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INVITED REVIEW

# Phototransduction in mouse rods and cones

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**Abstract** Phototransduction is the process by which light triggers an electrical signal in a photoreceptor cell. Image-forming vision in vertebrates is mediated by two types of photoreceptors: the rods and the cones. In this review, we provide a summary of the success in which the mouse has served as a vertebrate model for studying rod phototransduction, with respect to both the activation and termination steps. Cones are still not as well-understood as rods partly because it is difficult to work with mouse cones due to their scarcity and fragility. The situation may change, however.

**Keywords** Phototransduction · Mouse · Rods · Cones

## Introduction

Vertebrates rely on retinal rods and cones for conventional, image-forming vision. Rods are specialized for low-light vision. They are extremely sensitive and can signal the absorption of single photons. Cones mediate daylight vision. They are much less sensitive to light than rods, but have higher temporal resolution. The presence of typically more than one type of cones in the retina mediates color vision.

Great progress has been made in understanding rod phototransduction since the introduction of the suction-electrode recording technique in the late 1970s [1]. Individual amphibian and mammalian (including primate) photoreceptors can be recorded with this method. Bovine retina, on the other hand, has been a favorite preparation for studying phototransduction by biochemists because of the abundance of tissue available. The mouse, however, has become an increasingly popular animal model for study in the past decade through the advent of gene-targeting techniques. When combined with electrophysiology, mouse genetics provides unmatched power in elucidating the in vivo functions of key phototransduction proteins, most of which have been knocked out, overexpressed, or mutated in rods, yielding a rich body of information on the mechanisms underlying the amplification, recovery, and adaptation of rod photoresponses (Table 1).

We shall first give a brief description of the structure and the development of mouse photoreceptors, followed by a summary of recent studies on rod phototransduction with emphasis on information gleaned from mouse models. Finally, a recent advance in studying mouse cones will be mentioned.

## Structure of mouse rods and cones

Rods constitute ~97% of mouse retinal photoreceptors, with cones accounting for the remainder [2]. The mouse photoreceptors are broadly similar to primate photoreceptors in physical dimensions (Table 2). The outer segment is about 1.4 μm in diameter and 24 μm in length for rods, and, correspondingly, about 1.2 μm and 13 μm for cones. These dimensions are significantly smaller than those of amphibian photoreceptors, which explain physiologists' longtime preference for the latter.

Rods and cones have four primary structural/functional regions: outer segment, inner segment, cell body, and

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**Table 1** List of major proteins involved in mouse rod phototransduction that have been knocked out, overexpressed, or mutated

| Proteins | Manipulation | Phenotype | References |
|---|---|---|---|
| Rhodopsin | Knockout | No ROS formation, no light response | [3, 4] |
| Transducin $G\alpha_{t1}$ | Knockout | No light response | [64] |
| Transducin $G_{\gamma 1}$ | S70L mutation | Deficit in light adaptation | [73] |
| $PDE_{\beta}$ | Mutation | Rd mouse, retina degeneration | [83–87] |
| $PDE_{\gamma}$ | Knockout | Reduced PDE activity, retina degeneration | [82] |
| $PDE_{\gamma}$ | Overexpression | Reduced gain, accelerated shutoff | [156] |
| $PDE_{\gamma}$ | W70A mutation | Reduced sensitivity, slow shutoff | [90] |
| CNGB1 | Knockout | No light response, retina degeneration | [106] |
| GRK1 | Knockout | Slow shutoff, larger amplitude | [118] |
| GRK1 | Overexpression | Normal light response | [56] |
| Arrestin | Knockout | Slow shutoff | [132] |
| Recoverin | Knockout | Slow shutoff, increased sensitivity | [131] |
| RGS9-1[2] | Overexpression | Accelerated shutoff | [56] |
| RGS9-1 | Knockout | Slow shutoff | [148] |
| Gβ5-L | Knockout | Same as RGS9-1 knockout | [151] |
| R9AP | Knockout | Same as RGS9-1 knockout | [150] |
| GC1 | Knockout | Normal dark current, cone dystrophy | [164] |
| GCAP1&2 | Knockout | Larger amplitude, delayed recovery | [169] |

[1] Due to space limitation, not all the genetically engineered mouse lines are listed. For those included, only the most salient phenotypes are listed, please refer to the text for more complete description.
[2] RGS9-1 overexpression was achieved by overexpressing R9AP, which resulted in overexpression of all three components of the GAP complex, RGS9-1, Gβ5-L and R9AP [56].

synaptic terminal. The outer segment is filled with a dense stack of membrane disks, spaced at intervals of about 28 nm. The disks carry the visual pigment (rhodopsin in rods and cone pigment in cones) and other transduction components either as transmembrane or peripheral membrane proteins. Visual pigment is the most abundant protein in the outer segment. The importance of visual pigment as a major structural component is demonstrated by the rhodopsin-knockout mouse, the rod outer segments of which fail to form [3, 4]. The rod photoreceptors of this mouse rapidly degenerate, followed suit by cones. The packing density of pigment molecules on the disks is remarkably uniform across different vertebrate species, being ~25,000 $\mu m^{-2}$, corresponding to a concentration of ~3.5 mM [5]. The total number of pigment molecules in the outer segment can thus be calculated roughly from its envelope volume. The dense stack of disks greatly increases the probability of photon capture. An interesting difference between rods and cones is that the rod disks (except for the nascent disks at the base of the outer segment) are completely internalized and therefore physically separate from the plasma membrane, whereas the cone disks remain as foldings of the plasma membrane. The open cone disks offer a much larger surface area for rapid fluxes of substances between the cell exterior and interior, such as chromophore transfer for pigment regeneration and fast calcium dynamics during light adaptation.

The inner segment contains the endoplasmic reticulum and the Golgi apparatus. It is also packed with mitochondria immediately adjacent to the outer segment in order to meet the high demand for metabolic energy associated with phototransduction. All proteins destined for the outer segment must pass through a narrow connecting cilium between the outer and the inner segments.

The synaptic terminal transmits the light signal to the second-order neurons in the retina: the bipolar and horizontal cells. In darkness, there is a steady inward current (the "dark current") through a cation conductance on the outer segment membrane, which depolarizes the rod or cone and maintains a steady synaptic release of glutamate. Light closes this cation conductance (the

**Table 2** Physical dimensions of the outer segment of mouse rods and cones

| | Rods | | | Cones | | |
|---|---|---|---|---|---|---|
| | Mouse | Primate | Salamander | Mouse | Primate | Salamander |
| Length (μm) | 23.6 | 25 | 22 | 13.4 | 13 | 8 |
| Diameter (μm) | 1.4 | 2 | 11 | 1.2 | $3_{base}$, $1_{tip}$ | $4_{base}$, $2.5_{tip}$ |
| Volume ($\mu m^3$) | 36 | 40 | 2,000 | 14 | 30 | 70 |
| References | [2] | [183] | [184] | [2] | [101] | [101] |

Salamander and primate photoreceptors are included for comparison purpose.

"light-sensitive" conductance, consisting of cGMP-gated channels) to stop the dark current and produce a membrane hyperpolarization as the response. This hyperpolarization decreases or terminates the dark glutamate release. The signal is further processed by other neurons in the retina before being transmitted to higher centers in the brain.

## Development of mouse photoreceptors

Rods and cones renew their outer segments continually [6, 7]. Newly formed disks at the base of the outer segment progressively displace previously synthesized disks toward the apical end. The disks reaching the apex of the outer segment are shed and phagocytosed on a daily basis by the adjacent retinal pigment epithelium (RPE) [8]. The rate of formation and disposal of the disks are roughly equal so that a constant outer-segment length is maintained in the adult retina.

The mouse rod outer segment (ROS) changes little in diameter during development, but it elongates at a rapid and almost linear rate from postnatal day (P) 11 to 17, reaching adult length by P19–25 [9]. The increase in ROS length parallels the almost linear rise in rhodopsin content from P8 to P23 [10]. At the peak rate of growth during P13–P17, ~120 disks are synthesized per day compared with 75 disks in the adult retina [6, 9].

The dark current recorded in developing mouse rods increases roughly in direct proportion to the length of the mouse ROS between P12 (around the time of eye-opening in neonate) and P45 (adult) [11]. The kinetics of the dim–flash response changes rather little during development and the flash sensitivity of rods increase by approximately 1.5-fold, reflecting the presence of a small percentage (~1%) of free opsin (i.e., devoid of chromophore) in neonatal rods even after overnight dark adaptation. The constitutive activity of this small amount of opsin mildly triggers adaptation mechanisms and, therefore, causes a small reduction in the sensitivity of the cell (see below). A similar small, age-dependent increase in rod sensitivity was found for rat. Previously, a 50-fold increase in rod sensitivity was reported for rat from neonate to adulthood [12], but now this appears to be incorrect.

## Rod response activation

The inward dark current through the light-sensitive, cGMP-gated channels is composed of ~85% $Na^+$ and ~15% $Ca^{2+}$ in rods ([13], see also [14]). An outward current presumably through potassium channels completes the loop at the inner segment and cell body. The $Na^+$ is steadily pumped out of the cell via a Na–K ATPase at the inner segment, and the $Ca^{2+}$ is extruded via the Na/Ca,K exchanger (NCKX) residing also in the outer-segment plasma membrane.

Upon absorbing a photon, the rhodopsin molecule becomes enzymatically active (R*) and catalyzes the activation of the G protein transducin to G*. Transducin, in turn, activates the effector phosphodiesterase (PDE) to PDE*. PDE* hydrolyzes the diffusible messenger cGMP. The resulting decrease in the cytoplasmic free cGMP concentration leads to the closure of the cGMP-gated channels and the membrane hyperpolarization. Channel closure also leads to a reduction in the cytoplasmic free $Ca^{2+}$ concentration owing to stoppage of the $Ca^{2+}$ influx but continued extrusion of $Ca^{2+}$ by NCKX. This decrease in intracellular $Ca^{2+}$ triggers negative feedback to produce light adaptation.

The details of the activation phase of rod phototransduction are now well-established. A quantitative description that reproduces the activation kinetics of the rod response under physiological conditions is achieved [15–18]. We shall discuss below the major proteins mediating the activation phase in mouse rods—visual pigment, transducin, the effector PDE, and the cGMP-gated channel. The focus will be on studies with combined approaches from mouse genetics and physiology.

### Visual pigments of mouse rods and cones

Mouse has a single rod pigment, rhodopsin, and two cone pigments: S- and M-cone pigments, with maximal spectral sensitivity at 360 and 508 nm, respectively. Mouse is unusual in that individual cones express both S- and M-cone pigments, with the M-pigment level decreasing in a gradient from dorsal to ventral retina [19].

Mouse rhodopsin and cone pigments belong to the superfamily of G-protein coupled receptors. A high-resolution (2.8 Å), three-dimensional structure of the ground state of bovine rhodopsin was determined in 2000 by Palczewski et al. [20]. The future challenge is to solve the structure of cone pigments, which is more unstable than rhodopsin.

Visual pigments from most vertebrate species, including mammals, use 11-*cis*-retinal (denoted A1), while those from many water-based animals use 11-*cis*-3,4-dehydroretinal (denoted A2), as their natural ligand [21, 22]. The chromophore is covalently bound via a Schiff-base linkage to a conserved lysine residue (K296 in mammalian rhodopsin) in the seventh transmembrane helix. In darkness, the 11-*cis*-retinal acts as a powerful antagonist to lock rhodopsin predominantly in an inactive state because free opsin can weakly activate the transduction cascade [23–25]. The antagonistic role of 11-*cis*-retinal was clearly demonstrated in RPE65-null mice. RPE65 is the key isomerase in the RPE visual cycle, which is important for regenerating

rod and cone pigments. *Rpe65*$^{-/-}$ retina has virtually no 11-*cis*-retinal [26]. Photoreceptors degenerate due to the constant activation of phototransduction by the large amount of free rod opsin. This degeneration can be prevented by deleting the transducin α-subunit, which blocks the activation of the downstream cascade [27]. In a separate experiment, K296 is mutated to glutamic acid, producing an opsin with no chromophore-binding site [28]. Although the K296E opsin constitutively activates transducin in vitro, the constitutive activity of the mutant opsin in vivo was turned off due to phosphorylation by rhodopsin kinase followed by arrestin-binding (see "R* termination").

Even with 11-*cis*-retinal attached, rhodopsin occasionally undergoes spontaneous (thermal) activation in the dark, producing responses identical to those triggered by photons [29]. The spontaneous activation of visual pigment molecules sets an ultimate limit on visual sensitivity [30–33]. In a toad rod, the rate of thermal activation of rhodopsin was measured to be 0.031 $s^{-1}$ at 22°C, corresponding to an average wait of 2,000 years for the spontaneous activation of a given rhodopsin molecule to occur, based on a total of $2\times10^9$ rhodopsin molecules per cell [34]. This great stability makes it possible for rods to pack many rhodopsin molecules to the rod disks so as to increase its photon-capture ability while keeping the dark noise within a manageable level. In wild-type mouse rods, it is rather difficult to measure the discrete noise arising from the thermal activation of rhodopsin because of the relatively small amplitude of the single-photon response. However, the measurement has been achieved with *GCAPs*$^{-/-}$ rods [35], the single-photon response of which is nearly five times that of the wild type as a result of the elimination of the $Ca^{2+}$-mediated negative feedback on guanylate cyclase (see below). The rate is ~0.012 at 36°C [35]. Red cone pigment is much more prone to spontaneous isomerization than rhodopsin [36, 37], but it is difficult to measure the rate from native cones [37]. The *GCAPs*$^{-/-}$ mouse will be a useful tool for accurately measuring the rate of thermal activation of mammalian cone pigments by expressing them in *GCAPs*$^{-/-}$ rods.

Photon absorption by 11-*cis*-retinal triggers the *cis*-to-*trans* isomerization of the retinoid. This isomerization rapidly converts the ligand from a powerful antagonist to a powerful agonist, leading to the formation of a series of spectrally distinct intermediates of rhodopsin in the order of bathorhodopsin, lumirhodopsin, metarhodopsin I (Meta I), and metarhodopsin II (Meta II) within a few millisecond (reviewed in [38]). Meta II is the active form of rhodopsin (R*), which in turn decays to the inactive Meta III. The Meta-II state of cone pigment decays 50 times more rapidly than that of rhodopsin [39–41]. Despite this difference, rhodopsin and transgenic red cone pigment signal identically downstream when compared side-by-side in the same *Xenopus* rod or cone [37]. The same was found for rhodopsin and transgenic red cone pigment in mouse rod [42]. Thus, not only do rod and cone pigments interact with a given transducin identically, but the shutoff mediated by a given pigment kinase and arrestin is also similar and precedes the Meta-II decay of rod and cone pigments, rendering the decay of Meta II non-rate-limiting under normal conditions (see "R* termination").

Retinal absorbs maximally in the UV range ($\lambda_{max}$~ 380 nm) when in solution or bound to opsin in the unprotonated form. The absorption shifts into the visible region when the Schiff-base is protonated. Like other vertebrate pigments, mouse rhodopsin and M-cone pigment are protonated; on the other hand, mouse S-cone pigment is unprotonated, explaining its absorption in the UV-region [43]. The positively charged Schiff-base is stabilized by the counterion E113 (residue number according to mouse rhodopsin) in rhodopsin and M-cone pigment [20, 44–46]. Rhodopsin activation involves a "counterion switch" mechanism in which E181 located in the extracellular loop II transfers a proton via a hydrogen-bonded network to the primary counterion, E113, during the formation of Meta I. Therefore, E181 replaces E113 as the counterion to stabilize the protonated Schiff-base in the transition stage before its eventual deprotonation [47].

Because the mouse S-cone pigment is not protonated and the nearby E113 is neutral, the interesting questions are: does a protonation event occur during light activation, and does it involve a counterion switch mechanism? Remarkably, after the 11-*cis* isomerization, the *all-trans*-retinal picked up a proton in the Lumi state [48] and a counterion switch occurs from E108 (E113 in rhodopsin) to E176 (E181) during the Lumi to Meta I transition, in close analogy to rhodopsin [48, 49]. Thus, the counterion switch appears to be a general mechanism for the activation of all visual pigments.

The decay of R* eventually leads to the departure of all-*trans*-retinal from the protein. The all-*trans* chromophore is converted back to 11-*cis*-retinal through a cascade of enzymatic reactions called the visual cycle in the adjacent RPE, before being used again for the regeneration of visual pigments (for example, see review [50]).

Visual pigment is a major structural component of rods and cones. It is not surprising that genetic deletion of mouse rhodopsin results in rods without proper outer-segment formation [3, 4]. Is half the amount of normal rhodopsin enough for maintaining a healthy ROS? In *rho*$^{+/-}$ mouse, rods are properly formed despite only half of the normal level of rhodopsin being present. However, a progressive mild degeneration of the rods does occur. Interestingly, the activation rate of the photoresponse of *rho*$^{+/-}$ rods is twice that of normal [51]. The explanation for this observation was originally proposed that a lower

rhodopsin concentration reduces protein crowding on the disk membrane, thereby increasing rhodopsin's diffusion coefficient and its rate of encounter with transducin. Thus, this finding would point to the diffusional encounter of transducin by photoexcited rhodopsin as the rate-limiting step in the activation of the rod photoresponse. However, Liang et al. [52] subsequently reported that rods in *rho*$^{+/-}$ mouse are not completely normal in that rhodopsin exists in small raft-like structures, as well as in large and organized para-crystals. In addition, there is an approximate 40% reduction in the ROS volume, in the rhodopsin content and in the 11-*cis*-retinal level in these cells. These authors suggested that the observed acceleration of phototransduction in *rho*$^{+/-}$ rods was not due to a lower density of rhodopsin on the disk surface but to the structural changes in the whole ROS.

## Transducin

Photoactivated pigment binds the transducin heterotrimer and catalyzes the exchange of GTP for GDP on the α-subunit. Gα-GTP (G*) dissociates from R* and its native partners, Gβγ, and interacts with the cGMP PDE to carry the signal forward. Released R* is free to activate additional transducin molecules. Transducin activation by R* represents the first amplification step in the phototransduction cascade.

The estimated rate of transducin activation by a single R* has ranged from 10 to over 3,000 $s^{-1}$ at room temperature (for review, see [16]). More recently, a rate of ~120 $s^{-1}$ was reported to be more consistent with biochemical, light-scattering, and electrophysiological measurements ([53] but see [54]). The rate is roughly doubled in mammalian rods due to a difference in body temperature. Until recently, it was believed that over a hundred transducins are activated during the lifetime of a single R* in mammalian rods (e.g. [55]). This number is now revised to be ~20 in mouse rods, based on the shorter lifetime of R* (80 ms) and 240 $s^{-1}$ activation rate of transducin by R* [56].

Transducin is present at 10% the amount of pigment. Rods and cones have different isoforms of transducin, being $G\alpha_{t1}G\beta_1\gamma_1$ in rods and $G\alpha_{t2}G\beta_3\gamma_8$ in cones [57–60]. The C-terminal of the γ subunit is farnesylated and the N-terminal of the α subunit is acylated [61–63]. These lipid modifications help anchor the holo-transducin to the disk membrane. The importance of transducin for conveying the signal from R* to PDE was confirmed by gene-targeting experiments, in which rods of $G\alpha_{t1}$-null mice (*gnat1*$^{-/-}$) were found to lose all light sensitivity [64]. The *gnat1*$^{-/-}$ mouse line has proven to be a valuable tool for blocking rod phototransduction in many studies [27, 65–68]. It was also used successfully to delineate two apoptotic pathways in light-induced retinal degeneration [69]. Bright light triggers apoptosis of photoreceptors through a mechanism requiring the activation of rhodopsin but not transducin signaling. In contrast, low-intensity light induces apoptosis that is predominantly dependent on transducin signaling.

Almost a decade ago, rod transducin was found to undergo light-dependent redistribution [70, 71]. Great progress has been made in the past few years by using mouse (or rat) models for study. Both $G\alpha_{t1}$ and $G\beta_1\gamma_1$ subunits are present predominantly in the ROS in darkness, but translocate in bright light, with slightly different time courses, to the inner segment and the inner nuclear layer [72, 73]. This phenomenon has been suggested to contribute to light adaptation of rods [72], but others argue that the main function of transducin translocation is to provide protection for rods in bright light when rods contribute little to vision [74]. In contrast, $G\alpha_{t2}$ has been found not to translocate from the cone outer segment (COS) in bright light [74–76]. This might be consistent with the need for cones to function in bright light [74]. Incidentally, there is so far no published finding about any translocation of the corresponding Gβγ ($G\beta_3\gamma_8$) in cones.

$G\gamma_1$ in rods and $G\gamma_8$ in cones are farnesylated with a 15-carbon chain, but many other Gγ subunits are geranylgeranylated with a 20-carbon chain. What is the significance of this difference? Knock-in mice expressing geranylgeranylated instead of farnesylated $G\gamma_1$ exhibited impaired properties in light adaptation because the stronger attachment by geranylgeranylation attenuated the light-dependent translocation of $G\gamma_1$from ROS to the inner region [73]. Thus, it appears that the selective farnesylation is important for the regulation of visual sensitivity by providing sufficient but not excessive anchoring of $G\beta_1\gamma_1$ to the membrane.

## The cGMP PDE

PDE is the third component of vertebrate phototransduction. It is a tetrameric protein consisting of two equally active catalytic subunits, α and β, and two identical γ subunits [77, 78]. PDE is anchored to the disk membrane by isoprenylation of the C termini of the two catalytic subunits [79–81]. The density of PDE is ~1–2% of rhodopsin. Thus, the first three components of phototransduction are present in the ratio of 100R:10G:1PDE.

In the dark, the two γ subunits act as inhibitory subunits by binding to the two catalytic subunits and preventing the hydrolysis of cGMP. In the light, Gα-GTP encounters PDEγ and sterically displaces the latter (still associated with PDEαβ), therefore relieving its inhibitory effect on the catalytic subunits and permitting the hydrolysis of cGMP to proceed.

In contrast to the amplification achieved during transducin activation by R*, the activation of PDE by G* constitutes no gain, i.e., with an efficiency approaching at most unity. It is the catalytic power of PDE* that provides the second amplification step. It was reported that PDE* hydrolyzes cGMP at a rate close to the limit set by aqueous diffusion, with a $K_m$ of ~10 μM and a $K_{cat}$ of 2,200 $s^{-1}$ [53].

One might have expected that the deletion of PDEγ from mouse rods would unleash the full catalytic power of PDEαβ. However, Tsang et al. [82] found that, in the absence of PDEγ, the PDEαβ dimer actually lacked hydrolytic activity, and the photoreceptors of the mutant mouse rapidly degenerated. Thus, the inhibitory PDEγ subunit appears to be necessary for the integrity of the catalytic PDEαβ subunits. The degeneration might be caused by an abnormally high cGMP concentration due to the lack of hydrolysis. A related example is the *rd* mouse, which is one of the best-known models for retinal degeneration. The rod cells in the *rd* mouse begin to degenerate at about P8, followed by cones; by 4 weeks, virtually no photoreceptors are left [83, 84]. Degeneration in this mouse model is preceded by the accumulation of cGMP in the retina, correlated with deficient activity of the rod PDE due to a mutation in the β subunit [85–87]. It is worth noting that the *rd* mouse was instrumental in suggesting that inner retinal neurons could mediate non-image-forming vision [88, 89].

In addition to its inhibitory function, PDEγ accelerates the GTPase activating protein (GAP) activity of transducin for G* shutoff (see "G*-PDE* termination"). Mouse rods carrying the W70A point mutation of PDEγ, which impairs the $G\alpha_{t1}$–PDEγ interaction, showed a greatly reduced sensitivity to light and a slower recovery from the flash response than wild type [90].

### The cGMP-gated channel

The cGMP-gated channel belongs to the family of cyclic-nucleotide-gated (CNG) channels, which are nonselective cation channels (reviewed in [91, 92]). The channel is located on the plasma membrane and is the last component in the activation phase of phototransduction. In the dark, a basal concentration of one to several micromolars of cGMP keeps a small percentage of the CNG channels open [13]. The decline in cGMP concentration upon illumination leads to rapid closure of the channels with a sub-millisecond delay [93].

The rod CNG channel has a surprising 3CNGA1:1CNGB1 subunit composition [94–96], whereas the cone channel supposedly exhibits a 2CNGA3: 2CNGB3 stoichiometry [97]. CNGA1 and CNGA3 subunits form functional homomeric channels by themselves when heterologously expressed. Although CNGB1 and CNGB3 do not form functional channels by themselves, they confer several properties typical of native channels when co-expressed with A subunits: flickery opening behavior, increased sensitivity to L-*cis*-diltiazem, and weaker block by extracellular calcium (reviewed in [91, 98–100]). The combined amplification provided by pigment, PDE and CNG channels are very high, ensuring the high sensitivity of rods (for review see [16, 101]), including the ability of rods to signal the absorption of a single photon [102].

In humans, mutations in CNGA1 causes retinitis pigmentosa [103], while mutations in both CNGA3 [104] and CNGB3[105] cause achromatopsia. Mouse models carrying null mutations of CNGB1 [106] and CNGA3 [107] are available. CNGB1 was found to be crucial for the targeting of the native CNG channel in rods. Thus, only trace amounts of the CNGA1 subunit were found on the ROS in CNGB1-null mice and the majority of rod photoreceptors failed to respond to light [106]. CNGA3-deficient mice selectively lost their cone photoresponse with the rod pathway intact. Analogous to the case of rod transducin, CNGA3-null mice were used to block cone phototransduction in studying the intrinsically photosensitive retinal ganglion cells [65, 66].

## Phototransduction termination

After light activation, a timely recovery of the photoreceptor is essential so that it can respond to subsequently absorbed photons and signal rapid changes in illumination. This recovery from light requires the efficient inactivation of each of the activated components: R*, G*, and PDE*, as well as a rapid restoration of the cGMP concentration. The termination rates of the activation steps set the time course of the photoresponse.

In the past decade, knowledge gained from genetically engineered mouse lines has provided major advances in understanding the termination of the rod photoresponse. In the following sections, we shall discuss separately the events responsible for the inactivations of R*, G*, and PDE*, followed by the restoration of cGMP.

### R* termination

Activated rhodopsin (R*) is inactivated by a two-step process. First, R* is phosphorylated by rhodopsin kinase (GRK1), which lowers the activity of R*. Second, the protein arrestin binds to phosphorylated R*, capping its residual activity [108, 109].

Multiple serine/threonine residues at the C-terminal of rhodopsin (six in mice and seven in humans) provide the phosphorylation sites for GRK1. Cone pigments have more

potential phosphorylation sites at the C-terminal than rhodopsin. For example, human red cone pigment has ten such sites. Even though biochemical experiments originally suggested that rhodopsin is predominantly phosphorylated at only one serine residue after light exposure [110], subsequent recordings from transgenic mouse rods carrying phosphorylation site mutations suggested that the reproducible deactivation of R* requires at least three phosphorylation events [111]. In addition, all six sites need to be phosphorylated in order for the normal decline of the response to proceed.

Multiple phosphorylation events are also proposed to underlie the reproducibility of rod responses to single photons [111–113]. Despite the fact that events generated by single molecules are stochastic in nature, the single-photon response of rods shows remarkable reproducibility in amplitude and shape [102, 114, 115]. By averaging over multiple shutoff steps, the integrated R* activity varies less than otherwise controlled by a single step. This hypothesis is supported by a recent experiment using transgenic mouse rods carrying phosphorylation site mutations ([116]; however, see "G*-PDE* termination"). The authors showed that the reproducibility of the single-photon response varies in a graded and systematic manner with the number, but not the identity, of the phosphorylation sites. Each phosphorylation site provides an independent step in rhodopsin deactivation and that, collectively, these steps tightly control the lifetime of R*.

Much less is known about the role of the phosphorylation sites in cone pigments. The only in vivo experiment was done by Kefalov et al. [37], showing that transgenic frog rods expressing a human red cone pigment with all ten putative phosphorylation sites mutated gave a prolonged response. It suggests that the activated cone pigment is quenched by a similar two-step shutoff mechanism even though its active lifetime is much shorter than that of rhodopsin.

In addition to mutations of all of the C-terminal serine and threonine residues to alanine [111], rhodopsin phosphorylation can also be prevented by deleting the C-terminal region of the pigment [117] or ablating GRK1 [118]. As expected, rods from all three transgenic mouse lines showed similar properties of the single-photon response, with an amplitude reaching a plateau about twice that of the wild type and decaying stochastically to baseline after a long interval of 3 to 5 s. GRK1-mediated phosphorylation begins to reduce the activity of R* at ~100 ms after the flash, because this is the time point at which the transgenic response starts to deviate from the wild-type response (Fig. 1). As mentioned earlier, it was estimated in a recent study that the lifetime of R* is ~80 ms [56], suggesting that arrestin-binding occurs rapidly after phosphorylation of the pigment. Therefore, GRK1/arrestin-mediated shutoff occurs in rods even earlier than the fast Meta-II decay of cone pigment [37], which is of the order

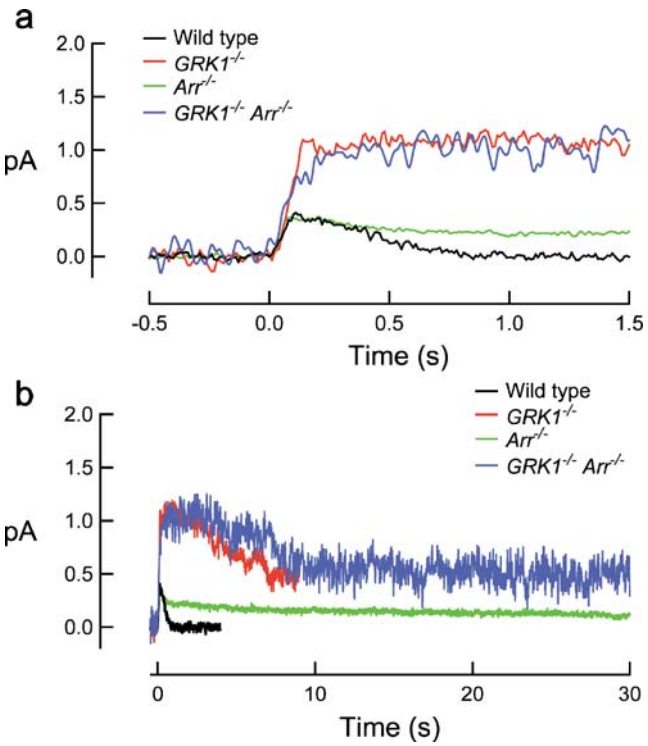


**Fig. 1** Form of the single-photon response from knockout mouse rods with deficient R* termination on fast (**a**) and slow (**b**) timescales. Flashes were delivered at $t=0$. Figure was kindly supplied by Dr. M. E. Burns

of 1 s after flash [39–41]. The GRK1-mediated shutoff likely happens even faster in cones (see below).

Mouse and rat are unusual in that the same GRK1 is present in both rods and cones. In all other species studied, another pigment kinase (the so-called "cone pigment kinase"), GRK7, is present in cone photoreceptors [119–122]. Indeed, many animal species, including human, have both GRK1 and GRK7 in cones. This explains why Oguchi patients with a defective *GRK1* gene have normal daytime vision, whereas GRK1-null mice have prolonged cone photoresponses [123]. Interestingly, GRK7 was shown to have considerably higher specific activity than GRK1 and, is present at a much higher concentration in cones than GRK1 is in rods in fish [121, 124]. This difference has been proposed as a potential mechanism underlying the faster shutoff and lower sensitivity of cones than rods (recently reviewed in [125]).

Biochemical experiments suggest that GRK1-mediated phosphorylation of R* is regulated by recoverin (Rec) [126–129], which belongs to a family of calcium-binding proteins. The hypothesis is that, when intracellular free $Ca^{2+}$ concentration is high in the dark, Rec–$Ca^{2+}$ binds to GRK1 and inhibits R* phosphorylation. When $Ca^{2+}$ concentration decreases in the light, $Ca^{2+}$ dissociates from recoverin; consequently, the resulting affinity between

recoverin and GRK1 is reduced, and its inhibition on R* phosphorylation is released. However, this hypothesis was challenged by in vitro measurements suggesting that the extent of R* phosphorylation was unaffected by light adaptation and by changes in intracellular $Ca^{2+}$ [130]. This controversy was finally settled by recordings from $Rec^{-/-}$ mouse rods [131], which showed that Rec–$Ca^{2+}$ prolongs the dark-adapted flash response and increases the rod's sensitivity to dim steady light, probably by inhibiting the phosphorylation of R* by GRK1. Furthermore, $Rec^{-/-}$ rods had faster $Ca^{2+}$ dynamics, indicating that recoverin is a significant $Ca^{2+}$ buffer in the ROS.

In the second step for the deactivation of R*, arrestin binds to phosphorylated R* (R*–P) to cap its catalytic activity. In mouse, the dim–flash responses from rods of arrestin-knockout ($Arr^{-/-}$) mice do not differ greatly from the wild-type response until in its falling phase, when recovery reaches approximately halfway back to baseline [132]. Therefore, phosphorylation alone can reduce R*'s activity significantly. The response of $Arr^{-/-}$ rods on average recovers ~10 times more slowly than the response of rods lacking rhodopsin phosphorylation (Fig. 1), presumably reflecting the continuous activity of the phosphorylated meta-II state of R* until it decays to inactive meta III. In rods lacking both GRK1 and arrestin ($GRK1^{-/-}Arr^{-/-}$), the activation phase and peak amplitude of the dim–flash response resemble those of $GRK1^{-/-}$ rods but then decay slowly with a time constant similar to that of the $Arr^{-/-}$ response [133], reflecting the decay of non-phosphorylated R. Thus, it appears that phosphorylation does not influence the decay of R*.

At least two splice variants exist for rod arrestin: a full-length (p48) and a C-terminal truncated form (p44) [134]. P44 has a faster on-rate than p48 for binding R* and R*–P [135, 136]. In addition, p44 is more efficient than p48 in turning off R* in vitro [137, 138]. Although p48 is ~10 times more abundant than p44, it translocates from ROS to the rest of the cell in the dark, therefore largely, absent in dark-adapted ROS [71, 139–141]. This raises an interesting question about the roles of individual isoforms of arrestin in the intact rods. By selectively expressing the two isoforms in mouse rods lacking endogenous arrestin, it was found that both isoforms could quench the activity of phosphorylated R* rapidly. However, only p48 was able to quench the activity of non-phosphorylated R* [133].

Cones express their own arrestin called cone arrestin or X-arrestin [142]. Surprisingly, both the rod and cone forms of arrestin exist in mouse cones [143]. Recent photopic electroretinogram (ERG) recordings on cone-arrestin-knockout mouse have shown that cone arrestin is involved in the recovery of the cone response, but its exact function is still unclear [144], whereas rod arrestin seems to play no role in the cone response.

## G*-PDE* termination

Earlier, genetically engineered mouse lines were designed to disrupt R* termination. More recent studies have centered on G*-PDE* termination. G* is inactivated when its bound GTP is hydrolyzed to GDP. Although transducin has a slow intrinsic GTPase activity, the hydrolysis is greatly accelerated by a GAP complex. The complex is composed of a member of the family of regulator-of-G-protein-signaling proteins [RGS9-1, 145], the long form of Gβ5 subunit (Gβ5-L, [146]}, and a membrane-anchor protein (R9AP and [147]). RGS9-1 has a G protein γ-like (GGL) domain that binds to Gβ5-L and has an N-terminal DEP (Dishevell/Eg110/Pleckstrin) domain that interacts with R9AP. After hydrolysis, Gα-GDP dissociates from PDEγ, which reexerts its inhibition on the catalytic PDEαβ subunits (reviewed in [55]). The molecular reactions underlying this step are shown schematically in Fig. 2.

Even though only RGS9-1 has GAP activity, all three components of the GAP complex are obligatory partners because genetic ablation of any one of them in mouse resulted in an increased instability of the other two through a posttranscriptional mechanism [148–150]. This is why transgenic mouse rods lacking RGS9-1, Gβ5-L, or R9AP display a similar delay in the recovery phase of the flash response without much noticeable differences in the activation phase [148, 150, 151]. In addition, the GAP activity on transducin is enhanced by PDEγ (see "The cGMP PDE section; [90, 145, 152, 153]). This provides an elegant mechanism for ensuring that excitation does not normally decay until G* has bound to the effector, PDE. The same GAP complex is present in both rods and cones; however, its concentration is much higher in cones than in rods [154, 155]. This has been suggested to contribute to the faster response kinetics of cones than rods.

Overexpression of PDEγ in mouse rods can accelerate the shutoff of light response independent of the GAP complex, suggesting that the inhibitory sites on PDE α and β are accessible to excess PDEγ after endogenous PDEγ

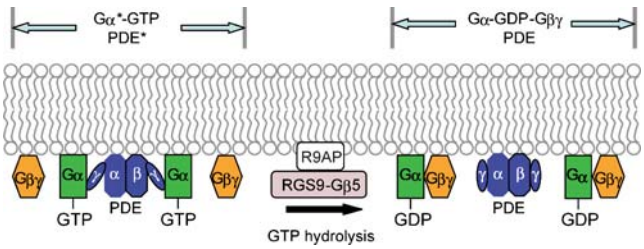


**Fig. 2** Schematic on the termination G*-PDE*. *Gα*-GTP* binds to PDEγ and activates PDE. The deactivation of Gα*-GTP is accelerated by the GAP complex, R9AP-RGS9-1-Gβ5L, in which RGS9-1 facilitates the hydrolysis of the bound *GTP* to *GDP*, leading to the reformation of the inactive heterotrimeric Gα-GDP-Gβγ and inactive PDE. An *asterisk* denotes the active state. This step was found to be the rate-limiting step of rod recovery [56]

has been displaced by G* upon illumination [156]. Overexpression also reduces the gain of transduction apparently through the "dominant negative effect" of interfering with the binding of G* to endogenous PDEγ.

Termination of phototransduction requires the shutoff of both R* and G*-PDE*, with the slower step determining the overall rate of response recovery. The identity of the rate-limiting step for rod recovery has been a long-standing question until recently [114, 157–160]. By overexpressing the GAP complex in mouse rods, Krispel et al. [56] showed that the recovery of rod response was dramatically accelerated, whereas overexpression of GRK1 had no effect. This experiment unequivocally demonstrated that the termination of G*-PDE* is the rate-limiting step. It is worthy to note that the same step is not necessarily the rate-limiting step in cone recovery because there is a much higher concentration of the GAP complex in cones. The same "overexpression" technique can be used in cones to answer this question.

Another implication of the above finding has to do with the question of the reproducibility of the single-photon response (see "R* termination"). Because G* deactivation is 2.5 times slower than R* deactivation [56], the slower G* termination dictates the recovery kinetics of the single-photon response of rods. In addition to the multistep involved in the shutoff of R*, the activation of ~20 transducin by one R* can provide the necessary averaging for an otherwise stochastic process.

### Restoration of cGMP

The free concentration of cGMP is determined by the PDE-mediated hydrolysis and guanylate–cyclase (GC)-mediated synthesis. For the photoreceptor to recover, not only is the light-stimulated cGMP hydrolysis by PDE required to terminate as discussed in the previous sections, but the dark level of cGMP also has to be restored. There are two GCs in mouse photoreceptors: GC1 (GC-E) and GC2 (GC-F). GC1 is present in both rods and cones [161, 162], and GC2 is present only in rods [163]. Mouse rods lacking GC1 can generate normal dark current, suggesting that GC2 can compensate for the loss of GC1 in synthesizing cGMP [164]. GC1 is important for cone function because cones degenerate in its absence [164]. Single knockout of GC2 and double knockout of both GCs are necessary for clarifying the functional differences between the two in rods.

GC activity is regulated by $Ca^{2+}$, mediated by the guanylate–cyclase activating proteins (GCAPs). This regulation is the most important negative feedback mechanism triggered by $Ca^{2+}$ in the light. For topics on other $Ca^{2+}$-feedaback effects and light adaptation, readers can refer to the following publications: [13, 101, 165, 166].

GCAPs belong to a large family of $Ca^{2+}$-binding proteins including calmodulin. Two GCAPs are present in mouse retinas, GCAP1 and GCAP2. Both GCAPs are expressed in mouse rods, but GCAP1 is primarily expressed in cones [167, 168]. In darkness, the relatively high $Ca^{2+}$ concentration promotes the formation of the $Ca^{2+}$-bound form of GCAPs, which inhibits GCs. When the $Ca^{2+}$ concentration declines during the light response, the dissociation of $Ca^{2+}$ allows GCAP to activate GC, thereby quickly restoring the basal cGMP concentration.

The two *GCAP* genes were knocked out in mouse simultaneously by taking advantage of their tail-to-tail chromosome localization [169]. The flash response of $GCAPs^{-/-}$ rods showed a larger amplitude and delayed decline compared to the wild type, consistent with a delay in cGMP synthesis during recovery when the associated $Ca^{2+}$ feedback was removed. The power of $Ca^{2+}$-mediated regulation through GCAP can be appreciated from the fact that the single-photon response of $GCAPs^{-/-}$ rods is five times that of the wild type vs the twofold increase when pigment phosphorylation is prevented. By comparing the light response of $GCAPs^{-/-}$ rods with that of the wild-type rods, Burns et al. [35] found that the activation of GC resulting from a change in $Ca^{2+}$-dependent GCAP activity occurs within ~40 ms after the flash and in a highly cooperative manner, with a Hill coefficient of 4. Therefore, the effect occurs much earlier than pigment phosphorylation, which is 80–100 ms after the flash (see "R* termination"). The rapid feedback to GC has dual effects on photoreceptors, decreasing the dark-adapted flash sensitivity [169] and speeding the restoration of the dark current [35].

What are the functional differences between the two GCAPs? In vitro, GCAP1 activates GC1 more efficiently than GC2 [170, 171], whereas GCAP2 activates both GC1 and GC2 with similar efficiency [163, 172, 173]. In vivo, expression of GCAP1, the major form in cones, in $GCAPs^{-/-}$ retina restored normal response kinetics of cones as expected [174]. A more interesting situation is in mouse rods where the two GCAPs coexist. Expression of bovine GCAP2 in $GCAPs^{-/-}$ rods restored the recovery of rods to saturating flashes but failed to restore the recovery kinetics of responses initiated by subsaturating flashes and particularly failed to rescue the fast initial phase of recovery [169]. In contrast, the expression of GCAP1 in $GCAPs^{-/-}$ retina restored the normal response kinetics of rods [175], suggesting that GCAP1 is responsible for the initial rapid phase of response recovery. More work is needed to clarify the role of GCAP2 in rods.

## Mouse model of cone phototransduction

Recently, there have been substantial advances in the understanding of cone transduction with fish as the model [120, 121, 124, 176, 177]. On the other hand, despite the

enormous success in studying rod phototransduction by a combination of mouse genetics and suction-electrode recording in recent years, the usage of mouse as a model system for studying cone phototransduction has until recently been limited to ERG studies. This is mainly due to the rarity of cones (~3%) and the fragility of the COS.

This hurdle was overcome recently by Pugh et al. [67, 178], who have developed a new configuration for recording from mouse cones. The conventional suction pipette recording, which involves drawing the ROS into the suction pipette ("OS in"), is not tolerated well by the more fragile COS. Instead, they drew a portion of the inner segment ("OS out") of a cone photoreceptor in a retinal slice into the suction pipette, allowing long, stable recordings. Previously, it was shown that the same information could be obtained by recording from either outer or inner segment of amphibian rods and cones [179] as expected from the nature of the circulating current.

To overcome the difficulty of identifying the ~3% cones in mouse retina, Pugh and colleagues used three different mouse lines. The first lacks the neural leucine zipper transcription factor (Nrl) [180], which drastically alters the cell fate of rod photoreceptors by turning them into cone-like photoreceptors [178, 181]. The second expresses EGFP in mouse cones, which facilitates/verifies their identification [182]. The third lacks the rod transducin α-subunit ($gnat1^{-/-}$), which blocks rod phototransduction [64].

In the case of the EGFP mouse line, background light is required to suppress the rod response so that the cone response can be isolated. As a result, the cone response is slightly light-adapted, therefore slightly faster and smaller for a given test flash intensity than that from $gnat1^{-/-}$ or $Nrl^{-/-}$ cones. When this factor is taken into consideration, the light response properties of mouse cones recorded from the three mouse lines are very similar and are as expected from mammalian cones (Fig. 3, Table 3) [67]. Prominent among these features is that mouse cones are far more tolerant than mouse rods to bleached pigment. The dark current recovers substantially in both S- and M-type cones after strong flashes that bleach a substantial fraction of the pigment. One surprising finding, however, is that the inactivation of M pigment is more retarded than that of S pigment in the absence of GRK1, suggesting the existence of a GRK1-independent inactivation mechanism for the S pigment. $Nrl^{-/-}$ cones differ from the wild type in certain respects. Their outer segments are shorter, more disordered, and undergo a slow degeneration [181]. In addition, in contrast to the wild type, $Nrl^{-/-}$ cones express a much higher percentage of S-opsin. Thus, transgenic mice expressing EGFP in their cones and $gnat1^{-/-}$ mice are better than $Nrl^{-/-}$ mice for studying cone physiology, although there is still the caveat of a variable mixture of M and S pigments in mouse cones, which makes the comparison of sensitivity across cells difficult. This is especially true considering that the S pigment seems to be inactivated differently from the M pigment. In addition, mouse cones lack the so-called cone pigment kinase GRK7, which is present in the cones of many other species, including human (see "R* termination").

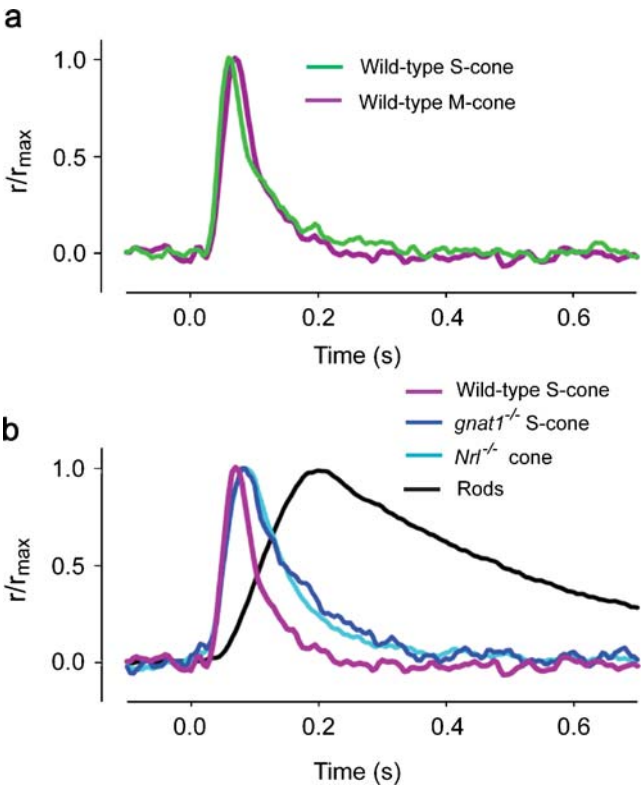


**Fig. 3** Flash responses of mouse cone photoreceptors from different genotypes. **a** Comparison of the average response of S-cones to 361-nm flashes and M-cones to 510-nm flashes. **b** Comparison of the average flash responses to 361-nm flashes of wild-type S-cones, $gnat1^{-/-}$ S-cones, $Nrl^{-/-}$ cones, and rods recorded under the same "OS out" conditions. Each trace is scaled to unity at its peak. Data from Fig. 4e,f of [67]. Reproduced from **The Journal of General Physiology, 2006, 127: 359–374.** Copyright 2006 The Rockefeller University Press

**Table 3** Kinetic and sensitivity parameters of mouse rods and cones

| | $I_d$ (pA) | $t_p$ (ms) | $\tau_D$ (ms) | $I_{1/2}$ (photons $\mu m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| S-cone ($n$=21) | 6±1 | 73±5 | 73±10 | (1.8±0.6)×$10^5$ |
| M-cone ($n$=8) | 8±2 | 63±5 | 68±18 | (2.5±0.9)×$10^5$ |
| Rods ($n$=26) | 20±6 | 205±10 | 235±20 | 350 |

Data from Table 1 of [67]. Reproduced from The Journal of General Physiology, 2006, 127: 359–374. Copyright 2006 The Rockefeller University Press.

$I_d$ Dark current, $t_p$ the time to peak of the dim-flash response, $\tau_D$ the dominant recovery time constant, $I_{1/2}$ flash strength that elicited a half-maximal response, uncorrected for pigment bleaching for cones.

## Concluding remarks

In the past decade, great progress has been made in using mouse models to elucidate the mechanisms of activation and termination of the rod phototransduction pathway. Although there are still questions to be answered about the rod pathway such as the reproducibility of the single-photon response, the current frontier of phototransduction research lies in cones, which, for human vision, are far more important than rods. The recent success in recording from single mouse cones ushers in a new era in research on vertebrate cone phototransduction. Many long-standing questions, e.g., the mechanisms for the enormous ability of cones to adapt to light and the differences between rods and cones in sensitivity and kinetics, can now be addressed with a combination of mouse genetics and electrophysiology.

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