized, so without dark PDE activity, cGMP would accumulate and open all of the cGMP-gated channels. For reasons that will be addressed later, the rod has a lot of channels but only a small percentage are normally open in darkness. If the rod were to contain too much cGMP, the floodgates would open and the cell would fill up with Na^+ and Ca^{2+} since both go through the channel. This scenario is thought to occur in some forms of retinal disease, resulting in photoreceptor death and blindness.

The last component of the cascade, the channel, is very, very noisy because it opens and closes very rapidly. **Figure 5C** shows a single channel that keeps flickering open and closed. Why use such a noisy channel? The channel has a greater tendency to open when cGMP is bound, and cGMP tends to stay bound to the open channel. For the channel to respond to changes in cGMP concentration, it must revert to the closed state to give the cGMP a chance to come off. Once the channel is closed, if the concentration of cGMP is high, another cGMP will bind and re-open the channel. If the concentration of cGMP is low, the channel will stay closed since there will be fewer nucleotides around. Thus channel chatter ensures that the response to a decrease in cGMP levels will be quick.

A high off-rate constant of the closed channel for cGMP is also helpful, but then a lot of channels are needed in order to get enough of them to open at reasonable levels of cGMP. That's one reason the rods have lots of channels, but as noted above the channel is a great source of noise. To keep the noise from being problematic, the rod does something clever. The channel is rather non-selective for cations…Na^+, Ca^{2+}, Mg^{2+} and even K^+ will permeate. Most of the current is carried by Na^+ since there is so much

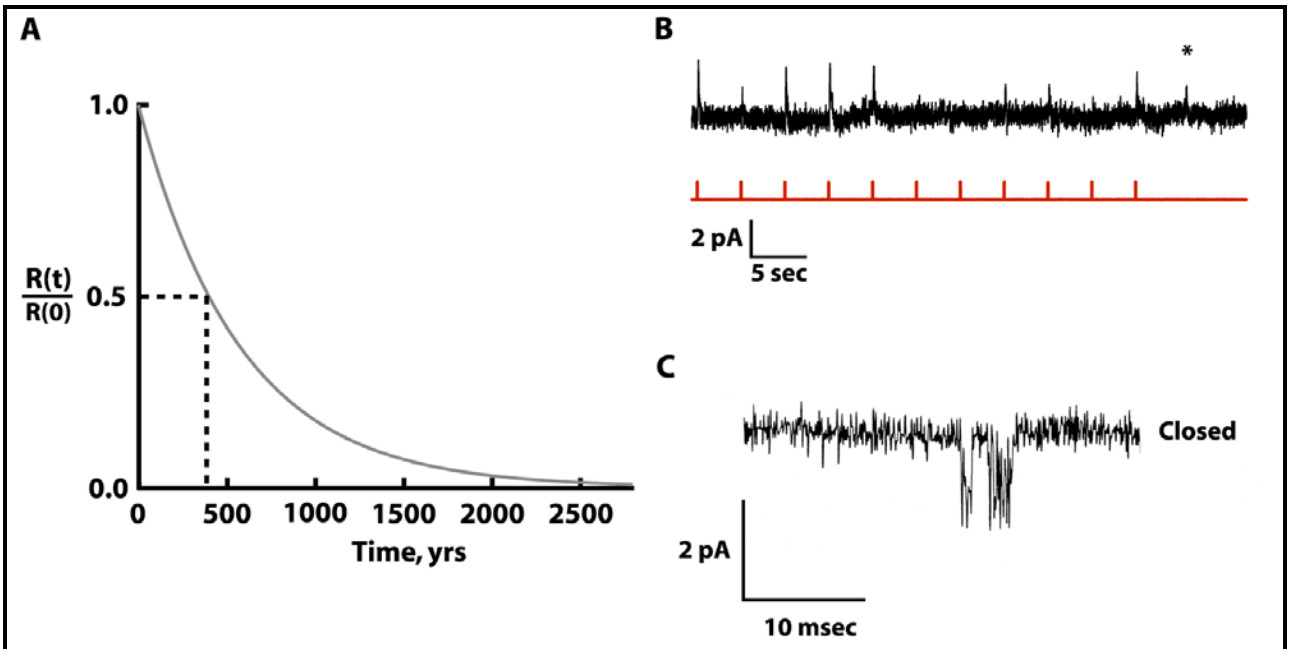

Figure 5. Noise in the system. **A.** Exponential decay of rhodopsin in darkness due to thermal isomerization. At 37°C, rhodopsin has a half-life of about 400 years. **B.** Dim flash responses of a mouse rod. The stimulus is so dim that not all the flashes elicit a response, but in some instances there is a "response" when no stimulus is presented (*). The line beneath the response shows the timing of flashes. T. Isayama, Massachusetts Eye & Ear Infirmary and Harvard Medical School. **C.** Current fluctuations of a single rod cyclic nucleotide-gated channel in saturating cGMP. The "fuzziness" in the open state results from unresolved transitions back to the closed state. Courtesy of G. Matthews, State University of New York at Stony Brook.

of it around, and the negative membrane voltage favors its entry. Na^+ is well behaved and passes through promptly. On the other hand, divalents, such as Ca^{2+} and Mg^{2+}, take their time in going through, sometimes even stopping. When they stop, nothing else can get by because the channel is a single-file pore. This "divalent block" reduces the single channel conductance. Consequently, to get the proper amount of current the rod uses a large number of channels and averages out the noise.

The general trend as we go further and further along the transduction pathway, is for molecules to become less and less stable. The effects of the noise generated by these elements are minimized and even put to good use as in the case of PDE and the channel.

Having dealt with the background noise in our pin drop analogy, we next see that if the pin should fall on a soft surface, it would barely make any sound at all. It would be far better if the pin were to fall onto a microphone connected to an amplifier and loud speakers. In biology, a strategy for achieving amplification is through the use of a biochemical cascade. A stimulus activates an enzyme, which in turn activates another enzyme, and so on. In rods, the first amplifying step is when R* activates transducin. After turning on transducin, it turns on another, and another (**Fig.6A**). All told, R* turns

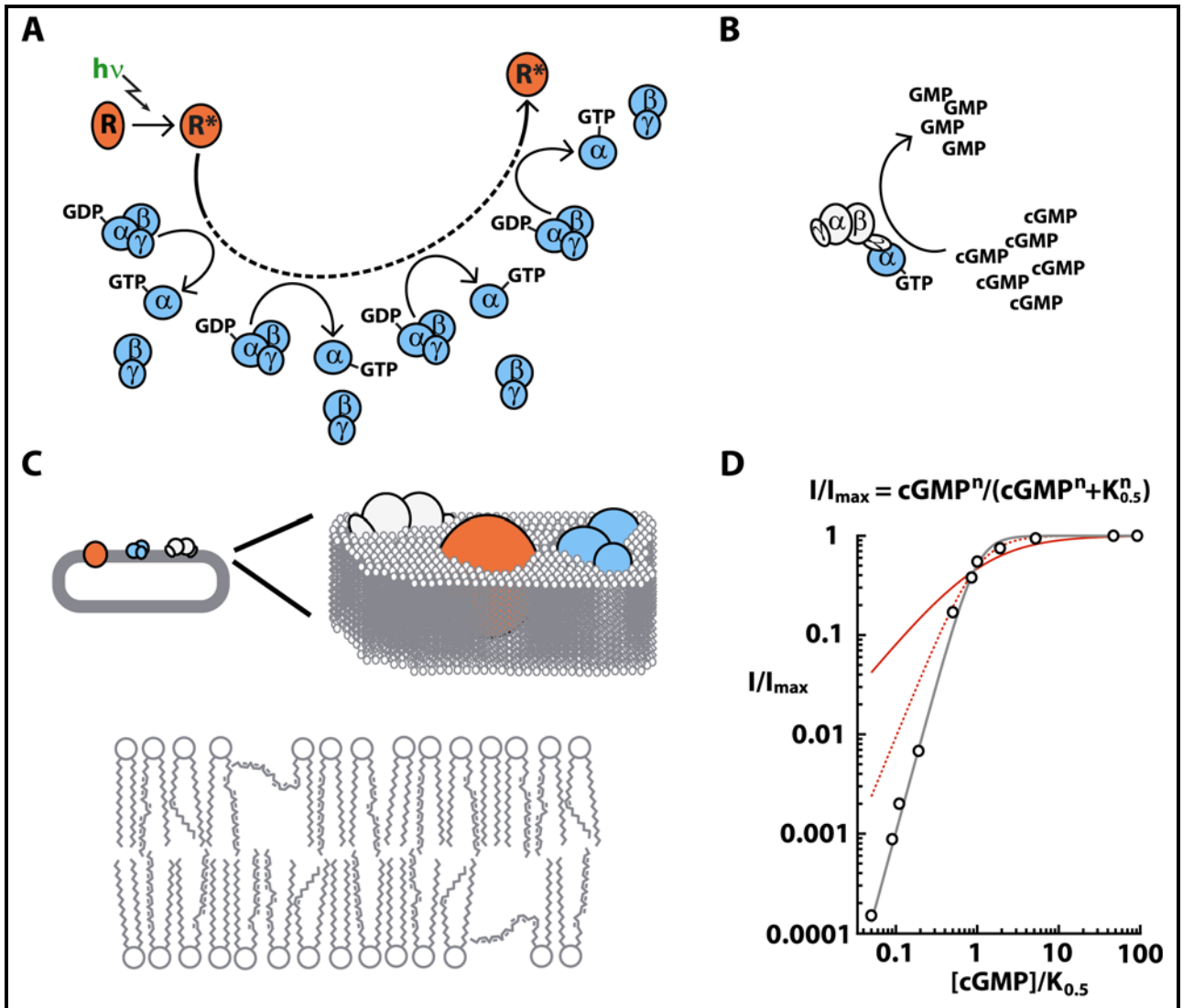

Figure 6. Amplification in the phototransduction cascade. **A.** Rhodopsin to transducin. A single R* can activate close to a hundred transducin molecules. **B.** Phosphodiesterase. Each transducin α-GTP can bind to one PDE γ subunit, relieving inhibition on one of two PDE catalytic subunits (α or β) that hydrolyzes cGMP to 5'-GMP at the diffusion limit. A second transducin α-GTP is required to activate the other PDE catalytic subunit. **C.** Protein interaction on the disk membrane. Transduction is improved by anchoring R* (orange), transducin (blue), and PDE (gray) to the phospholipid bilayer to ensure timely collisions (**top**). The high unsaturated fatty acid content of the disk membrane makes them very fluid because kinky double bonds disrupt the tight, orderly packing of the fatty acids (**bottom**). **D.** Cyclic GMP-gated channels. The dose-response relation for channel opening increases as the third power of cGMP concentration for [cGMP] < $K_{0.5}$ in response to a change in cGMP concentration, and the effect is enhanced as cooperativity rises. For comparison, solid and dashed red lines depict relations with Hill coefficients of n=1 and n=2, respectively, where more channels are open at a lower [cGMP], but increases in cGMP levels produce less dramatic effects. A. Zimmerman, Brown University.

on close to a hundred transducins. Transducin activates PDE stochiometrically. But each PDE catalytic subunit enzymatically hydrolyzes cGMP as fast as cGMP can bind to it (**Fig.6B**).

With a multi-step cascade, amplification increases over time. In this regard, it is significant that rhodopsin, transducin and PDE are all attached to the disk membrane (**Fig.6C**). Conversion of a 3-dimensional reaction volume to a 2-dimensional surface not only improves the overall probability for collisions between reactants, it also improves the odds for an effective collision. Imagine that R* and transducin have to be oriented in a specific manner in order to bind. In solution, right off the bat, many R* and transducin pairs will be in exactly the wrong orientation when they collide. But in the membrane, R* and transducin can be fixed to increase the odds that they are facing the right direction.

Membranes, however, can be more viscous than aqueous solutions, hindering the diffusion of membrane proteins. To minimize these effects, disk membranes are very special; they are low in cholesterol and have a high content of unsaturated fatty acids, properties that make the membrane very fluid (**Fig.6C**). As a result, the disk membrane is one of the most fluid in the body with a consistency like that of olive oil. The lateral diffusion rate for rhodopsin ranks among the fastest for any integral membrane protein. This feature is important because the rate of transducin activation is limited by how fast R* collides with transducin, which turns out to be about once every few milliseconds. These two factors -- the restriction of the transduction components to the membrane and the membrane's fluidity -- both optimize signal amplification.

Additional amplification comes from the way in which the channel responds to changes in cGMP concentration. The dose response curve for the channel (current versus concentration of cGMP) (**Fig.6D**) follows the Hill equation:

$$I/I_{max} = cGMP^n/(cGMP^n + K^n),$$

where K is the concentration at which half the channels are open and n is the Hill coefficient. When n>1, there is cooperativity, that is the binding of one cGMP enhances additional binding and channel opening. For the native channel, n is about 3 (**Fig.6D, gray line**). The minimal amount of cGMP that must be present to open the channel is increased, but channel opening is very sensitive to changes in cGMP. Put another way, since multiple cGMP molecules are required to open the channel, it will close when only one or two cGMP molecules leave the channel, making it easily shut down by absorption of light. Therefore the signal transduced by the disk membrane-bound components is further magnified at the plasma membrane by the cooperative nature of cGMP binding to the channel.

Ultimately, a single photon closes about 200 channels and thereby prevents the entry of about a million Na^+ ions into the rod.

That brings us to the next problem. In the pin drop analogy, a loud sound can result from one pin dropped from a tall height, but it can also result from multiple pins hitting the same surface simultaneously from a lower height. How can we differentiate between the two? By making the signal from each hit the same.

The size of the single photon response is determined by the shutoff of the phototransduction cascade. Rhodopsin shuts off after binding to arrestin (**Fig.7A**). Arrestin is a very large protein, whose sheer bulk prevents any transducins from getting close enough to bind. A one step shutoff would be stochastic, that is sometimes it would happen quickly, sometimes slowly. The rod improves on this by phosphorylating R* (**Fig.7A**) at serine and threonine residues on the C terminus of the rhodopsin molecule that are targeted by the enzyme rhodopsin kinase (**Fig.2A**). There are multiple phosphorylation sites, and each added phosphate simultaneously reduces R*'s activity and increases R*'s affinity for arrestin. Rieke and colleagues determined that a multi step shutoff averages out the variability in the timing and confers response reproducibility.

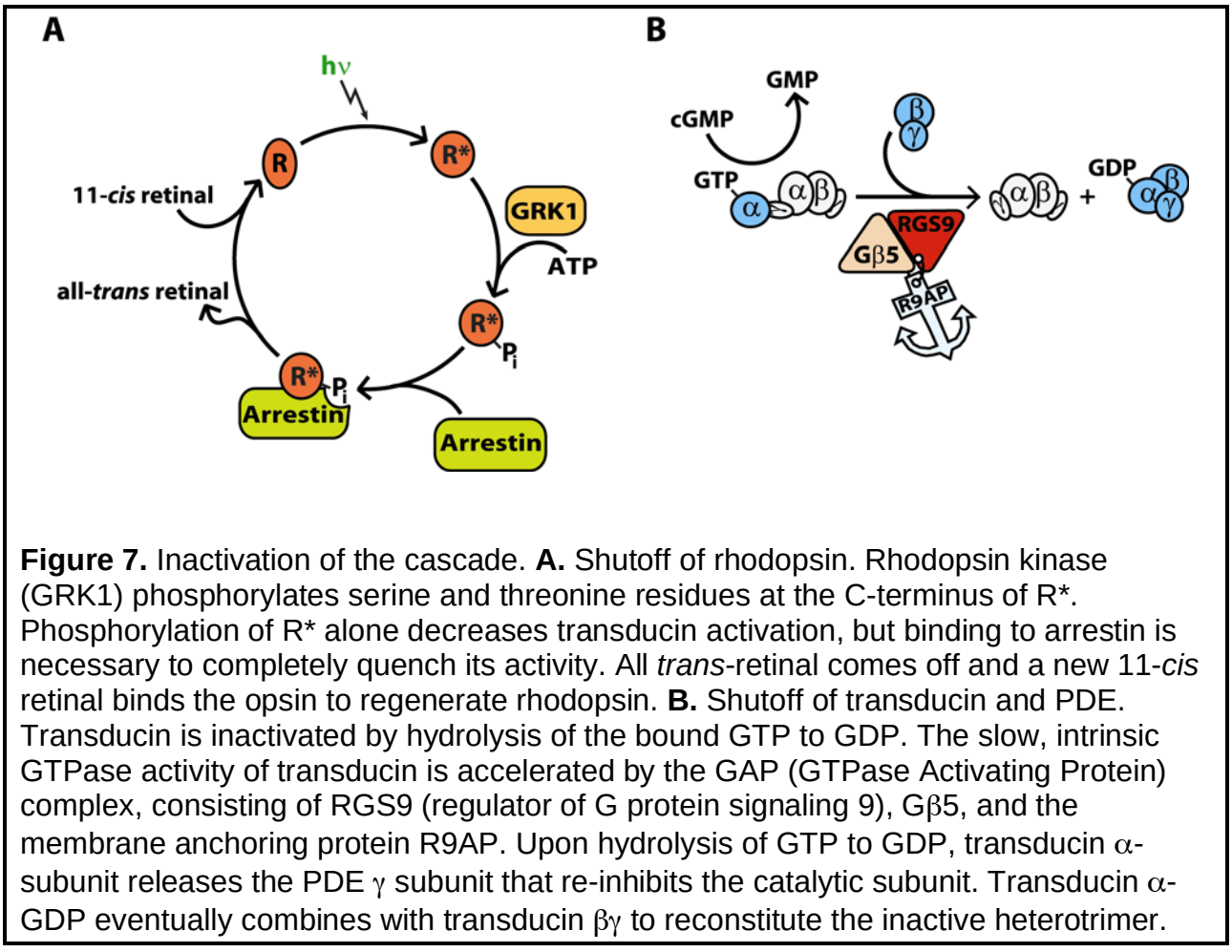

Figure 7. Inactivation of the cascade. **A.** Shutoff of rhodopsin. Rhodopsin kinase (GRK1) phosphorylates serine and threonine residues at the C-terminus of R*. Phosphorylation of R* alone decreases transducin activation, but binding to arrestin is necessary to completely quench its activity. All *trans*-retinal comes off and a new 11-*cis* retinal binds the opsin to regenerate rhodopsin. **B.** Shutoff of transducin and PDE. Transducin is inactivated by hydrolysis of the bound GTP to GDP. The slow, intrinsic GTPase activity of transducin is accelerated by the GAP (GTPase Activating Protein) complex, consisting of RGS9 (regulator of G protein signaling 9), Gβ5, and the membrane anchoring protein R9AP. Upon hydrolysis of GTP to GDP, transducin α-subunit releases the PDE γ subunit that re-inhibits the catalytic subunit. Transducin α-GDP eventually combines with transducin βγ to reconstitute the inactive heterotrimer.

Transducin has a built-in shutoff mechanism, namely hydrolysis of GTP. When that happens, transducin lets go of PDEγ, which resumes its inhibition of a PDE catalytic subunit (**Fig.7B**). Transducin βγ subunits recombine with transducin α-GDP and the heterotrimer returns to its resting state (**Fig.7B**). GTP hydrolysis is slow intrinsically, but it is accelerated by the GAP (GTPase Activating Protein) complex (**Fig.7B**). To ensure that transducin does not shut off before activating PDE, transducin and the GAP

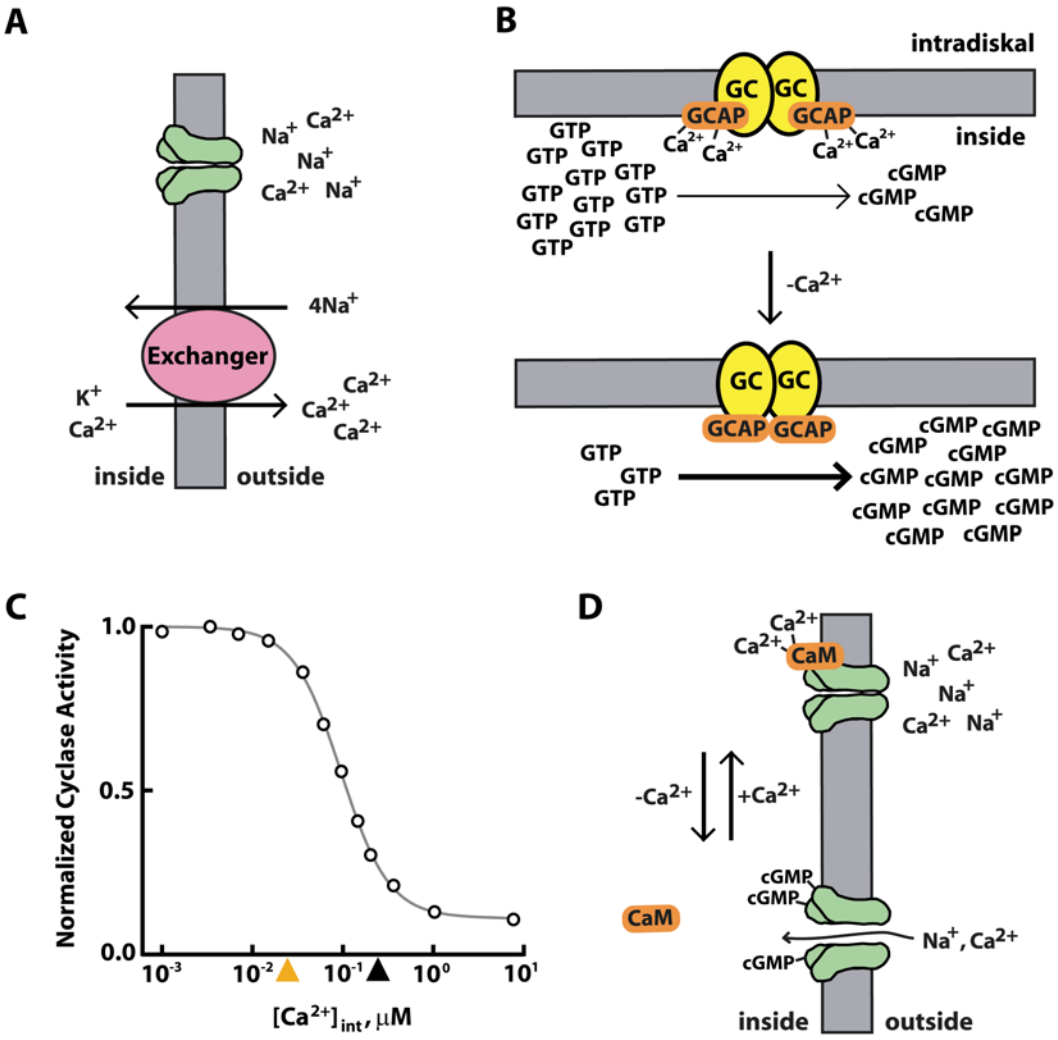

Figure 8. Calcium feedback. **A.** $Na^+/K^+,Ca^{2+}$ exchange. In response to light, cGMP-gated channels in the outer segment membrane shut, hyperpolarizing the cell. An exchanger in the outer segment couples the removal of a Ca^{2+} to the movement of one K^+ and four Na^+ down their electrochemical gradients. **B.** Production of cGMP. In the dark, Ca^{2+} binds to GCAPs (guanylate cyclase activating proteins) that are attached to and inhibit guanylate cyclase (GC), causing the cyclase to synthesize cGMP at a slow rate (**top**). GCAP activates cyclase in the absence of Ca^{2+}, and cGMP production increases (**bottom**). **C.** Ca^{2+} dependence of guanylate cyclase activity. At dark-adapted Ca^{2+} levels (black arrowhead), cyclase operates at a low level, but visual transduction causes a drop in Ca^{2+} to light-adapted levels (orange arrowhead) and elicits a GCAP-mediated increase in cyclase activity according to a Hill function: $A = (A_{max}-A_{min})/(1 + ([Ca^{2+}]/K_{m\ Ca})^n)+A_{min}$, where A is guanylate cyclase activity, $K_{m\ Ca}$ is 100, and n is 1.7. Courtesy of A. Dizhoor and I. Peshenko, Salus University. **D.** Cyclic nucleotide-gated channel. As $[Ca^{2+}]_{int}$ decreases during light activation, Ca^{2+}-calmodulin (CaM) or a related protein releases from the channel, causing an increase in the channel's affinity for cGMP. The increased affinity promotes nucleotide binding and channel opening. As $[Ca^{2+}]_{int}$ is restored, CaM binds the channel once more and reduces its affinity for cGMP.

complex have a low affinity for each other, until transducin α-GTP binds PDEγ.

Full recovery requires that the intracellular cGMP concentration be brought back up to dark-adapted levels. As noted above, cGMP is synthesized continuously from GTP by guanylate cyclases. Retinal guanylate cyclases are transmembrane proteins that exist as homodimers, whose activity is kept low by the action of GCAPs (guanylate cyclase activating proteins). One GCAP is bound to each subunit, preventing full activity. Thus recovery can take a pretty long time because the basal rate of cyclase activity is pretty slow. The rod, however, has a solution - feedback.

Remember that a trickle of Ca^{2+} enters the rod through the cGMP-gated channels. Ca^{2+} is removed by a $Na^+/K^+,Ca^{2+}$ exchanger molecule in the outer segment plasma membrane (**Fig.8A**). When the channels close, Ca^{2+} ceases to enter, but extrusion through the exchanger continues, so $[Ca^{2+}]_{int}$ falls. In the dark, GCAPs bind Ca^{2+} and inhibit cyclase activity. A decrease in $[Ca^{2+}]_{int}$ causes Ca^{2+} to dissociate from GCAPs, allowing them to dimerize. Dimerization of GCAPs leads to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis (**Fig.8B,C**). The cGMP-gated channel is a second target for feedback. In the dark, Ca^{2+}-Calmodulin (CaM) or a related protein binds the channel and reduces its affinity for cGMP (**Fig.8D, bottom**). During visual transduction, the decrease in $[Ca^{2+}]_{int}$ causes CaM to be released, increasing the channel's affinity for cGMP (**Fig.8D, top**) so that during recovery, the channel reopens at lower levels of cGMP, i.e. sooner.

Putting all the steps together: high amplification of signal and a low level of background noise allow the rod to detect single photons. Deactivation mechanisms ensure that each photon produces a similar response. In our eye, the single photon response reaches an amplitude of about one pA, it peaks in less than 200 msec, and recovers completely after about a sec (**Fig.9A**). The variance in size is less than 1/5 of the mean amplitude. That's pretty good! But what happens when the rod absorbs two photons at once?

Light enters the rod from the synaptic terminal end, passes through the inner segment and finally reaches the outer segment. When a photon gets absorbed, it causes a local decrease in cGMP levels and the closure of channels in an annulus surrounding the site of absorption (**Fig.9B**). The width of the annulus is about 5% of the rod outer segment length. With two photons, the Poisson nature of absorption makes it highly unlikely that both will be absorbed at the same disk. Therefore each photon absorption closes a separate subset of the rod's channels (**Fig.9B**).

The increasing hyperpolarization produced by closure of more channels does not, however, increase the inward current carried by the remaining open channels because the channels are outwardly rectifying (**Fig.9C**). The basis lies in the voltage dependence of divalent block (discussed earlier) and a weak effect of voltage on channel gating. With regard to the latter property, it is interesting to note that cGMP-gated channels bear structural similarity to voltage-gated potassium channels. The consequence of outward rectification is that under physiological conditions, the current depends strictly on the number of open channels and not on the membrane voltage. Then the reduction in dark current produced by two photons is simply twice that

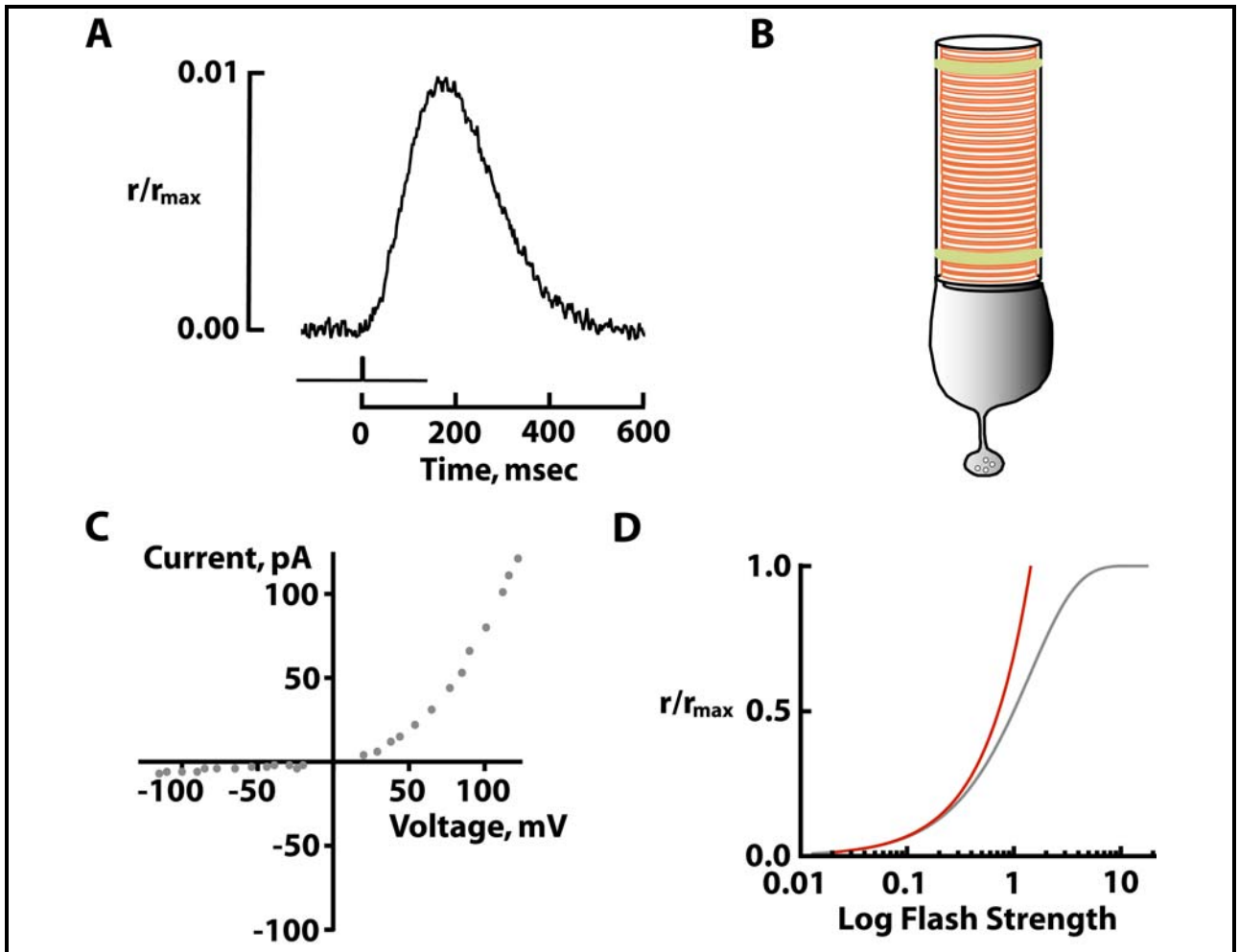

Figure 9. Absorption of 2 photons. **A.** A single photon response. The response to a single photon is presented as a fraction of the saturating response amplitude (14 pA). The line beneath the response shows the timing of the flash. Courtesy of J. Schnapf, University of California San Francisco. **B.** Spatial distribution of rhodopsin photoisomerization. Two photons are unlikely to be absorbed in the same disk, and therefore produce local effects independent of each other (green annuli). **C.** Current-voltage relations. The ohmic or linear behavior at high voltages becomes outwardly rectifying at physiologically relevant voltages, meaning that in this range the dark current is nearly independent of membrane potential. Measurements were made on a membrane patch excised from a toad rod outer segment with symmetric monovalent solutions plus 1 mM Ca^{2+} and 1.6 mM Mg^{2+} on the extracellular face, but zero divalents, 0.15 mM EDTA and 1 mM cGMP on the intracellular face. Courtesy of G. Matthews, State University of New York at Stony Brook. **D.** Stimulus-response relation for the rod. The response to multiple photon absorptions can be described by a saturating exponential function (gray trace). A plot of normalized response and flash strength (i) on a log scale indicates that at high photon counts, the response saturates because all the channels are closed. A linear relation (red trace) is shown for comparison.

produced by one photon. The rod responds linearly, in other words it functions as a photon counter (**Fig.9D, red trace**).

When there are lots of photons, the effects of individual rhodopsin isomerizations begin to overlap, so there are diminishing returns and the rod response diverges from linearity. The behavior follows a saturating exponential:

$r/r_{max} = 1\text{-}exp(-ki)$,

where i is flash strength and k is a constant equal to the quotient of ln(2) divided by the flash strength giving rise to a half maximal response (**Fig.9D, gray trace**). Bright flashes close all of the channels and saturate the rod's response ($r/r_{max} = 1$). Suprasaturating flashes reduce cGMP further, keeping the rod saturated for a longer time before recovering, because more time is needed to re-synthesize cGMP to a level that reopens the channels.

In summary, the rod converts light into an electrical signal by triggering a G protein-coupled receptor cascade. In this way, the rod generates highly amplified, reproducible signals on a fairly quiet background that enable it to count single photons. The cones operate in a similar manner, but with their own set of phototransduction components, and with a level of sensitivity lower than that of the rods. Both rods and cones contribute to the detection of visual information. Rods function in dim light situations as photon counters that can detect very small amounts of light. Cones, although not as sensitive, respond faster, respond to intensities over a broader range, and lay the basis for color vision…but those are topics for another resource page.

For further information, we recommend:

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