

Current understanding of signal amplification in phototransduction

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The studies of visual signal transduction, or phototransduction, have played a pivotal role in elucidating the most general principles of G protein signaling, particularly in regards to the concept of signal amplification, i.e., the process by which activation of a relatively small number of G protein coupled receptors is transformed into a robust downstream signaling event. In this essay, we summarize our current quantitative understanding of this process in living rods of lower and higher vertebrate animals. An integration of biochemical experiments *in vitro* with electrophysiological recordings from intact rod photoreceptors indicates that the total number of G protein molecules activated in the course of a light response to a single photon is ~16 in the mouse and ~60 in the frog. This further translates into hydrolysis of ~2000 and ~72000 molecules of cGMP downstream of G protein, respectively, which represents the total degree of biochemical amplification in the phototransduction cascade.

A lot has been said about the critical role that the studies of visual signal transduction, or phototransduction, have played over the past 3 decades in elucidating the most general principles of G protein signaling (see refs. 1–4 for recent reviews). One of these principles is the concept of amplification, i.e., the process by which activation of a relatively small number of G protein coupled receptors is transformed into a robust downstream signaling event. In the most extreme case of dark-adapted rod photoreceptor cells responding to single photons, a single activated molecule of the GPCR, rhodopsin, causes an electrical response sufficient to convey light absorption to downstream retinal neurons. While it has been recognized for decades that the degree of signal amplification in this cascade is exceptionally high, our quantitative understanding of this process in living rods has been achieved only recently.

Signal amplification takes place at multiple stages of the phototransduction cascade (Fig. 1A; reviewed in ref. 5). At the first step, photoexcited rhodopsin (or metarhodopsin-II, usually called R*) catalyzes sequential GDP/GTP exchanges on multiple molecules of the G protein, transducin. Each active, GTP-bound form of the α -subunit of transducin ($G\alpha$) interacts with the downstream effector, cGMP phosphodiesterase (PDE; also known as PDE6), allowing each PDE to hydrolyze multiple cGMP molecules and thus producing the second amplification

step. The resulting cGMP reduction in the photoreceptor cytoplasm causes a closure of the cGMP-gated cation channels in the photoreceptor plasma membrane, producing an electrical response. The latter represents yet another signal amplification step because cGMP gates these channels cooperatively, with a small fractional decrease in cGMP concentration producing up to a 3-fold larger relative reduction in inward current. Once these sequential amplification steps of the phototransduction cascade had become identified, a great interest of the field was then focused on determining how many transducin molecules are actually activated by a single R* and how many cGMP molecules are hydrolyzed by a single PDE. We refer the readers to a comprehensive review describing the history of these studies⁶ and concentrate here primarily on our current understanding.

The ability of a single R* to activate a large number of transducin⁷ and PDE⁸ molecules was recognized very early. Perhaps the largest number was reported by Bownds and colleagues who showed that one R* can activate as many as 37000 transducin molecules in an *in vitro* preparation of frog rods.⁹ This represents nearly the entire transducin content of an individual disc, the double lipid bilayer membrane that serves as the principle signaling compartment in the outer segment. However, such high-gain transducin activation by a single R* occurs only if R* artificially remains active over a prolonged period of time, such as in *in vitro* biochemical assays. In contrast, R* in intact photoreceptors is deactivated very rapidly through a two-step mechanism consisting of R* phosphorylation and subsequent arrestin binding. Therefore, the most interesting and physiologically relevant question is how many transducin molecules are activated by a single R* under physiological conditions.

To answer this question, one first needs to know the rate at which an R* activates transducin and the average time over which this activation persists (i.e., R* lifetime) in a living rod. The most accurate measurements of transducin activation rate were conducted in suspensions of frog rod photoreceptor membranes and yielded the rate of ~150 molecules/R*/s.¹⁰ In mammals, this rate is estimated to be ~400 molecules/R*/s due to the difference in temperature.¹¹ Mechanistically, this high rate of

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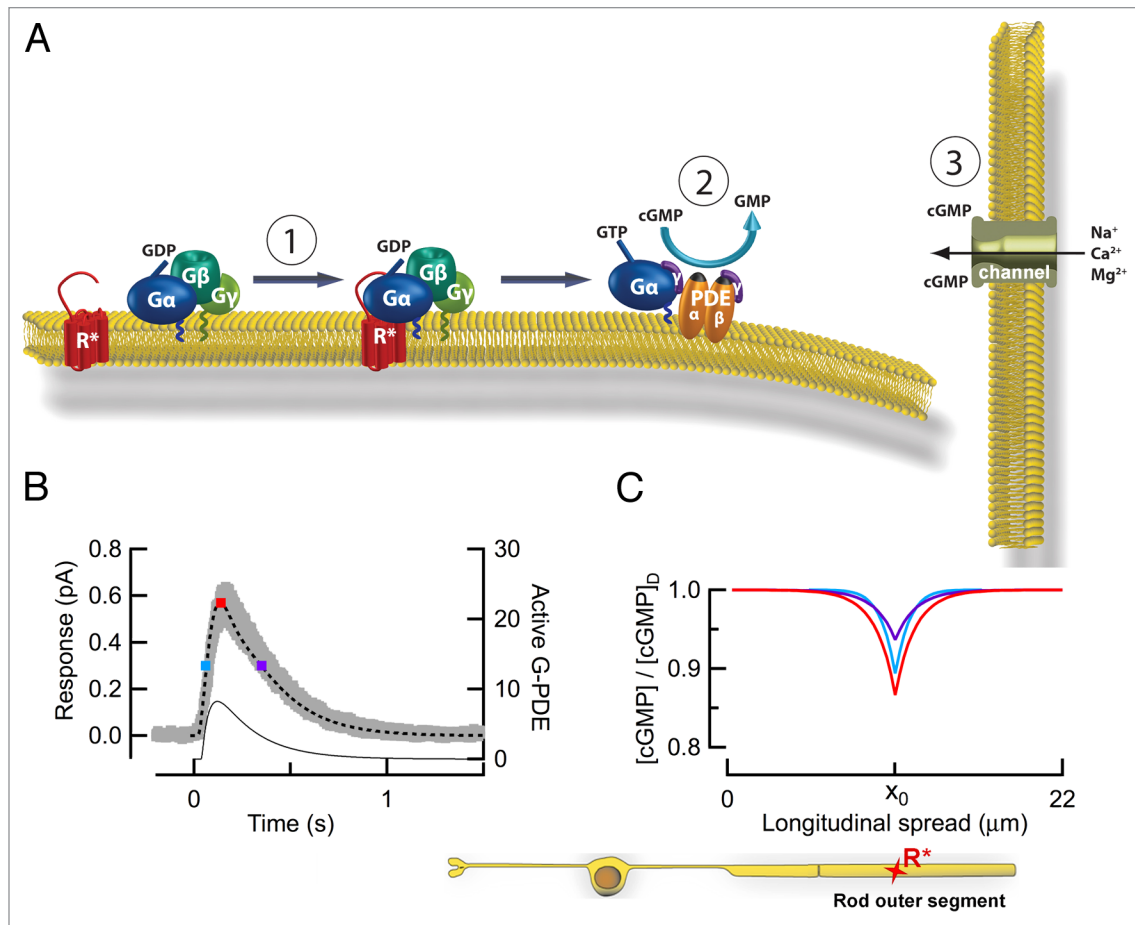


Figure 1. Signal amplification in rod phototransduction. **(A)** Three distinct biochemical stages amplify the signal generated by a single activated rhodopsin molecule, R^* : (1) high rate of transducin activation ($G\alpha\beta\gamma$); (2) high rate of cGMP hydrolysis by each activated PDE molecule; and (3) cooperative gating of the cGMP-sensitive ion channels by cGMP. **(B)** The time course of the electrical response to a single photon (thick gray trace) is compared with the time course and number of the active transducin-PDE complexes (thin black trace). **(C)** The spatial profile of the change in cGMP concentration relative to its dark level, at 3 times indicated at the time points in panel B. A schematic representation of the rod cell is shown beneath the graph. Note that the number of active transducin-PDE complexes at any time is quite small (Panel B) and the relative change in cGMP is likewise rather modest. Panel A is adapted with permission from ref. 2; panel B is adapted with permission from ref. 14.

activation is possible due to the very high density of transducin and PDE molecules on the disc membrane, and their high rates of lateral diffusion achieved by the unique lipid composition of photoreceptor membranes.¹²

The second piece that must be known in order to estimate the number of transducins activated by a single R^* in a living rod is how long this R^* remains active. A recent determination of R^* lifetime was performed in living mouse rods using genetic perturbations to control photoresponse kinetics and determined to be ~ 0.04 s.^{13,14} Therefore, the total number of transducins activated in the course of a mouse single photon response is $\sim 400 \times 0.04 = 16$. The lifetime of R^* in amphibian rods is estimated to be ~ 0.4 s,¹⁵ so that the number of transducins activated by a single R^* is $\sim 150 \times 0.4 = 60$.

Let us now consider the second amplification step in phototransduction, which arises from high rate of cGMP hydrolysis by activated PDE. This enzyme is among a handful of the most efficient enzymes whose k_{cat}/K_m ratios exceed 10^8 $M^{-1}\cdot s^{-1}$. Measurements in frog rod photoreceptor

membranes revealed that each catalytic subunit of PDE is characterized by the k_{cat} of ~ 2200 s^{-1} and the K_m of ~ 10 μM . Assuming that free cGMP concentration in the dark-adapted amphibian rod is 4 μM ¹⁶ and the average lifetime of activated PDE is 2 s,^{15,17} we calculate the total number of cGMP molecules hydrolyzed by one activated PDE as ~ 1200 . Combined with transducin activation gain, this calculation demonstrates that activation of single rhodopsin results in the hydrolysis of ~ 72000 molecules of cGMP. For mouse rods with ~ 10 -fold shorter lifetimes of both R^* and activated PDE,^{13,18} corresponding calculations yield ~ 2000 molecules of cGMP hydrolyzed downstream from a single R^* .

In terms of generating the single photon response, it is critical to consider not just the degree of biochemical amplification, but also the spatiotemporal dynamics of cGMP concentration changes, which was recently investigated for mouse rods.^{14,19} The authors concluded that fewer than 10 transducins are active at any given time (Fig. 1B) and the change in cGMP concentration is sufficiently spread along the axis of the rod outer segment

(Fig. 1C), so there is no local saturation of the signal. The latter is important because it ensures that full amplification available from the cooperative gating of the cGMP-gated channels contributes to the electrical response. An additional new insight from these studies is that the size of the photoresponse is determined by interplay between signal amplification and powerful feedback regulation of cGMP synthesis by Ca²⁺-dependent guanylate cyclase, thoroughly described in preceding studies (reviewed in refs.20, 21). Cyclase feedback serves to attenuate responses driven by longer-living R*s to a greater extent than those driven by shorter-living R*s, ultimately yielding responses with stereotyped time courses and amplitudes.¹⁹ This is thought to provide the visual system with more reliable single photon detection.

Another interesting feature of rod photoreceptors is that bright light causes a massive translocation of transducin from the light-sensitive outer segment compartment to the rest of the cell.²² This reduces transducin activation rate and the overall gain of phototransduction, which may spare the rod from excessive signaling under conditions when visual input is dominated by cones. Recent literature suggests that transducin translocation

may also serve to prevent adverse effects of constant exposure to bright light and thus be neuroprotective (reviewed in refs. 2, 23–26).

In summary, vertebrate phototransduction remains a beautiful model system for understanding G protein signaling under physiological conditions. However, it is fairly unique in regards to the high speed and gain of G protein activation by a GPCR. Most other G protein pathways have a far lower gain and sometimes even a one-to-one correspondence between a GPCR and its activated G protein. Ultimately, the degree of signal amplification in any pathway is determined by the cellular context in which it functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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