

Vertebrate Rods and Cones

The two kinds of vertebrate photoreceptors, rods and cones, have an **outer segment** with sensory membrane elaborated from a modified cilium and containing the visual pigment and all of the enzymes and channels required for transduction (Figure 9.11). The area of sensory membrane is greatly increased by numerous invaginations, which in rods detach from the plasma membrane as disks (see Figure 2.4), but in cones remain accessible to the extracellular solution. The repeat distance between disks or membrane invaginations is about 30 nm and is rather uniform from species to species. The number of disks or invaginations is therefore mostly a function of the length of the outer segment; typical values are 1100 rod disks and 750 cone invagi-

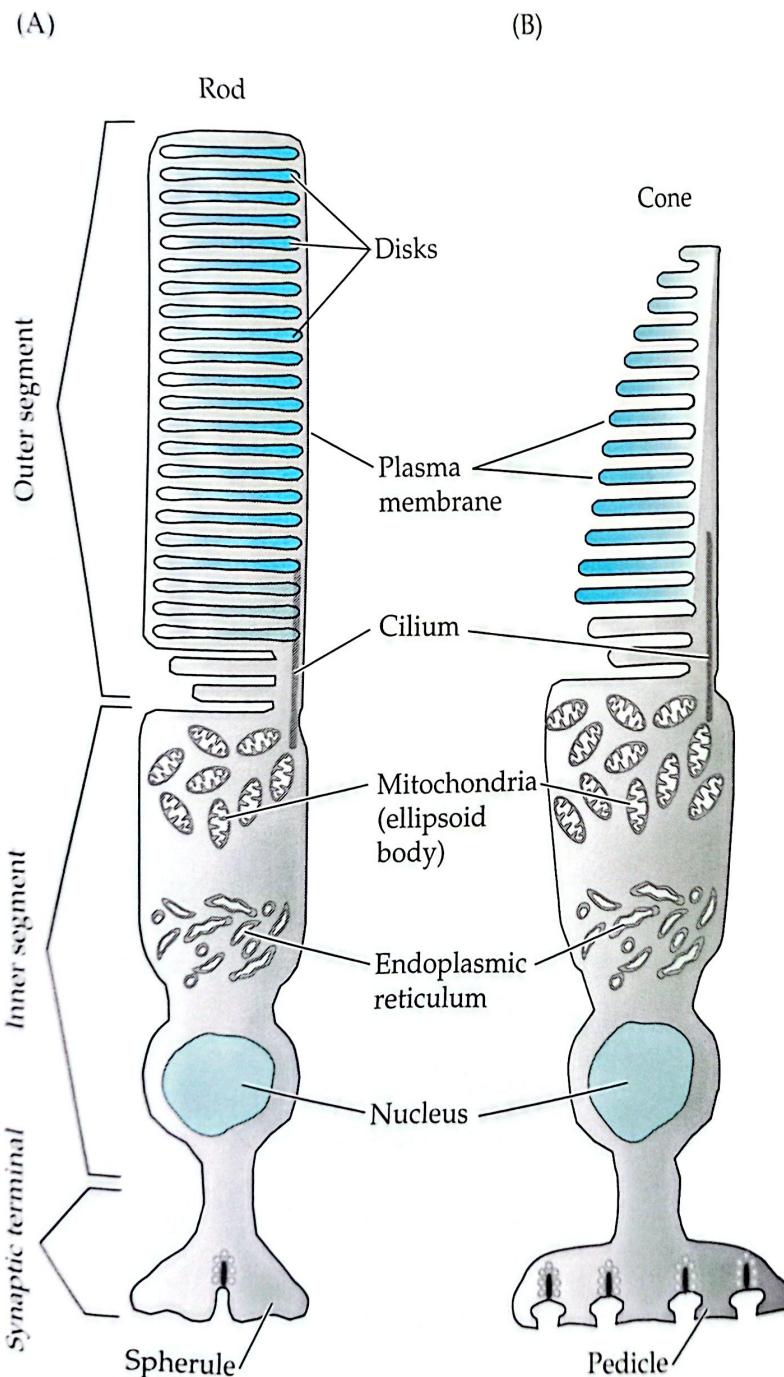


Figure 9.11
Vertebrate rods and cones

Principal structural features of vertebrate photoreceptors. (A) Rod. Note the outer segment composed of disks. (B) Cone. The outer segment has membrane infoldings instead of disks. (After Fain, 1999.)

nations for the amphibian *Necturus* (Brown et al., 1963) and 1200 invaginations for a monkey cone (Dowling, 1965).

The disks of rods, though independent of one another and separated from the plasma membrane by 10–20 nm, are nevertheless interconnected by fine filamentous material (Figure 9.12). The very edge of the disk forms a specialized structure, called a **rim**, that is known to contain protein not found in the rest of the disk. The molecules **peripherin** and **rom-1** are localized to the rim and seem to play an important role in the formation of the disks (see Molday, 1998). This part of the disk also contains the **ABCR/Rim protein**, a transporter that moves retinal across the disk membrane (see McBee et al., 2001). These are probably not the only pumps or transport molecules in the disks, since there is evidence that the pH inside the disk is not the same as that in the cytosol (Chen et al., 2002), suggesting that H^+ might also be transported.

The metabolic part of the cell, called the **inner segment**, is also highly organized. In the region just adjacent to the outer segment, there is a high concentration of mitochondria forming a condensed region called the **ellipsoid body**, which is visible in the light microscope. The plasma membrane just adjacent to the mitochondria contains a high concentration of Na^+/K^+ ATPase (Stirling and Lee, 1980). The endoplasmic reticulum and nucleus lie below the ellipsoid body, and at the proximal end of the cell there is a presynaptic terminal called a **spherule** for rods and a **pedicle** for cones.

Photoreceptors are secondary receptor cells; they lack voltage-gated Na^+ channels and do not generate Na^+ -dependent action potentials. The change in membrane potential produced by light is communicated at specialized synapses onto second-order horizontal and bipolar cells. As for insect photoreceptors (see Figure 2.12A) the presynaptic terminals contain dense bodies that in rods and cones are called **synaptic ribbons** and resemble the pres-

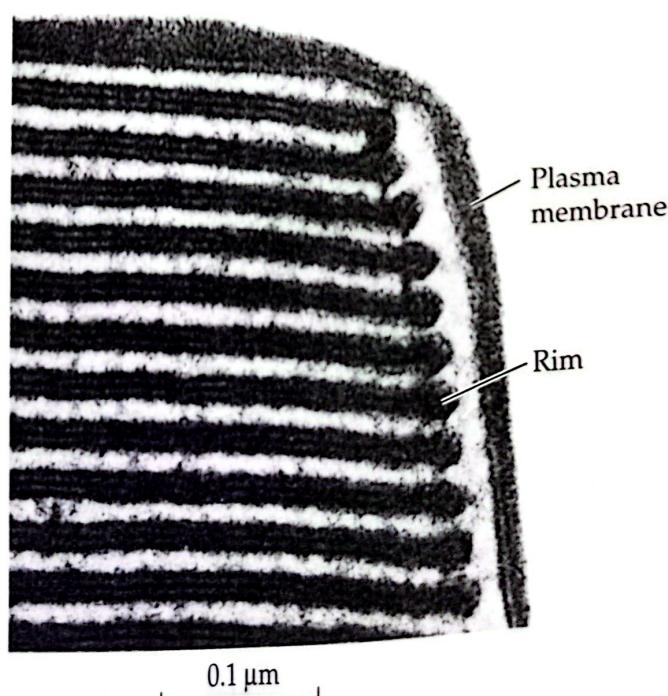


Figure 9.12
Low-power electron micrograph of a rod outer segment showing the disk rim
Note fibrous protein between adjacent disks and between rim and plasma membrane. (From Fain and Schröder, 1985.)

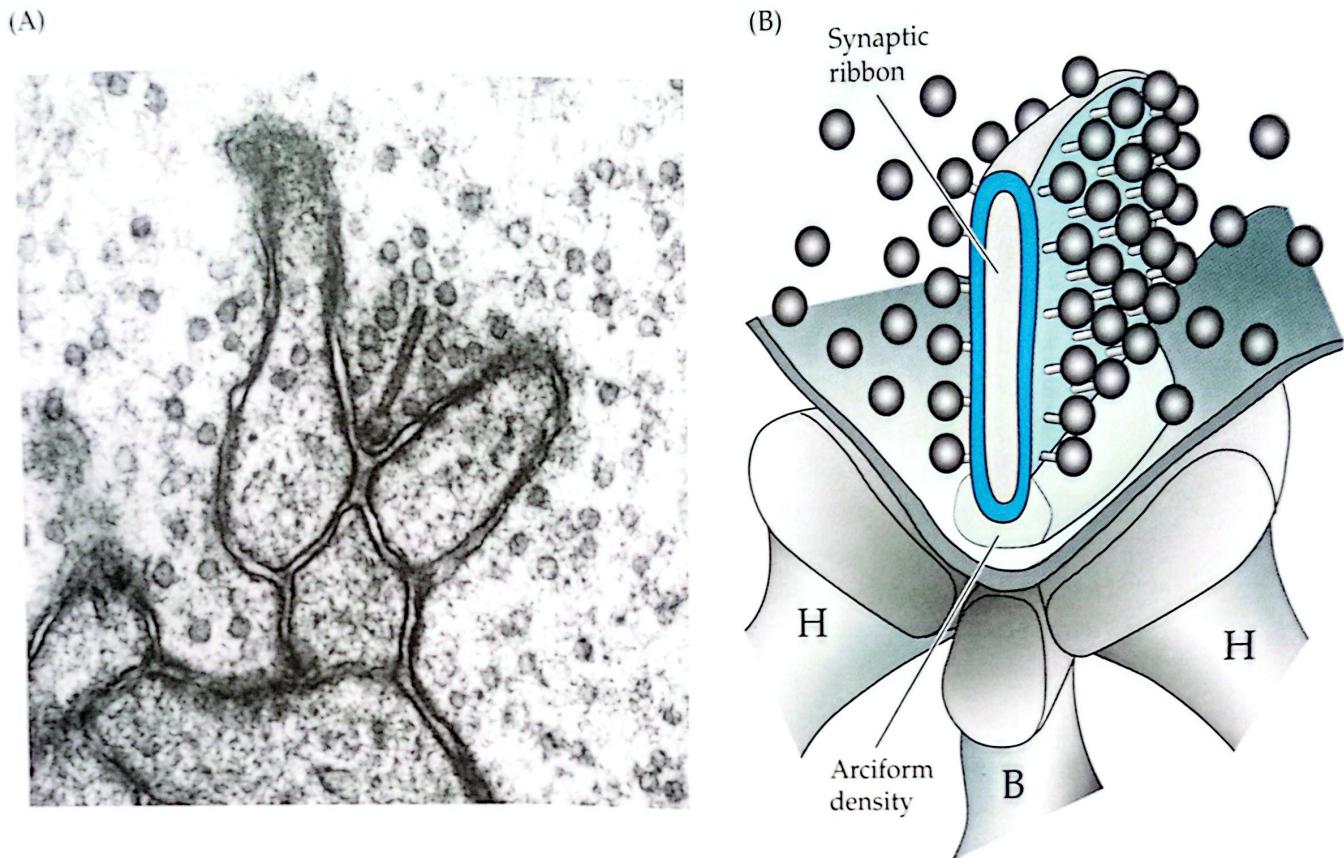


Figure 9.13

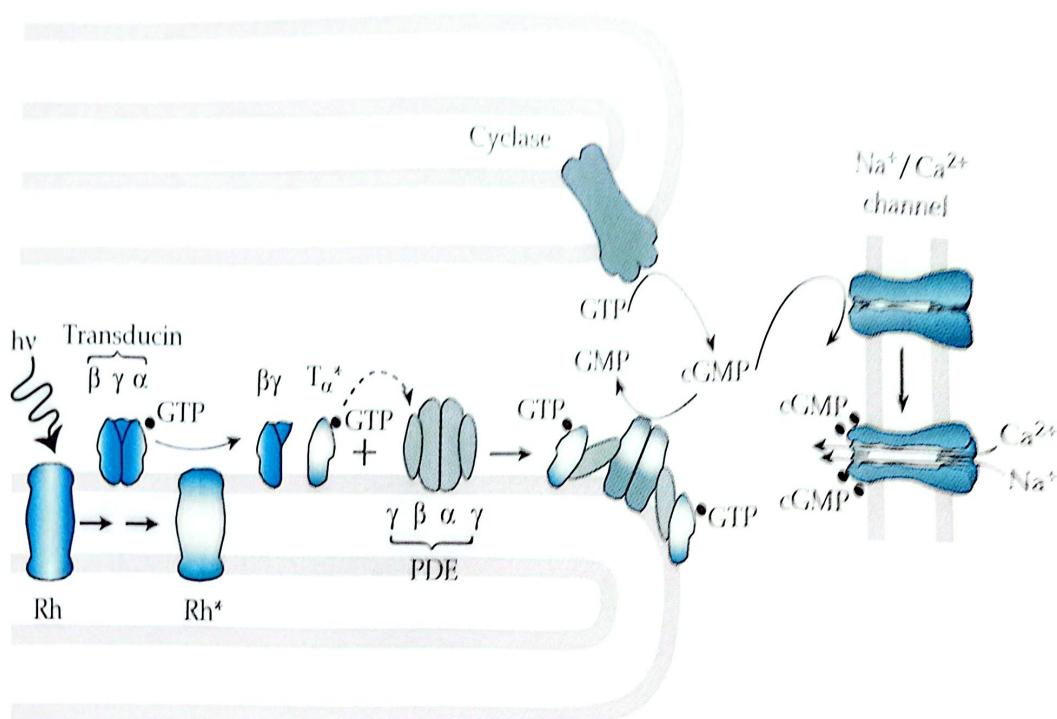
Photoreceptor synapse (A) Electron micrograph of a synapse of a primate cone. Magnification 70,000 \times . (B) Schematic drawing of a photoreceptor synapse. Abbreviations: H, horizontal cell process; B, bipolar cell process. (A, courtesy of S. J. Schein.)

naptic structures of electroreceptors (see Figure 2.12B) and hair cells (see Figure 6.2). In cross section the ribbons appear as dense rods surrounded by a halo of synaptic vesicles, which in some sections appear to be connected to the ribbons by fine filamentous material (Figure 9.13A). When the ribbons are followed through many serial sections, their shape can be reconstructed (Figure 9.13B), and they can be seen to occupy much of the synaptic ending and bind hundreds of synaptic vesicles (Rao-Miroznik et al., 1995). The ribbons contain a variety of molecules, including motor proteins (Morgans, 2000), and may actually convey the vesicles to their release sites just adjacent to another specialized structure, the **arciform density**. The synaptic transmitter is almost certainly glutamate (see, for example, Copenhagen and Jahr, 1989).

Transduction in Vertebrate Photoreceptors

The mechanism of activation in a vertebrate photoreceptor is now fairly clear (Figure 9.14A; see also Hoffmann, 2000; Ebrey and Koutalos, 2001; Arshavsky et al., 2002). The formation of Rh^* produces a change in the confor-

(A)



(B)

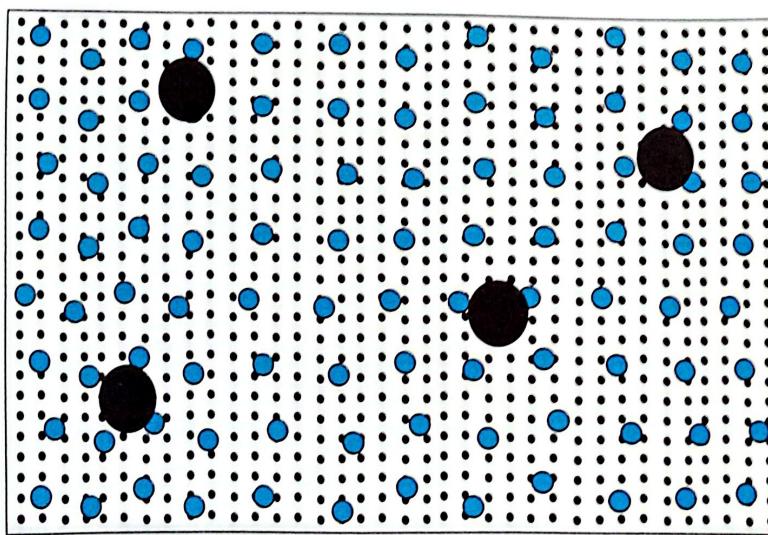


Figure 9.14
Vertebrate phototransduction (A) Transduction cascade. (B) Relative proportions of transduction proteins. The small black dots are rhodopsin molecules, the blue dots are transducin, and the large black dots are PDE molecules. Distribution of molecules would be much less regular in actual disk membrane. Disk membrane of this dimension would have adjacent to it an average of one free cGMP molecule. (A after Pugh and Lamb, 1993; Fain, 1999; B after Bownds and Arshavsky, 1995.)

mation of the parts of the rhodopsin molecule that are exposed to the cytoplasm, particularly the carboxyl tail and the loops connecting helices III/IV and V/VI. This opens a binding site for a heterotrimeric G protein called

transducin. Transducin binding triggers a change in the conformation of the guanosine nucleotide-binding site on the α -subunit (see Hamm, 1998), GDP falls off this binding site, and GTP then binds. Activated T_α -GTP separates from $\beta\gamma$ and might actually come off the disk membrane, diffusing between the disks within the cytoplasm (Kuhn et al., 1981).

The T_α -GTP associates with effector enzyme, which for vertebrate photoreceptors is a cyclic nucleotide PDE and not a PLC β . PDE is a tetramer with α and β catalytic subunits and two inhibitory γ subunits (see Figure 4.6B). In the inactive enzyme, the γ subunits prevent the α and β from hydrolyzing cGMP, probably by preventing access to the catalytic binding sites. The conformation of the γ subunits changes when T_α -GTP is bound, exposing the catalytic binding sites and greatly increasing the activity of the enzyme. Each catalytic subunit also contains a high-affinity noncatalytic binding site for cGMP, and binding of cGMP to these sites can affect the nature of the interaction of γ with α and β (D'Amours and Cote, 1999). The affinity of cGMP for these noncatalytic sites is so great, however, that cGMP comes on and off too slowly to make much of a contribution to the photoreceptor light response (Calvert et al., 1998).

For every 1000 rhodopsin molecules in the disk membrane, there are about 100 transducins and four PDE molecules (Figure 9.14B). As we saw in Chapter 2, rhodopsin can diffuse freely within the surface of the disk, and this is likely to be true also for the PDE and inactive transducin heterotrimer. A single Rh^* during its 1- to 2-second lifetime can collide randomly with many transducin molecules and produce many hundreds of molecules of T_α -GTP. Each T_α -GTP can bind to and activate only a single PDE catalytic subunit, but activated PDE can hydrolyze several thousand cGMP molecules before its activity is terminated. The overall gain of transduction for a rod is therefore of the order of 10^6 cGMP molecules hydrolyzed per Rh^* .

The sensory cascade of vertebrate photoreceptors relies upon random collisions of membrane proteins instead of a highly organized transducosome/signalplex as in arthropod photoreceptors. Nevertheless, transduction in rods and cones is surprisingly fast and efficient. Single quantum voltage responses in rods are of the order of 1 mV (Fain, 1975; Schneeweis and Schnapf, 1995)—smaller than those for arthropod receptors but sufficiently large to produce a reliable detection. The minimum latency of the photoreceptor for a bright light flash is only 7 milliseconds for both rods and cones (Cobbs and Pugh, 1987; Hestrin and Korenbrot, 1990), not much different from that for *Drosophila* photoreceptors (see Figure 9.10B). The rate of transduction can be made even faster if the rhodopsin concentration of the disk membrane is decreased to increase the frequency of encounter of Rh^* with transducin (Calvert et al., 2001). The packing density of rhodopsin in the disk is not optimized for speed—the responses are apparently fast enough without this—but is determined instead by the concentration necessary to maximize the probability of light capture by the photopigment.

Ion Channels of Rods and Cones

Figure 9.15 gives the principal types of ion channels and transporters in a vertebrate photoreceptor (see Molday and Kaupp, 2000). The cyclic nucleotide-gated channels are mostly in the plasma membrane of the outer segment (see Kaupp and Seifert, 2002). In both rods and cones the channels are heteromers, with α subunits (CNGA1 for rods and CNGA3 for cones; see

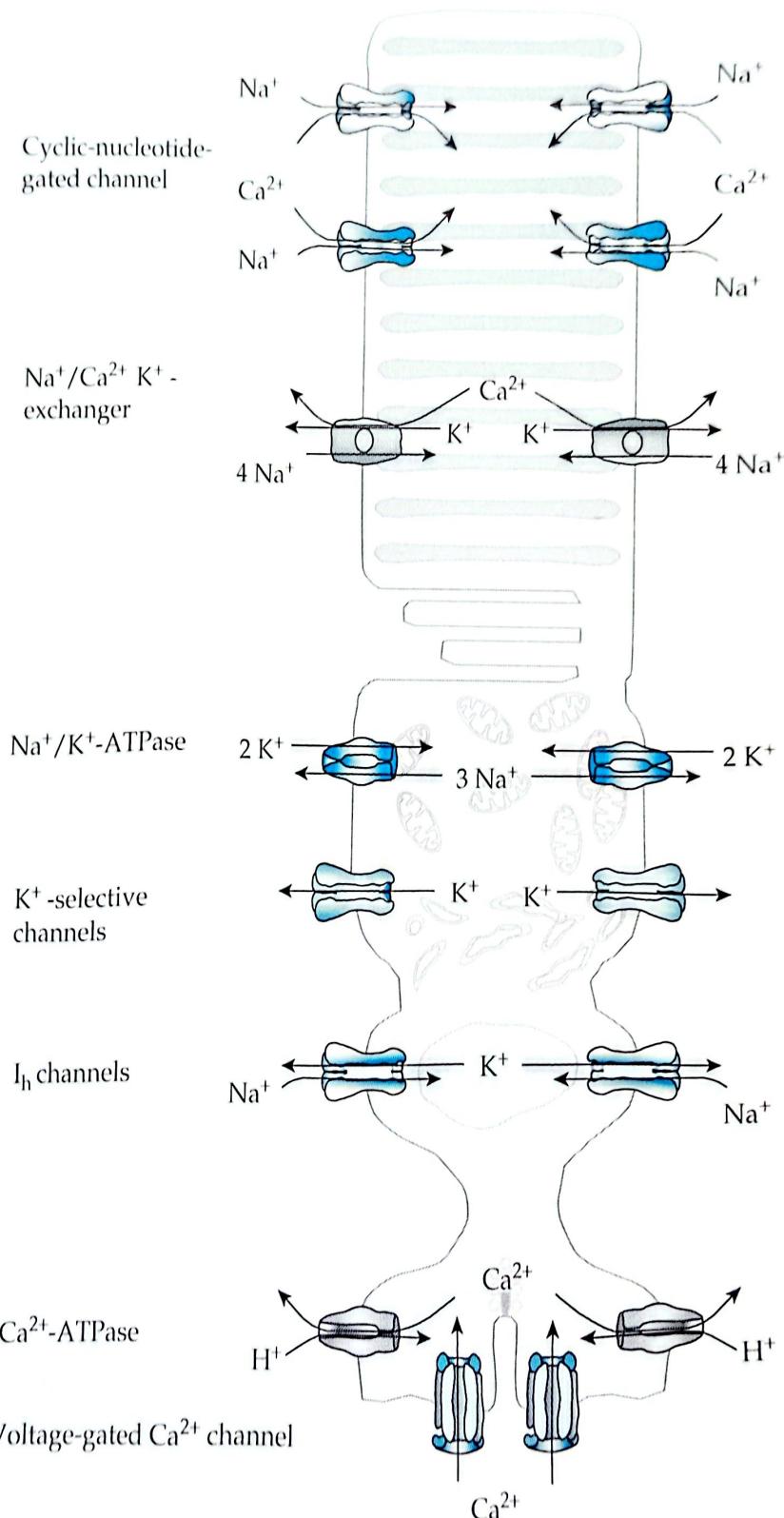


Figure 9.15
Channels and transporters in membrane of vertebrate rod Major proteins responsible for ion conduction and transport are different in the inner and outer segment.
 (After Fain, 1999.)

Bradley et al., 2001) having the structure illustrated in Figure 4.11B, and β subunits (CNGB1 for rods and CNGB3 for cones) with a different, though related amino acid sequence. All of these channel subunits have six transmembrane domains, a P region forming the channel pore, and a cytoplasmic carboxyl terminus with a binding site for cyclic nucleotide. In rods, but apparently not in cones (Gerstner et al., 2000), the β subunit also has a large cytoplasmic amino terminal region, called the **glutamic-acid-rich part**, or **GARP**, that may play some role in protein–protein interactions in the outer segment (Korschen et al., 1999). The stoichiometry of the rod channel is $\alpha_3\beta$ (Weitz et al., 2002; Zheng et al., 2002; Zhong et al., 2002). Channels in both rods and cones are quite selective for cGMP over cAMP, unlike the channels in olfactory receptors, which bind both nucleotides nearly equally; but the olfactory channels bind both cAMP and cGMP with considerably greater affinity than rod channels bind either.

The channels in rods and cones, like those in lizard parietal eye (see Chapter 4), are rather nonspecifically permeable to monovalent cations and actually more permeable to Ca^{2+} than to Na^+ . Since the Na^+ concentration is much higher than the Ca^{2+} concentration in the extracellular medium, only of the order of 10–15% of the current entering the cyclic nucleotide-gated channels in a rod is carried by Ca^{2+} . Cones have channels that are different from those in rods, and nearly twice as much of the current, or about 20–30%, is carried by Ca^{2+} (Perry and McNaughton, 1991; Ohyama et al., 2000). As we shall see, this Ca^{2+} influx plays an important role in the physiology of the light response.

The entering Ca^{2+} is removed by an active transport molecule that uses the energy of both the Na^+ and K^+ gradients to move Ca^{2+} out of the cell (Cervetto et al., 1989; Lagnado and McNaughton, 1990): Four Na^+ ions move inward and one K^+ moves outward for every Ca^{2+} ion that is extruded. This means that four charges are moved inward and three charges are moved outward for each cycle of the transporter, so that like the Na^+/K^+ ATPase, the $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$ exchanger is electrogenic. The inward current carried by the transporter can actually be recorded (see Figure 9.20B), and this provided one of the first important clues for the role of Ca^{2+} in the physiology of the photoreceptor (Yau and Nakatani, 1984; Hodgkin et al., 1987). Remarkably, the exchange molecules are tightly bound to the channel with a fixed stoichiometry of two exchangers per channel (Schwarzer et al., 2000).

The inner segments of both rods and cones have a high concentration of Na^+/K^+ ATPase and an assortment of channels that are not directly activated by light (see Molday and Kaupp, 2000). There are K^+ channels that provide the principal K^+ permeability of the cell and make an important contribution to the resting membrane potential (Beech and Barnes, 1989), as well as channels, called I_h , that are permeable to both Na^+ and K^+ and activated by hyperpolarization. Finally, at the synaptic terminal there are voltage-gated Ca^{2+} channels near the release sites that regulate the exocytosis of the vesicles. It is of some interest that the Ca^{2+} entering the rod or cone at the

synaptic terminal is removed, not by a Na^+/K^+ - Ca^{2+} transporter, but rather by a Ca^{2+} ATPase (Krizaj and Copenhagen, 1998; Morgans et al., 1998), and the concentration of Ca^{2+} is apparently regulated rather independently in the two halves of the cell (Krizaj and Copenhagen, 1998).

The Photocurrent

In darkness, both Na^+ and Ca^{2+} enter the rod through the cyclic nucleotide-gated channels of the outer segment, producing a current called the **dark current** (Penn and Hagins, 1969). The large resting conductance of the cell to Na^+ produced by the cyclic nucleotide-gated channels has the consequence that the resting membrane potential is of the order of -35 mV , more depolarized than for most neurons and intermediate between the equilibrium potential for K^+ (E_K , -80 to -90 mV) and the reversal potential for the cyclic nucleotide-gated channels (near zero; see Chapter 4). Light decreases the probability of opening of the cyclic nucleotide-gated channels and reduces the influx of Na^+ (and Ca^{2+}) into the outer segment. This causes the membrane potential of the rod to hyperpolarize.

Figure 9.16A shows the change in membrane potential produced by a series of brief light flashes of increasing intensity, recorded from a salamander rod with an intracellular microelectrode. As the cyclic nucleotide-gated channels close in the light, the membrane potential moves in a negative direction closer to E_K . The brighter the light, the larger the hyperpolarization. In very bright light there is an additional rapid relaxation in the voltage (arrow in Figure 9.16A). The reason for this is that hyperpolarization activates the I_h channels (Figure 9.15), and since these channels are only a little less permeable to Na^+ than to K^+ , the I_h current has a reversal potential near the dark resting membrane potential of the photoreceptor. As the I_h channels are activated by hyperpolarization during the light response, they cause the membrane potential to depolarize rapidly back toward the resting potential, producing the “nose” at the beginning of the response. The I_h conductance is specifically blocked by low concentrations of extracellular Cs^+ , and Cs^+ eliminates the rapid relaxation of the voltage response (Fain et al., 1978; Hestrin, 1987).

The current through the cyclic nucleotide-gated channels can be measured by pulling the outer segment into a suction electrode connected directly to a current-measuring amplifier. This method measures the current entering the outer segment, which is equal to the current passing through the cyclic nucleotide-gated channels, since these channels are mostly localized to the outer segment, and since they seem to be the only functioning channels in this part of the cell. Suction-electrode recordings of light responses are shown in Figure 9.16B from the same cell for which the voltage responses are given Figure 9.16A. They show that a sustained current flowing into the outer segment in darkness is decreased when the rod is illuminated with a brief flash. Bright light closes all of the cyclic nucleotide-gated channels and reduces the current entering the outer segment to zero (Baylor et al., 1979).

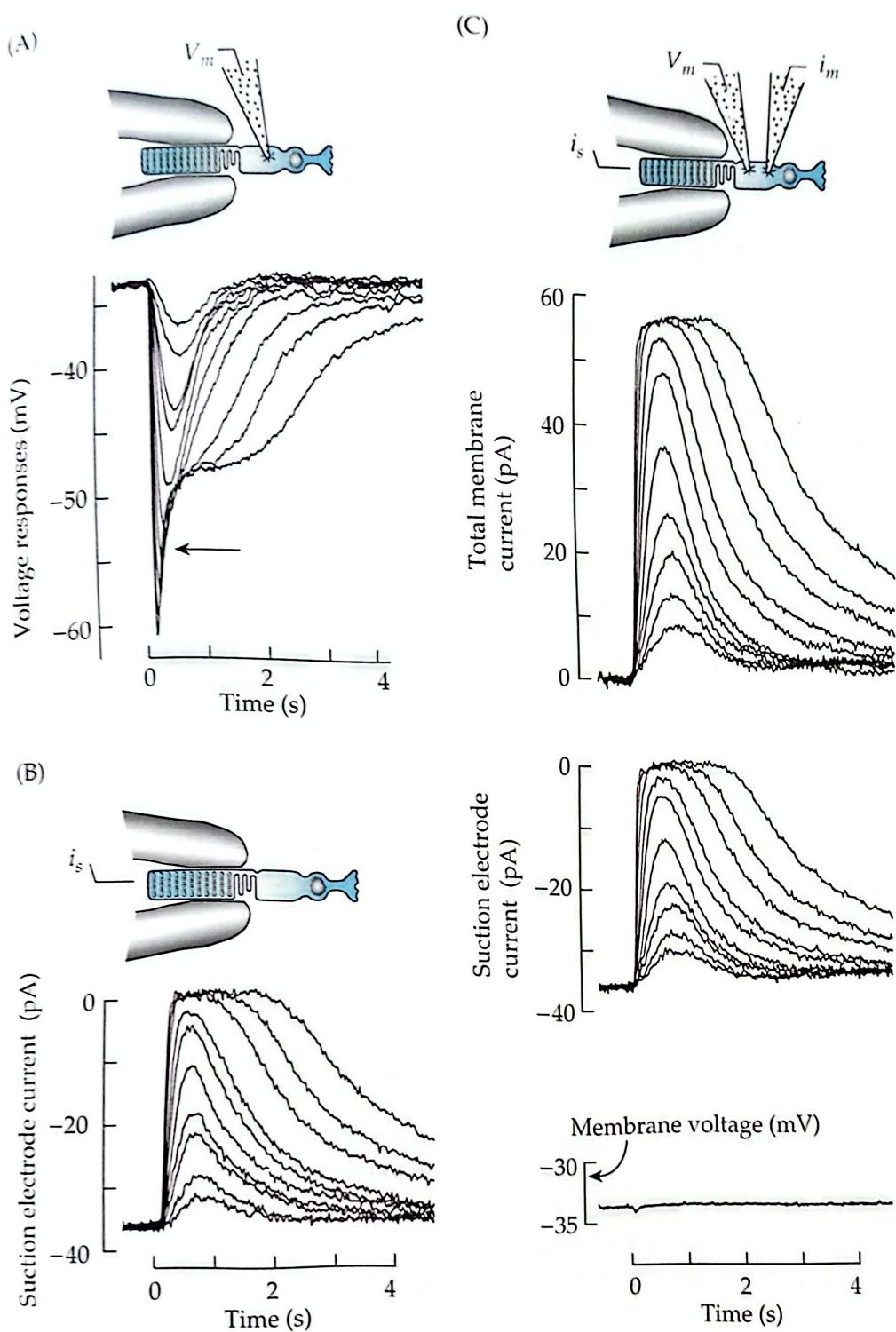


Figure 9.16
Electrical recording from vertebrate rod The responses of salamander rod to a series of brief flashes of increasing intensity from (A) intracellular recording, (B) suction-electrode recording, and (C) voltage clamp. (After Fain, 1999, with data reprinted from Baylor and Nunn, 1986.)

One disadvantage of suction-electrode recording is that it does not voltage clamp the cell. Since as stated in Equation 3.8,

$$\Delta i_m = \Delta g(V_m - E_{rev})$$

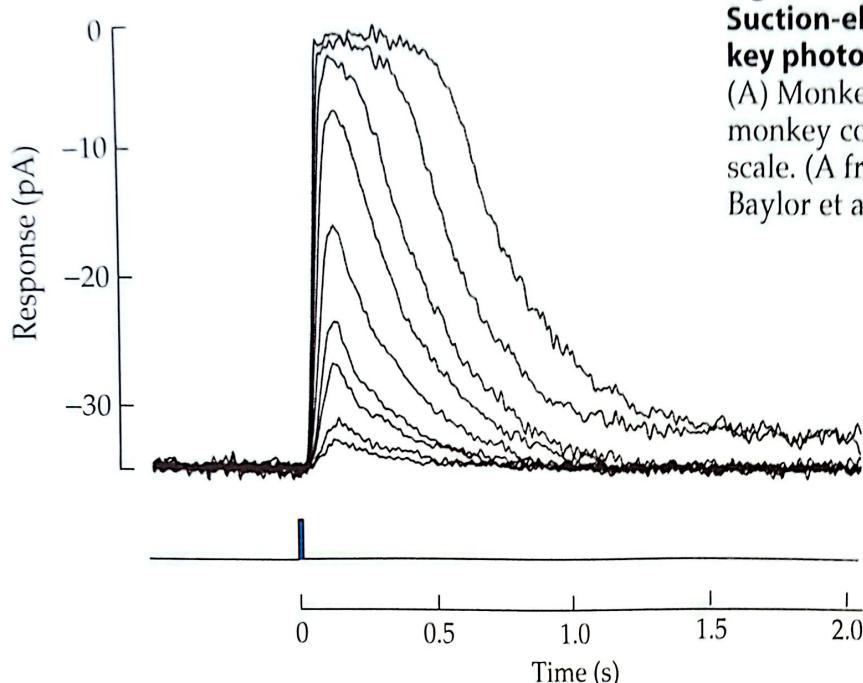
and since for the cyclic nucleotide-gated channels E_{rev} is near zero, $\Delta i_m \sim \Delta g(V_m)$, and changes in membrane voltage like those shown in Figure 9.16A might influence the wave form of the currents. This possibility was examined by Baylor and Nunn (1986), who recorded outer segment currents with a suction electrode from rods that were simultaneously voltage clamped (Figure 9.16C). The upper traces show the total membrane currents measured with voltage clamp. The current is initially zero, since in darkness the cell is at steady state and the current entering the outer segment through the cyclic nucleotide-gated channels is exactly balanced by current leaving the inner segment, mostly through K^+ channels. Since the inner segment currents are unaffected by illumination, the time course of the voltage-clamp current in the upper traces reflects the time course of the decrease of the outer segment conductance.

The lower traces show suction-electrode recordings made simultaneously from this same rod under voltage clamp. The amplitude of the suction-electrode current is smaller than that for the voltage-clamp current, in part because current is lost through the seal between the suction pipette and the cell and in part because not all of the outer segment was brought into the pipette. What is remarkable, however, is that the relative amplitude and time course of the suction-electrode currents in Figure 9.16C are quite similar to those for the currents recorded in Figure 9.16B, for which no voltage clamp was used. The reason for this is that the change in conductance (Δg in Equation 3.8) is voltage dependent. The voltage dependence isn't very large but is enough to compensate for the change in the driving force ($V_m - E_{rev}$) as the voltage changes during the light response. This has the effect that the photoreceptor current $\Delta g(V_m - E_{rev})$ shows very little dependence on voltage, with the happy consequence that suction-electrode recording, which is much easier than voltage clamping, gives a faithful representation of the change in outer segment conductance.

Suction-electrode recordings have been made from the receptors of many species, even from the very small rods and cones of mammals. Figure 9.17 gives responses of a monkey rod and cone, each to a series of brief flashes of increasing intensity. Note the difference in time scale: Responses of cones reach peak amplitude and decay much more quickly than those of rods. Cones in primates, and to some extent also in other species, show a prominent after effect, so for a brief period the probability of opening of the cyclic nucleotide-gated channels is actually greater just after the light response than in darkness. Although the minimum latency is nearly the same for rods and cones, rods are typically 10–100 times more sensitive to light.

We still do not know why rod and cone responses show these differences in sensitivity and time course. There are many possibilities. The two photoreceptor types have a different anatomy (Figure 9.11) and contain molecularly different species of most of the proteins involved in sensory transduction (see

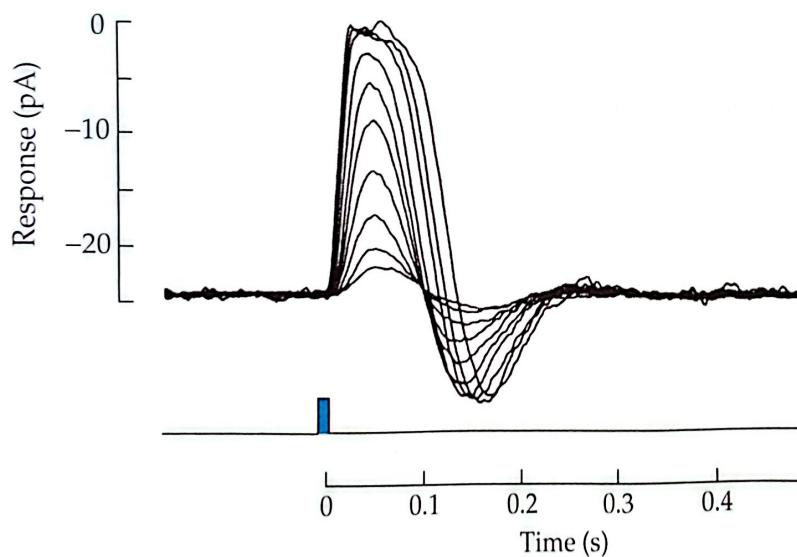
(A) Rod

**Figure 9.17**

Suction-electrode recordings from monkey photoreceptors (*Macaca fascicularis*)

(A) Monkey rod. (B) Long-wavelength monkey cone. Note the difference in time scale. (A from Baylor et al., 1984; B from Baylor et al., 1987.)

(B) Cone



Ebrey and Koutalos, 2001), including the photopigment, transducin, PDE, cyclic nucleotide-gated channel, and Na^+/K^+ - Ca^{2+} transporter (Szerencsei et al., 2001). The photopigment and PDE appear to have greater intrinsic noise in cones, with the consequence that cones seem to behave as if they were illuminated by a steady adapting light when none is actually present (Rieke and Baylor, 2000). We shall see shortly that adapting light can have a dramatic effect on the wave form and sensitivity of the light response (see Figure 9.21D). Cone channels have a larger component of inward Ca^{2+} current in darkness and can extrude Ca^{2+} more quickly via Na^+/K^+ - Ca^{2+} exchange (Sampath et al., 1999). These differences in Ca^{2+} homeostasis may play an important role in re-

sponse decay (see Miller et al., 1994). Finally, there is evidence that the gain of transducin activation and the time course of rhodopsin phosphorylation may differ in rods and cones, and this could also be responsible at least in part for the differences in response sensitivity and wave form (Tachibanaki et al., 2001).

Shutting Down the Light Response

Activation of transduction must be followed by turn-off. The faster turn-off can occur, the more rapidly the cell can detect another stimulus, and the more accurately the visual system can distinguish temporal changes in illumination. All the steps in transduction must be returned to their initial condition: Rh^* must be inactivated; T_α must come off the inhibitory subunits of the PDE and recombine with $T_{\beta\gamma}$; the cyclic nucleotide concentration must be restored to its dark level; and the channels must reopen. The steps in turn-off are highly orchestrated and can be modulated, particularly by changes in Ca^{2+} concentration.

The inactivation of Rh^* occurs as for other G protein-coupled receptors (see Figure 4.4). **Rhodopsin kinase** phosphorylates serine and threonine residues on the carboxyl terminus of rhodopsin, and an arrestin protein then binds to phosphorylated rhodopsin, sterically inhibiting the binding of transducin. Receptor phosphorylation (Bownds et al., 1972; Kuhn and Dreyer, 1972) and arrestin binding (Kuhn, 1978) were first discovered in vertebrate photoreceptors and have been extensively characterized. The carboxyl terminus of rhodopsin in different species contains either six or seven serine and threonine groups that can all be phosphorylated (Wilden and Kuhn, 1982). Turn-off seems to proceed by multiple phosphorylation (Mendez et al., 2000) beginning at the most C terminal serine, which is the favored site for the kinase (McDowell et al., 1993; Ohguro et al., 1993; Papac et al., 1993), and then proceeding inward (Kennedy et al., 2001). If rhodopsin phosphorylation is prevented, either by genetically altering rhodopsin to remove its carboxyl tail (Chen et al., 1995; Mendez et al., 2000), or by disrupting the gene for rhodopsin kinase (Cideciyan et al., 1998; Chen et al., 1999a), photoresponses turn off abnormally and are greatly prolonged (as shown for the trace labeled “Rh truncation” in Figure 9.18A).

Rods have two molecular forms of arrestin, one much more abundant than the other but coded by splice variants of the same gene (Palczewski et al., 1994). Both bind preferentially to phosphorylated rhodopsin and inhibit the binding of G protein, effectively terminating the lifetime of Rh^* . In mice in which the arrestin gene has been disrupted, photoresponses again turn off abnormally and become prolonged (Xu et al., 1997), but the effect is not as great as that produced by removing the C terminus of rhodopsin and preventing phosphorylation (Figure 9.18A). The reason for this seems to be that phosphorylation even without arrestin can produce some inhibition of transducin binding (Wilden et al., 1986). The phosphorylation of rhodopsin and binding of arrestin happen rather quickly, with a time constant in a dark-

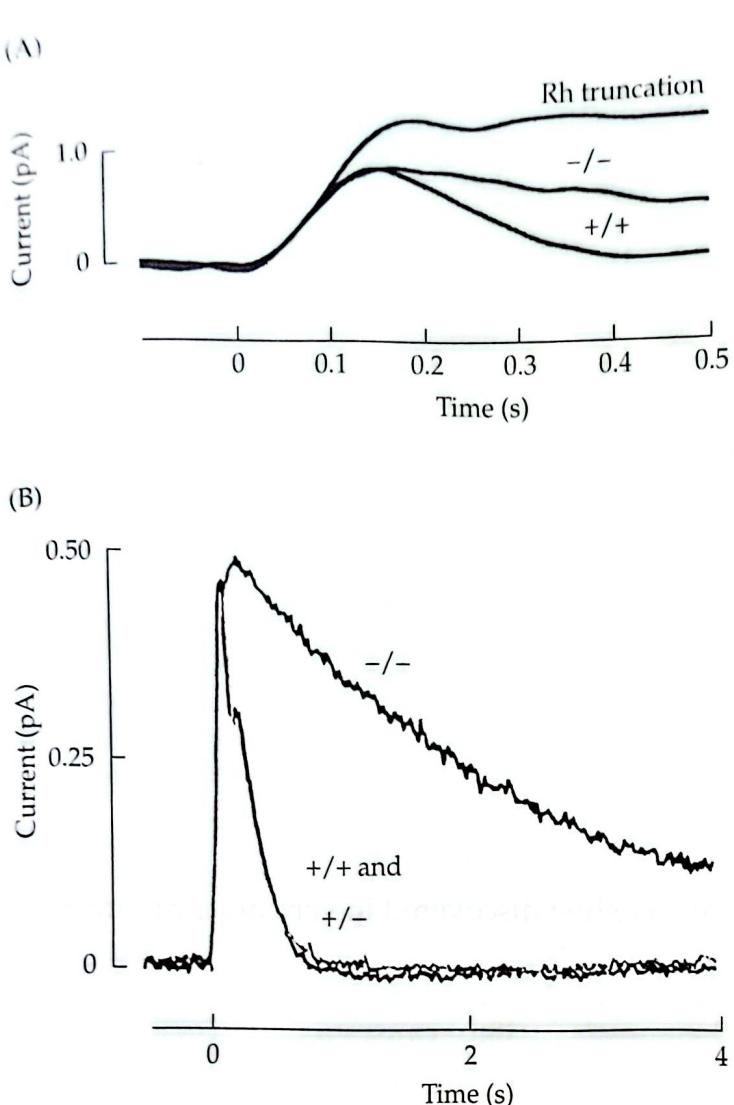


Figure 9.18
Mechanism of turn-off for photoreceptor transduction (A) Average suction-electrode response of rod to a single photon in a wild-type mouse (+/+), in a mouse in which the gene for arrestin has been disrupted (-/-), and in a mouse in which the gene for rhodopsin had been altered so that the C terminus of the molecule had been truncated, removing sites for protein phosphorylation (*Rh truncation*). (B) Same as for part (A) but from a mouse lacking photoreceptor RGS9 protein (-/-), and from wild-type (+/+) and a mouse heterozygous for the *rgs9* gene (+/-). (A from Xu et al., 1997; B from Chen et al., 2000.)

adapted amphibian rod of the order of 0.5 second, and there is now general agreement that rhodopsin turn-off is not rate-limiting for the turn-off of the cascade, at least for rods (Matthews, 1997; Sagoo and Lagnado, 1997; Tsang et al., 1998; Chen et al., 2000).

As for other heterotrimeric G proteins (see Figure 4.5), the inactivation of T_α -GTP and restoration of the $T_{\alpha\beta\gamma}$ complex require the hydrolysis of GTP to GDP on the T_α guanosine nucleotide-binding site (see Arshavsky et al., 2002). Although transducin by itself can hydrolyze GTP, the rate is rather slow. It is greatly accelerated by GTPase-activating proteins (GAPs), and in particular by the protein **RGS-9** (He et al., 1998), which is abundant in rods and found at an even higher concentration in cones (Cowan et al., 1998). RGS-9 is present in the outer segment in a tight complex with a G protein β subunit, called **G β 5L** (Makino et al., 1999; He et al., 2000), which is not the same as the β subunit of transducin. The rate of turn-off is further accelerated by the PDE γ subunit (Arshavsky and Bownds, 1992; Tsang et al., 1998), though the PDE γ seems to have little effect on its own and requires the RGS-

9 protein (Chen et al., 2000). In mice in which the gene for the RGS-9 protein has been disrupted (Chen et al., 2000), the recovery of the light response is again greatly retarded (Figure 9.18B).

To return the cyclic nucleotide concentration to its level in darkness, both rods and cones use membrane-bound guanylyl cyclases, of which two different forms are expressed in photoreceptors (Yang et al., 1995). Both have the structure of the membrane-bound guanylyl cyclases used as receptors for hormones (see Figure 4.2) or in olfactory cells (see Chapter 7). They seem to occur in the outer segment as homodimers and, in rods, are integrated into the disk membrane (Yang and Garbers, 1997). Like other membrane-bound guanylyl cyclases (Wedel and Garbers, 1997), these proteins have an “extra-cellular” ligand-binding domain, but this domain for the rod protein has no known ligand and is located inside the disk (Figure 9.19A). This part of the protein is connected by a single membrane-spanning domain to the cytosolic catalytic part of the protein where cGMP is synthesized.

The activity of the guanylyl cyclase is tightly regulated by cytoplasmic Ca^{2+} concentration (Koch and Stryer, 1988). In the dark there is a large influx of Ca^{2+} into the outer segment through the cyclic nucleotide-gated channels, which is balanced by efflux via the $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$ transporter. When the photoreceptor is illuminated, the channels close, and this decreases the entry of Ca^{2+} into the outer segment. The transporter seems not to be directly affected by light (Nakatani and Yau, 1988b; Koutalos et al., 1995) and continues to extrude Ca^{2+} until the decrease in intracellular Ca^{2+} concentration lowers the rate of the transporter enough for the cell to reach steady state. This has the effect that the closing of the channels by light causes a decrease in the Ca^{2+} concentration (Figure 9.20), from a dark level of about 400–600 nM to as low as 5–10 nM when the channels are all closed (see Fain et al., 2001).

The decrease in Ca^{2+} concentration alters the rate of the guanylyl cyclase via small-molecular-weight Ca^{2+} binding proteins, called **guanylyl cyclase-activating proteins**, or **GCAPs** (see Polans et al., 1996; Dizhoor, 2000). There are again two different molecular variants of GCAPs, but both appear to act in the same way. They associate with cytoplasmic binding sites on the guanylyl cyclase molecule near the disk membrane (Figure 9.19A). In the dark, when the Ca^{2+} concentration is high, the GCAPs inhibit the guanylyl cyclase. The decrease in Ca^{2+} concentration produced by the closing of the cyclic nucleotide-gated channels causes the Ca^{2+} to come off the GCAPs, stimulating the cyclase to synthesize cGMP.

This GCAP-dependent stimulation of the cyclase causes an accelerated return of cGMP concentration that reopens the channels and is largely responsible for the declining phase of the light response. If the genes for both GCAPs are disrupted (Mendez et al., 2001), the initial phase of the photocurrent is unaffected, but the channels continue to be closed for a longer time and reopen much more slowly (Figure 9.19B).

Virtually the same effect can be produced by inhibiting the light-dependent change in outer segment Ca^{2+} concentration. This can be done at least for

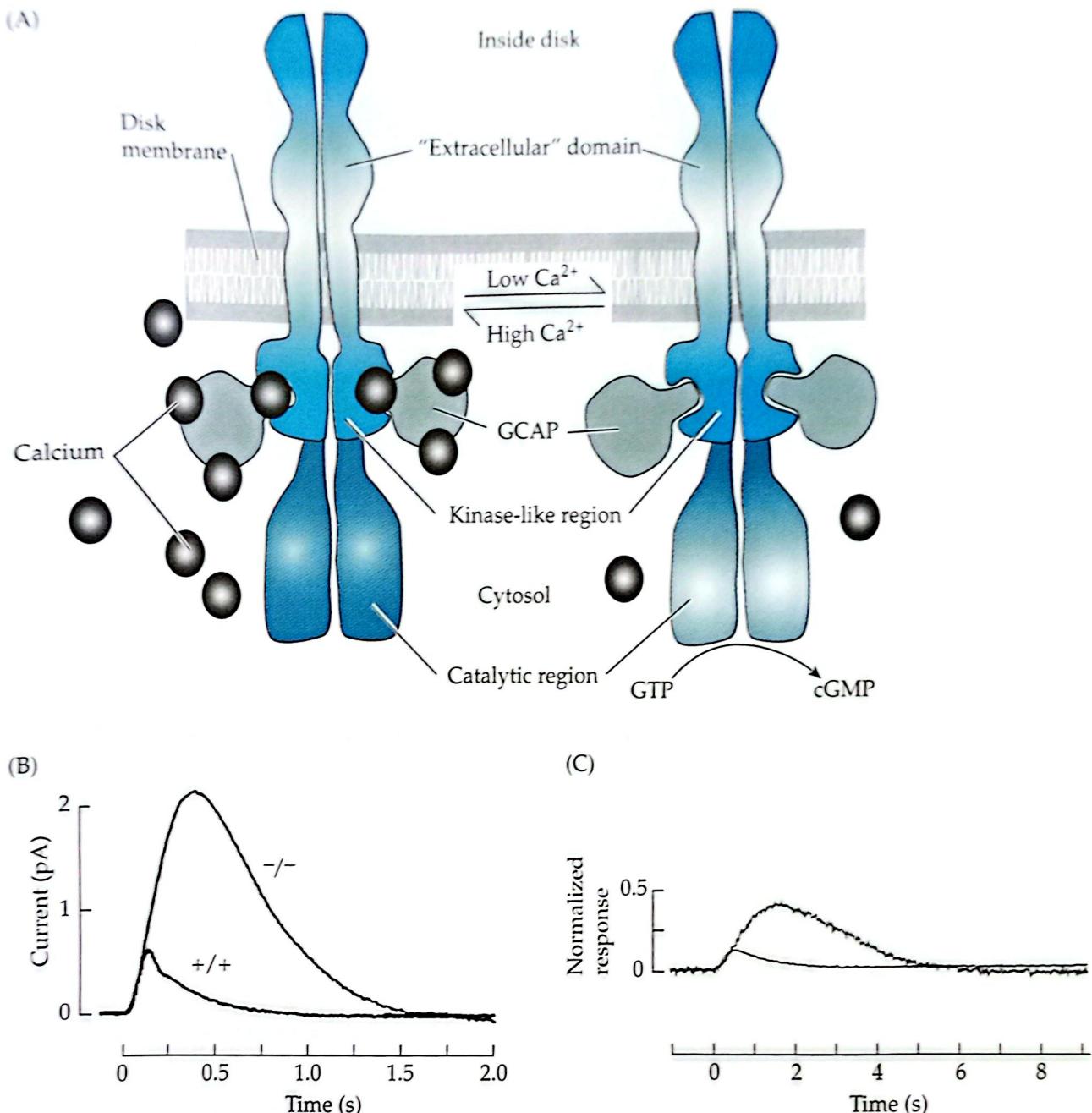


Figure 9.19
Role of Ca^{2+} -dependent regulation of guanylyl cyclase in response decay

(A) Schematic diagram of Ca^{2+} regulation of membrane-bound guanylyl cyclase in rod disk membrane. (B) Average single-photon response from a normal mouse rod (+/+) and from a rod for which both GCAP genes had been disrupted (-/-). (C) Small-amplitude responses normalized to peak response amplitude for a salamander rod in Ringer (smaller response) and in $0 \text{ Ca}^{2+}/0 \text{ Na}^+$ solution (larger response). (A after Polans et al., 1996; B from Mendez et al., 2001; C from Fain et al., 1989.)

a few seconds by rapidly perfusing the outer segments of rods or cones with a medium that lacks both Ca^{2+} and Na^+ (Matthews et al., 1988; Nakatani and Yau, 1988a). The removal of Ca^{2+} stops Ca^{2+} influx, whereas substituting an-

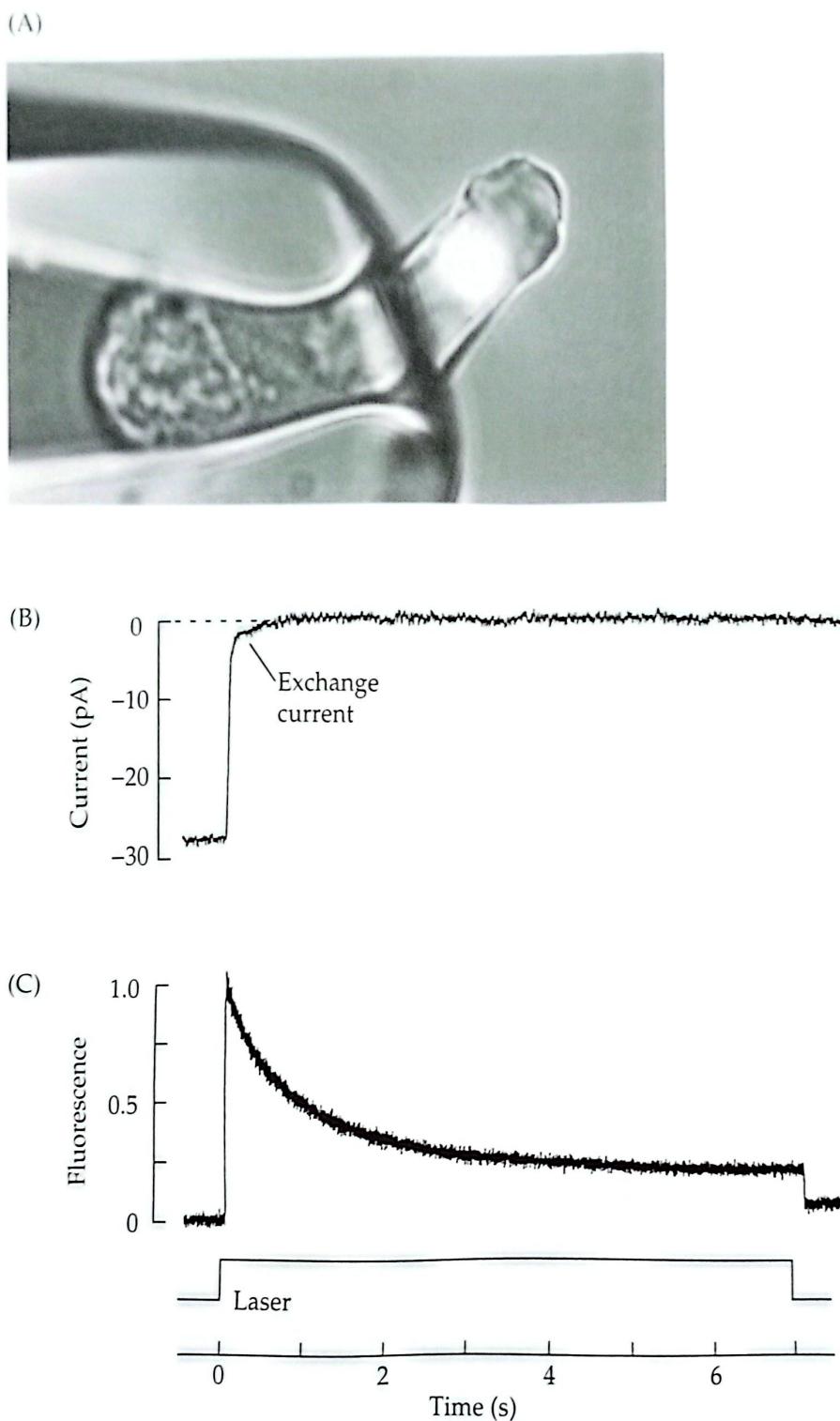


Figure 9.20
Light produces a decrease in photoreceptor intracellular Ca^{2+} concentration
 (A) Isolated salamander rod loaded with fluorescent Ca^{2+} indicator dye held with its inner segment in a suction pipette so that its outer segment could be illuminated with visible light from an argon laser. (B) Turning on of the laser produced a rapid decline in suction-electrode current due to the closing of channels. The more slowly declining component is inward current produced by the electrogenic $\text{Na}^+/\text{Ca}^{2+}-\text{K}^+$ transporter and gives the approximate time course of Ca^{2+} decrease. (C) From the same rod, the time course of Ca^{2+} decrease measured with a fluorescent indicator dye. (From Sampath et al., 1998.)

other ion, such as Li^+ or guanidinium $^+$, for Na^+ , stops the efflux of Ca^{2+} by the exchanger, since the exchanger requires extracellular Na^+ to function. The photoreceptors still have responses to light, since both Li^+ and guanidinium $^+$ can permeate the cyclic nucleotide-gated channels.

When both influx and efflux are blocked in this way, the Ca^{2+} concentration in the rod remains relatively constant (Fain et al., 1989; Matthews and Fain, 2001), and a light flash given to the photoreceptor in this solution produces a response wave form that is prolonged in much the same way as the disruption of the GCAP genes (Figure 9.19C). These experiments show that the change in outer segment Ca^{2+} concentration causes an acceleration of the return of outer segment current (see also Torre et al., 1986), and this effect of Ca^{2+} is almost entirely due to regulation of cyclase activity via the GCAP proteins (see also Burns et al., 2002).

Light Adaptation

Vertebrate photoreceptors adapt in the presence of steady light, a process called **light adaptation**. Constant stimulation decreases sensitivity and resets the operating range of a photoreceptor, much as maintained hair bundle deflection does for a hair cell (see Figure 6.12). This can be seen in Figure 9.21. In part (A) of this figure, a rod in darkness was stimulated with brief flashes at intensities that increased systematically by about a factor of 4. The peak amplitude of these responses became larger as the light intensity increased, (as in Figures 9.16 and 9.17) and trace out a **response-intensity curve**, giving peak amplitude as a function of flash intensity. In Figures 9.21B and 9.21C these same flash intensities were repeated for this same rod but in the presence of two different steady background lights. The backgrounds themselves produced a decrease in the outer segment current that slowly declined and reached a steady plateau level. Flashes superimposed on this background produced a further closing of the channels. Note, however, that the sensitivity of the rod was decreased in the presence of the background, such that the whole operating range of the photoreceptor was shifted to higher flash intensities.

The decrease in sensitivity is perhaps clearer in Figure 9.21D. Here small-amplitude responses of a rod to brief flashes have been superimposed. Since the intensities of the flashes in darkness and in the different backgrounds were not the same, the response amplitudes in each case have been divided by the flash intensities and plotted in units of sensitivity, that is, photocurrent per unit light intensity. The largest response was recorded without a background light, and the others are for backgrounds of progressively increasing intensity. As the background intensity increased, sensitivity declined, and the wave form of the response was also altered. At each of the progressively brighter background intensities the responses rose along approximately the same initial curve, but they began to decline at a progressively earlier time. These recordings show that one of the principal mecha-

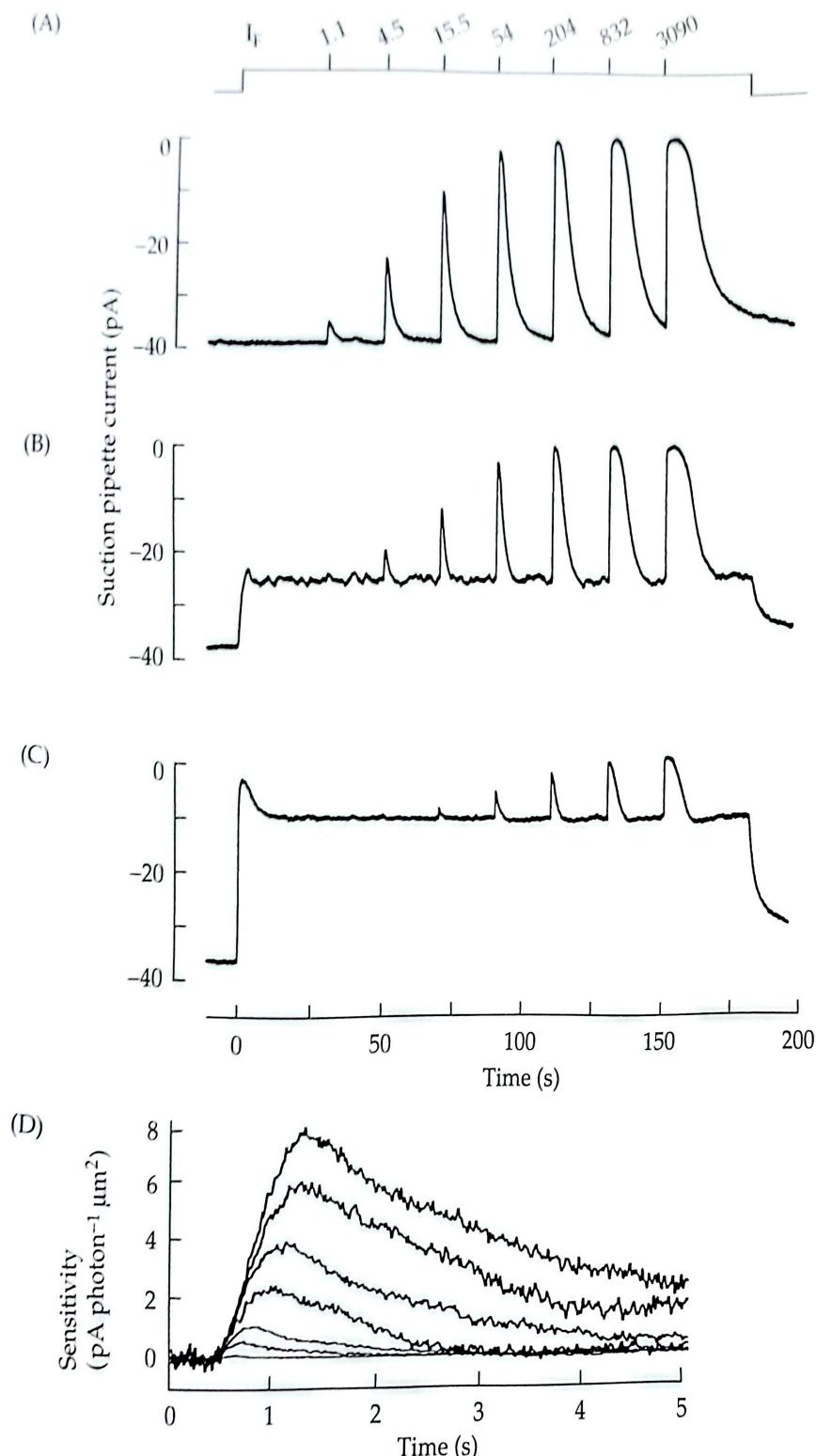


Figure 9.21
Light adaptation in a vertebrate rod (A–C) Suction electrode recording of responses of the same salamander rod to flashes given in darkness and in the presence of two steady background lights. Abbreviation: I_F , flash intensity in units of photons per μm^{-2} . Background intensities were 1.7 (B) and 37.2 (C) photons $\mu\text{m}^{-2}\text{s}^{-1}$. (D) Responses of a salamander rod in darkness (largest response) and in backgrounds of progressively increasing brightness. Responses have been plotted as sensitivities by dividing the current (in picoamperes) by the light intensity (in units of photons per μm^{-2}). (A–C from Matthews, 1990; D from Fain and Cornwall, 1993.)

nisms for the sensitivity decrease in rods and cones is an acceleration in the time course of response decay (Baylor and Hodgkin, 1974).

Light adaptation in rods requires a diffusible second messenger, since the sensitivity of the whole outer segment can be changed when rhodopsin molecules are activated in only a small fraction of the disks (see Fain, 1986). A messenger also seems to regulate sensitivity in cones, and there is now considerable evidence that in both kinds of photoreceptors this messenger is Ca^{2+} . One way of showing this is to perfuse the outer segment with a solution that lacks both Ca^{2+} and Na^+ , to reduce Ca^{2+} influx and efflux and minimize changes in outer segment Ca^{2+} concentration, as in the experiment of Figure 9.19C. Under these conditions adaptation is entirely eliminated (Matthews et al., 1988; Nakatani and Yau, 1988a). A large body of experimental work now shows that changes in Ca^{2+} are a necessary requirement for adaptation of the photoreceptor to light (see Fain et al., 2001).

What does the Ca^{2+} do? The answer to this question is still not completely clear, but one very important role of Ca^{2+} is certainly to modulate the activity of the guanylyl cyclase (see Figure 9.19). In the presence of a steady background light there is a steady increase in the rate of hydrolysis of cGMP by the PDE that produces a decrease in cGMP, a closing of the channels, and a decrease in the intracellular free Ca^{2+} concentration. As the Ca^{2+} falls, the cyclase activity increases until the synthesis of cGMP equals its rate of hydrolysis. This causes a fraction of the channels to reopen, preventing the background light from saturating the rod and allowing the receptor to continue to respond even in the presence of the maintained stimulation. The increase in the rates of the PDE and cyclase produce an accelerated turnover of cGMP that is responsible for much of the acceleration of the time course of decay of the response (Figure 9.21D), as well as much of the decrease in sensitivity (see Nikonov et al., 2000; Fain et al., 2001).

When the Ca^{2+} -dependent regulation of the cyclase is eliminated by disrupting the genes for the GCAP proteins, much, but not all of light adaptation is eliminated (Mendez et al., 2001). There are several other components of the transduction cascade that can be modulated by Ca^{2+} , but there is still considerable uncertainty as to which are the most important. There is, for example, evidence for a Ca^{2+} -dependent component of adaptation rather early in the transduction cascade (see, for example, Matthews, 1997), perhaps produced by regulation of rhodopsin kinase by a small-molecular-weight Ca^{2+} -binding protein (Kawamura, 1993), usually referred to as **recoverin**. Dialysis of recoverin into rods produces changes in response wave form (Erickson et al., 1998), as does disruption of the recoverin gene (Dodd, 1998), and these effects are of opposite sign. They are, however, surprisingly small (Dodd, 1998). Ca^{2+} has also been shown to modulate the light-dependent channels (see Molday and Kaupp, 2000), in rods by binding to calmodulin as in olfactory receptors (Hsu and Molday, 1993), and in cones apparently by binding to some other Ca^{2+} -binding protein (Hackos and Korenbrot, 1997). The effect for the rod channel is small and seems to make little contribution to adapta-

tion (Koutalos et al., 1995), but the one for the cone channel could be of greater significance (Rebrik and Korenbrot, 1998; Rebrik et al., 2000).

Pigment Renewal and the Recovery of Sensitivity after Bright Light

Light converts 11-cis retinal to all-trans retinal, and the chromophore must be reisomerized to its 11-cis form before rhodopsin can again be reactivated by photon absorption. In an arthropod photoreceptor the all-trans retinal remains covalently attached to the opsin, forming a thermally stable metarhodopsin that can absorb light in the visible part of the spectrum. In *Drosophila*, the rhodopsin of the R1–R6 photoreceptors containing 11-cis retinal absorbs maximally at 480 nm in the blue-green, and the corresponding metarhodopsin with all-trans retinal absorbs maximally at 570 nm in the yellow (see Minke and Hardie, 2000). All of the other pigments in *Drosophila*, including the UV-sensitive pigments, also have metarhodopsins that absorb in the visible, at wavelengths between 460 and 520 nm. When the metarhodopsin absorbs a photon, no light response is produced, but all-trans retinal is converted back to 11-cis. This doesn't immediately produce a form of rhodopsin that is capable of reactivating the cascade; in *Limulus* it can take 30–60 minutes after photoregeneration of the rhodopsin before the photopigment is ready to produce another light response (Levine et al., 1987). This is presumably the time required for unbinding of arrestin and perhaps also for rhodopsin dephosphorylation.

In vertebrates, some regeneration of pigment can be produced in a similar way by reisomerization with light (Hao and Fong, 1999; Chen et al., 2001b), but most of the 11-cis retinal is reformed by a process that is much more cumbersome (Figure 9.22; see also Fain et al., 1996; McBee et al., 2001). After the absorption of a photon, the Rh^{*} is phosphorylated and bound to arrestin. The all-trans retinal comes off the opsin molecule and is hydrolyzed to all-trans retinol in the outer segment by the enzyme **retinol dehydrogenase**. The all-trans retinol then leaves the photoreceptor and is carried in the extracellular space, perhaps in part by binding to **interphotoreceptor retinol-binding protein (IRBP)** (but see Ripps et al., 2000). The chromophore for rods is then deposited in an adjacent layer of cells called the **retinal pigment epithelium (RPE)**. The RPE contains an enzyme that isomerizes all-trans back to 11-cis and another that converts the 11-cis retinol to 11-cis retinal. The reisomerized chromophore then diffuses or is carried out of the RPE back to the photoreceptor, where it recombines with the opsin and regenerates rhodopsin. A similar process occurs for cones, but reisomerization of retinal seems to take place at least in part in retinal glial cells instead of, or in addition to, the RPE (Mata et al., 2002). Pigment regeneration is less efficient than in arthropods but has the virtue that in the dark after bright light exposure, *all* of the pigment can be regenerated. The only way an insect can regenerate

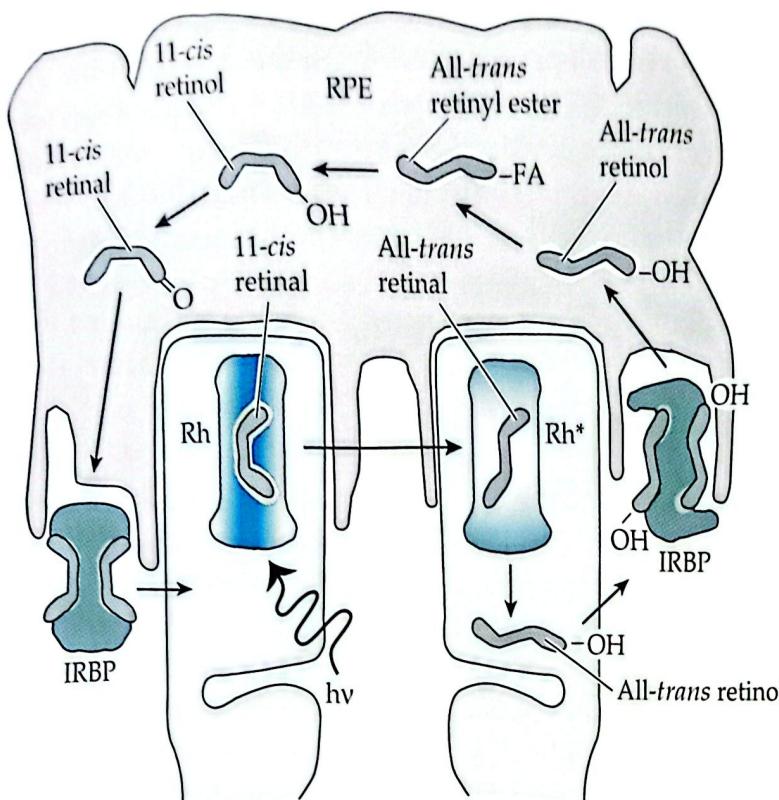


Figure 9.22
Regeneration of photopigment for a vertebrate rod Principal enzymes and transport proteins responsible for regeneration of rhodopsin in rod and retinal pigment epithelial cell. Abbreviations: RPE, retinal pigment epithelium; FA, fatty acid; Rh, rhodopsin; IRBP, interphotoreceptor retinol binding protein; $h\nu$, light. (After Bok, 1993, and Fain et al., 1996.)

all of its visual pigment in the dark is by complete resynthesis of the rhabdomeric membrane (see Figure 2.6).

The regeneration of rhodopsin after bright light exposure is rather slow: For human rods complete recovery can require as much as 30–35 minutes after the light is turned off. During this time the sensitivity of vision is considerably depressed. Part of the reason for this is that there is less pigment to absorb photons, since a fraction of the pigment has been bleached and there is then a somewhat smaller probability of absorption of a photon by the remaining, unbleached rhodopsin. This decrease is, however, much too small to account for the loss of sensitivity. Some other process must be occurring.

Stiles and Crawford (1932) first suggested that bleached pigment might desensitize the visual system by acting as an equivalent background light. In molecular terms this would mean that some component of bleached pigment can activate the transduction cascade much like light, producing an activation of the PDE, a decrease in Ca^{2+} concentration, and a modulation of the sensory cascade. It is, in fact, likely that virtually every bleaching intermediate can stimulate the cascade to some extent, including phosphorylated metarhodopsin and even opsin (see Liebrook et al., 1998; Fain et al., 2001). The question is, Which of these intermediates are the most important?

The answer seems to depend on the amount of visual pigment that has been bleached. For light that bleaches only a relatively small fraction of the pigment, the most important component of the equivalent background in a rod seems to be continued excitation of opsin by all-*trans* retinal (Hofmann et al., 1992; Jager et al., 1996). Sensitivity recovers as all-*trans* retinal is con-



verted to inactive all-*trans* retinol and the retinol leaves the photoreceptor. For large bleaches, all-*trans* retinal is converted to all-*trans* retinol before most of the pigment is regenerated (Kennedy et al., 2001). The photoreceptors remain desensitized, and the equivalent background seems then to be produced mostly by opsin itself, which stimulates the cascade, though with low probability (Cornwall and Fain, 1994). This remaining opsin continues to activate the cascade and depress sensitivity until all of the photopigment has been regenerated.

Summary

Photoreceptors respond to that part of the electromagnetic spectrum that we call light. They do this with the pigment rhodopsin, consisting of a retinal chromophore covalently attached to the protein opsin. From archaeobacteria to human, the mechanism of photon absorption is remarkably conserved. The chromophore is a derivative of vitamin A, called retinal, and the absorption of light produces an isomerization; in archaeobacteria all-*trans* retinal is converted to 13-*cis*, whereas in animals 11-*cis* is converted to all-*trans*. For most visual pigments the chromophore in the dark is protonated and forms a salt bridge with an adjacent, negatively charged amino acid. Isomerization produces a change in the shape of the chromophore within the opsin-binding pocket that breaks the salt bridge, triggering a change in conformation of the rhodopsin to an active form that initiates the sensory cascade.

In archaeobacteria, phototransduction is much like chemotaxis. Light produces an alteration of the concentration of phosphorylated CheY protein, which acts as a second messenger controlling the flagellar motor. In all animals, the visual pigment is a G protein-coupled receptor that activates a trimeric G protein and triggers a metabotropic cascade. Several different G protein families and transduction cascades have been implicated in phototransduction, sometimes even in different cells of the same organism. The most thoroughly studied cascades are those that produce the depolarizing responses of the arthropods *Limulus* and *Drosophila*, and those for the hyperpolarizations of vertebrate rods and cones.

In both *Limulus* and *Drosophila*, the photopigment is contained within numerous microvilli that are collectively referred to as a rhabdomere, which greatly increase the surface area of the plasma membrane. Rh* activates a trimeric G protein with an α_q subunit to produce α_q -GTP. This then stimulates a PLC β , generating the two second messengers IP₃ and DAG. Despite many years of intense effort, it is still not clear which, if either of these second messengers is directly responsible for gating the opening of the ion channels. The channels, at least in *Drosophila*, appear to be members of the TRP family of proteins, of which three different forms are expressed in the photoreceptor: TRP, TRPL, and TRP γ . In *Limulus*, on the other hand, the channels may be gated by cyclic nucleotides.