CHAPTER 9

Photoreception

Our bodies are exposed to a constant shower of electromagnetic radiation, in part man-made but in the great majority coming from the sun. The spectrum of this radiation encompasses high-energy gamma rays with wavelengths of the order of an atomic nucleus, to low energy radio waves with wavelengths of many kilometers. There is, however, a narrow band, from the near ultraviolet (300 nm) to the near infrared (1100 nm), which encompasses nearly three-quarters of the sun's energy. This band of wavelengths is further restricted by absorption by ozone and water vapor in the atmosphere, with the result that nearly half of the radiation reaching the surface of the Earth is within a range of approximately 400–700 nm (Wald, 1959). It is therefore hardly surprising that sensory proteins appeared very early in the evolution of life to absorb photons in this particular part of the electromagnetic spectrum, which we call visible light.

Proteins cannot themselves absorb visible light, because amino acids have their peak absorption in the ultraviolet. A protein molecule must be joined to a prosthetic group called a chromophore, which in combination with the protein is able to absorb illumination at longer wavelengths. Bacteria and other single-celled organisms use several different molecules to absorb light, but one family of compounds in particular was exploited very early in evolution by prokaryotes and green algae, and then subsequently by every animal from the coelenterates (Suga et al., 2008) to arthropods, mollusks, and all vertebrates including *Homo sapiens* (see Fain et al., 2010). These are the stereo-isomers of retinal. The archaeobacteria use all-*trans* retinal (Figure 9.1A). Animals including vertebrates and man use the 11-*cis* isomer of this compound (Figure 9.1B), with a few minor variations: in some insects the 11-*cis* retinal contains an additional hydroxyl group on the third carbon of the ionone ring (and is called 3-hydroxy-11-*cis* retinal), and in fresh-water fish and some aquatic amphibians and reptiles there is an additional double bond in the ring, and the chromophore is called 3-dehydroretinal or sometimes 11-*cis* retinal$_2$ (see Fain, 2015).

Visible radiation has the fortunate property that the energy of the photon is too small to produce DNA mutation or tissue damage but is nevertheless in excess of 40 kcal mol^{-1} over the entire range of visible wavelengths, sufficiently large to exceed the activation energy of a chemical reaction. For both bacteria and animals, the absorption of a photon by retinal produces a *photoisomerization*, changing the chromophore from one isomer to another. In archaeobacteria, all-*trans* is converted to 13-*cis* (Figure 9.1A), whereas in animals, the 11-*cis* isomer is converted to all-*trans* (Figure 9.1B). These reactions change the shape of the chromophore and induce a change in the conformation of the protein to which the chromophore is attached, providing a signal that triggers light detection.

Proteins with chromophores are used as sensory pigments for a very good reason: the chemical change produced by photon absorption can be coupled to a metabotropic transduction cascade to produce an electrical response, whose sensitivity can be at the physical limit of a single quantum of light (see Figure 2.13). Pigments are not the only way of detecting electromagnetic radiation. Many organisms

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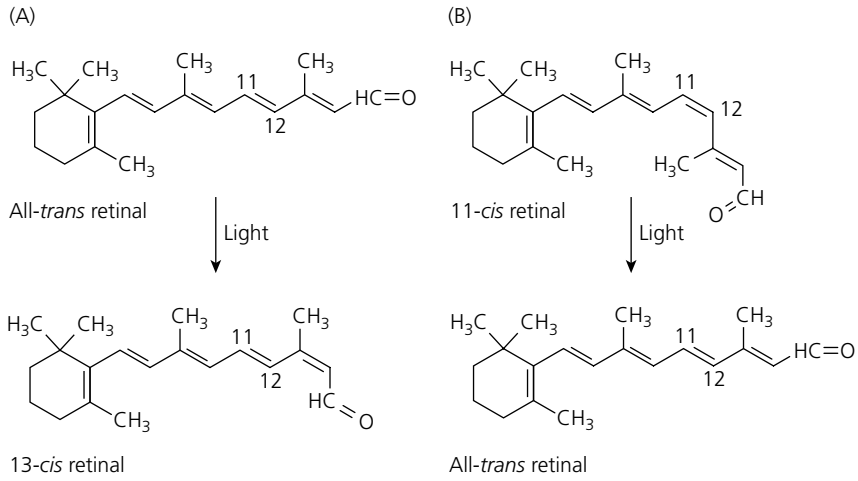

Figure 9.1 Retinal chromophores of visual pigments: photoisomerization. (A) In archaeobacteria, all-*trans* retinal is isomerized by light to 13-*cis* retinal. (B) In most animals, the chromophore is 11-*cis* retinal, which is isomerized by light to the all-*trans* form.

(including we ourselves) have receptors for infrared illumination, that is for heat, using quite a different mechanism. There are no chromophores or transduction cascades but rather proteins like TRPV1 (see Chapter 3 and Figure 3.6), which are ionotropic receptors with heat-sensitive channel gating. We defer a more complete description to Chapter 10.

Photopigment activation

Sensory proteins that use retinal chromophores are called *opsins*. As we saw in Chapter 4 (Figure 4.3A), the retinal in the X-ray crystallographic structure of vertebrate opsin lies in a binding pocket near the center of the opsin molecule. It is surrounded by seven α-helical, transmembrane domains of the protein, much as for epinephrine in the binding pocket of the β-adrenergic receptor. The retinal does not simply lie within the binding pocket but is *covalently attached* to the amino group of a lysine residue from the seventh of the α helices. Remarkably, in the sensory rhodopsins of archaeobacteria (see Spudich et al., 2000), the all-*trans* retinal chromophore also lies within a binding pocket in the center of the protein and is covalently bound, also to a lysine. Furthermore, the X-ray crystallographic structure of bacterial sensory rhodopsin has seven transmembrane α helices (Luecke et al., 2001) even though bacterial sensory rhodopsins are not G-protein receptors (bacteria don't *have* G-protein receptors). The lysine that combines with the retinal is again on the seventh of the α helices.

The terminal nitrogen of the lysine reacts with the aldehyde of the retinal to form a double bond called a Schiff base. In most of the rhodopsins (see Rao and Oprian, 1996; Sakmar, 1998; Okada et al., 2001a; Sakmar et al., 2002), including those of archaeobacteria (see Hoff et al., 1997; Spudich et al., 2000), this Schiff base is protonated. Single charges are rarely if ever found buried in the middle of a protein, but pairs of charges called *salt bridges* are quite common. In most vertebrate rhodopsins, a salt bridge is formed by the protonated Schiff base lysine and a negative charge contributed by a glutamate residue from the third transmembrane helix (Figure 9.2).

The protonation of the Schiff base has a large effect on the wavelength of light absorbed by the visual pigment. The absorption peak of free 11-*cis* retinal depends somewhat upon the solvent into which the retinal is dissolved but is at about 360–380 nm in the near ultraviolet (Knowles and Dartnall, 1977). When the chromophore combines with opsin, the resulting pigment can have its absorption maximum shifted by as much as 200 nm toward longer wavelengths. A significant fraction of this shift in wavelength is produced by protonation of

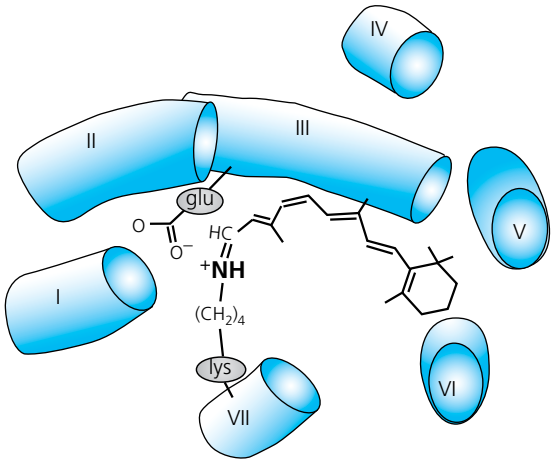

Figure 9.2 Attachment of chromophore to opsin. Retinal forms a covalent Schiff base attachment with a lysine from helix VII. This Schiff base in most visual pigments is protonated and exists as a salt bridge together with the negative charge of an acidic group from a glutamate in helix III.

the Schiff base (see Knowles and Dartnall, 1977). In humans and many other vertebrates, most of vision occurs between the wavelengths of 400 nm and 700 nm, from the blue to the deep red, as I have said. Vision in this part of the spectrum would hardly be possible if the absorption peak of our pigments were the same as that of the free chromophore.

The protonation of the Schiff base cannot be the only cause of the shift in wavelength; if it were, all pigments would have the same absorption peak. In human photoreceptors, there are four different pigments all structurally similar to one another with at least 40 percent identity of amino acid sequence (Nathans et al., 1986), all absorbing in somewhat different parts of the visible spectrum. The maximum absorption of our rod pigment is at about 500 nm in the blue-green, and our cone pigments absorb maximally at about 415–420 nm in the blue, 535–540 nm in the green, and 560–570 nm in the yellow (see Bowmaker et al., 1980; Nunn et al., 1984). Many amino acids in the vicinity of the chromophore can make subtle alterations in the electronic environment to tune the peak of absorption to different wavelengths. Furthermore, human middle and long wavelength cone pigments, and cone pigments in many other vertebrates, contain an extracellular binding site for Cl^-, whose negative charge also plays a significant role in moving the absorption maximum to longer wavelengths (see Ebrey and Koutalos, 2001; Stenkamp et al., 2002).

The protonation of the Schiff base has an important effect on the stability of the pigment. The sensitivity of sensory detection depends not only upon the sensitivity of transduction but also upon the noisiness of the receptor. Photoreceptors both in arthropods and in vertebrates are able to signal the absorption of a single quantum of light (Figure 2.13), but they would not be able to detect single photons reliably if rhodopsin even in darkness produced responses spontaneously. Because a single photoreceptor contains hundreds of millions of rhodopsins, even a very small rate of spontaneous conversion of the pigment to an active form would compromise the sensitivity of the cell. For this reason, rhodopsin in the dark is extraordinarily stable, with a half-life for spontaneous activation measured in the hundreds or even thousands of years (Yau et al., 1979; Baylor et al., 1980). Part of the reason for this stability is the formation of the salt bridge between the protonated lysine and the glutamate, because if this salt bridge is removed, for example by site-directed mutagenesis of the glutamate, the resulting visual pigment becomes spontaneously active (Robinson et al., 1992; Rao and Oprian, 1996; Spudich et al., 1997). The formation of the salt bridge cannot be the only cause of stability, because in some insects, birds, and even mammals (e.g. mouse), there are cones with photopigments sensitive to ultraviolet light, having a peak absorption close to that of free 11-*cis* retinal. In these pigments the Schiff bases appear *not* to be protonated (Shi et al., 2001; Fasick et al., 2002). There seem to be a variety of additional interactions probably including hydrogen bonds and hydrophobic forces within the protein structure which also contribute to stability (see Sakmar, 1998; Okada et al., 2001b; Sakmar et al., 2002; Filipek et al., 2003).

When rhodopsin absorbs a photon, the chromophore isomerizes and changes its molecular configuration. In vertebrate rhodopsin, the whole of the chromophore molecule rotates around the bond between carbons 11 and 12. This effect produces a rather large strain in the conformation of the protein component of the pigment. The strain is relieved by deprotonation of the Schiff base and movement of

the α helices, in vertebrate rhodopsin predominantly helices V and VI (see Figure 4.3B), producing an intermediate called metarhodopsin or Rh*. It is this form of rhodopsin that triggers activation of the sensory cascade.

Phototransduction

What happens next depends very much upon the organism, because the mechanism of transduction is different for bacteria and animals, and for vertebrates and many invertebrates. In archaeobacteria, the cascade is quite similar to the one for chemotaxis (see Chapter 7 and Figure 7.2). Sensory rhodopsin is coupled to a transduction protein called Htr, which is linked to a CheA histidine kinase (see Hoff et al., 1997). Just as for chemotaxis, the CheA protein controls the phosphorylation of a CheY second messenger which binds to the flagellar motor and regulates the rate of flagella reversals, or tumbles. Adaptation is produced by methylation (see Figure 7.3), but it is the Htr protein that is methylated rather than the visual pigment.

In all multicellular animals so far investigated, rhodopsin is coupled to a heterotrimeric G protein. Transduction is produced primarily, if not exclusively, by binding of the Gα subunit of the G protein to an effector enzyme. In insects, the Gα subunit is a member of the $G\alpha_{q/11}$ family and is coupled to a phospholipase C (PLC), encoded in *Drosophila* by the *norpA* gene. In vertebrates, the Gα subunit is a member of the $G\alpha_i/G\alpha_o$ family and is coupled to a phosphodiesterase (PDE6) (see Figure 4.5B). For *Drosophila*, the second messenger that gates the channels has not been identified. For vertebrates it is cGMP. In insects and other arthropods, light opens channels permeable to cations and produces a depolarization (Figure 1.5A). In vertebrate rods and cones, light *closes* channels that are also permeable to cations, producing a hyperpolarization (Figure 1.5B).

The variety of transduction mechanisms can perhaps best be illustrated by taking one particularly interesting example, the eye of the scallop *Pecten*. We described the peculiar anatomy of this eye in Chapter 2. It has two separate retinas, each containing its own population of receptor cells (Figure 2.11). Both have sensory membrane containing photopigment. In cells of the distal retina, the sensory membrane is elaborated from a modified cilium, whereas for cells in the proximal retina, the sensory membrane consists of microvilli (Barber et al., 1967). Hartline (1938) first showed that the physiology of the two retinas is also different. He recorded action potentials from the nerves coming from the retinas and discovered that cells in the proximal retina produce a burst of action potentials when the light goes on, but those in the distal retina are excited when light goes off.

The first intracellular recordings showed that light depolarizes the cells of the proximal retina much as in insects and other arthropods, whereas it hyperpolarizes the cells of the distal retina (Gorman and McReynolds, 1969; McReynolds and Gorman, 1970b). When the photoreceptors are voltage clamped (Figure 9.3A), light produces an inward (negative) current for the proximal cells but an outward (positive) current for the distal cells (Gomez and Nasi, 1997a). The inward current of the proximal cells has a reversal potential just positive of zero and is produced by an increase in permeability predominantly to Na^+ (McReynolds and Gorman, 1970b, 1974; Gomez and Nasi, 1996). The increase in Na^+ permeability causes the cell to depolarize—see Eq. (3.5). The outward current of the distal cells has a reversal potential near –70 mV, more negative than the dark resting membrane potential in these cells. It is produced predominantly by an increase in permeability to K^+, producing a hyperpolarization (McReynolds and Gorman, 1970b, 1974; Cornwall and Gorman, 1983a; Gomez and Nasi, 1994).

Although the sensory cascades for the two kinds of photoreceptors have not yet been elucidated in detail (see Nasi et al., 2000), they seem to have little in common. The photopigments have almost identical spectral absorption curves with maxima at about 500 nm (McReynolds and Gorman, 1970b; Cornwall and Gorman, 1983b). They are nevertheless distinct molecules with different amino acid sequences particularly in the interconnecting loop region between helices V and VI (Kojima et al., 1997). This is the part of the protein that is exposed to the cytoplasm and is thought to play an important role in activating the G protein. The proximal and distal retinas express different G proteins (Kojima et al., 1997; Gomez and Nasi, 2000): the proximal (depolarizing)

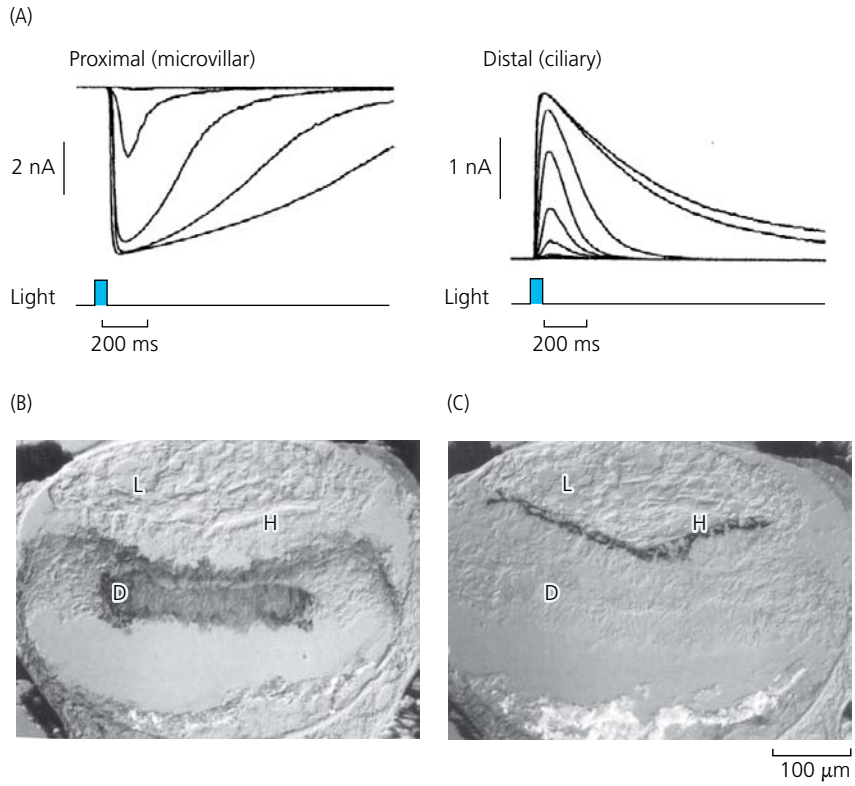

Figure 9.3 Phototransduction in the eye of the scallop. (A) Photocurrents of opposite polarity from voltage-clamped photoreceptors in the two retinal layers. The negative current recorded from the proximal microvillar photoreceptors ($V_m = -50$ mV) indicates an increase in conductance to Na^+ and other cations, whereas the positive current recorded from the distal ciliary photoreceptors ($V_m = -30$ mV) indicates an increase in conductance mostly to K^+. See Eq. (3.7). Anti-α_q (B) and anti-α_o (C) antisera were used for immunohistochemical localization of the G-protein α subunits. L, Lens; H, hyperpolarizing (distal) photoreceptor layer; D, depolarizing (proximal) photoreceptor layer. (A from Gomez and Nasi, 1997a; B and C from Kojima et al., 1997.)

photoreceptors express a $G\alpha_q$, like the depolarizing photoreceptors of *Drosophila* (Figure 9.3B), whereas the distal (hyperpolarizing) photoreceptors express a $G\alpha_o$ (Figure 9.3C).

The channels are also different. The most commonly observed channel openings for the depolarizing photoreceptors have a conductance of about 48 pS, whereas those for the hyperpolarizing photoreceptors have a conductance of about 26 pS. The channels of the hyperpolarizing photoreceptors can be activated by cGMP and its analogs (del Pilar Gomez and Nasi, 1995) and blocked by a variety of compounds known to block cyclic-nucleotide-gated channels (Gomez and Nasi, 1997b). It would therefore appear that the distal photoreceptors in *Pecten* have some kind of cyclic-nucleotide-gated channel. These channels are, however, different from those of parietal photoreceptors (Chapter 4) or olfactory receptor cells (Chapter 7), because they have very little permeability to Na^+ and are instead selectively permeable to K^+. The channels of depolarizing photoreceptors seem *not* to be gated by cyclic nucleotides (Nasi and Gomez, 1991). Their mechanism of activation is still uncertain.

Because transduction can be so variable in the different photoreceptors of the animal kingdom, and because only a few species have been examined in detail, we are not yet in a position to draw any

firm conclusions about the distribution of different G proteins, second messengers, and channels in the various phyla. The best we can do at present is to pick certain well-studied examples and explore them in detail. The choice is easy. The most thoroughly investigated photoreceptors are certainly those of two arthropods, the horseshoe crab *Limulus* and the fruit fly *Drosophila*; and the rods and cones of vertebrates.

The photoreceptors of arthropods

Like receptor cells in the proximal retina of scallop, all arthropod photoreceptors have sensory membrane produced by numerous microvilli which, together, form a structure called a *rhabdomere* (Figure 2.5). The organization of the rhabdomere varies considerably among different species. The ventral eye of the horseshoe crab *Limulus* and the compound eye of the fruit fly *Drosophila* provide useful contrasting examples.

Limulus is more closely related to spiders than to crabs. It has photoreceptors in many different places (Figure 9.4A). There are two large lateral eyes on either side of the body, homologous to the principal eyes of a lobster or dragonfly. There are in addition two median eyes on the top and in the center of the carapace, some of whose photoreceptors are sensitive to ultraviolet light (Nolte and Brown, 1969). There are even photoreceptors in the tail. The most useful cells for the study of transduction, however, have been the cells lying along the ventral nerve underneath the animal, located approximately in the center of the body. What these photoreceptors are doing in this unusual location is uncertain. Perhaps they help the animal detect changes in illumination when it is swimming—horseshoe crabs often swim upside down.

The ventral photoreceptors of *Limulus* are surrounded by glial cells and connective tissue, which can be peeled away to reveal the structure shown in Figure 9.4B (Stern et al., 1982). One part of the cell contains a dense skein of microvilli and is called the R (or rhabdomeric) lobe. It is somewhat analogous to the outer segment of a vertebrate rod or cone (Figures 2.7 and 9.13). The rest of the cell contains the nucleus and metabolic machinery of the photoreceptor and is called the A (or arhabdomeric) lobe. The A lobe is analogous to the rod or cone inner segment.

The microvilli are a few microns in length and mostly restricted to the outermost surface of the R lobe. At the base of the microvilli, there is an extensive network of smooth endoplasmic reticulum (ER), which serves as a store for calcium (Payne et al., 1988). As we shall see, there is abundant evidence of a role of IP_3-gated calcium release in the mechanism of transduction, and the smooth ER at the base of the microvilli may be functioning much like the ER of other cells, sequestering calcium and releasing it when IP_3 binds to IP_3 receptors in the vesicle membrane (see Figures 4.7 and 4.8). The whole of the cell is of the order of 50 microns wide and several hundred microns long, with a volume fifty to a hundred times greater than even the largest vertebrate photoreceptor. The cells in the other eyes of *Limulus* are also large and ample targets for poking with pipettes. That is why *Limulus* has been so attractive to physiologists. The photoreceptors of *Limulus* were the first of any species whose responses were recorded with intracellular microelectrodes (Hartline et al., 1952), the first to provide evidence of single-photon sensitivity (Yeandle, 1958), the first to be studied with voltage clamp (Millecchia and Mauro, 1969a), and the first to implicate IP_3 and Ca^{2+} as important messenger substances in photoreceptor transduction and adaptation (Lisman and Brown, 1975; Fein et al., 1984).

Drosophila photoreceptors are much smaller but have the great advantage that they can be manipulated by the powerful tools of fruit-fly genetics. The photoreceptors are found in three small ocelli at the top of the head and in the two large compound eyes (Figure 9.5A), which each have 700–800 units called ommatidia with a clearly defined structure (see Hardie and Postma, 2008; Yau and Hardie, 2009; Fain et al., 2010). At the top of each ommatidium there is a cornea and a fluid-filled pseudocone that together act as a lens focusing light onto the rhabdomeres of the photoreceptors (Figure 9.5B). Below these structures are the Semper (supporting) cells and eight photoreceptors, six of which (called R1–R6) contain the same visual pigment with peak absorption at 480 nm in the bluish green. The other two cells, R7 and R8, have rhabdomeres more centrally located, with R7 lying above R8 so that

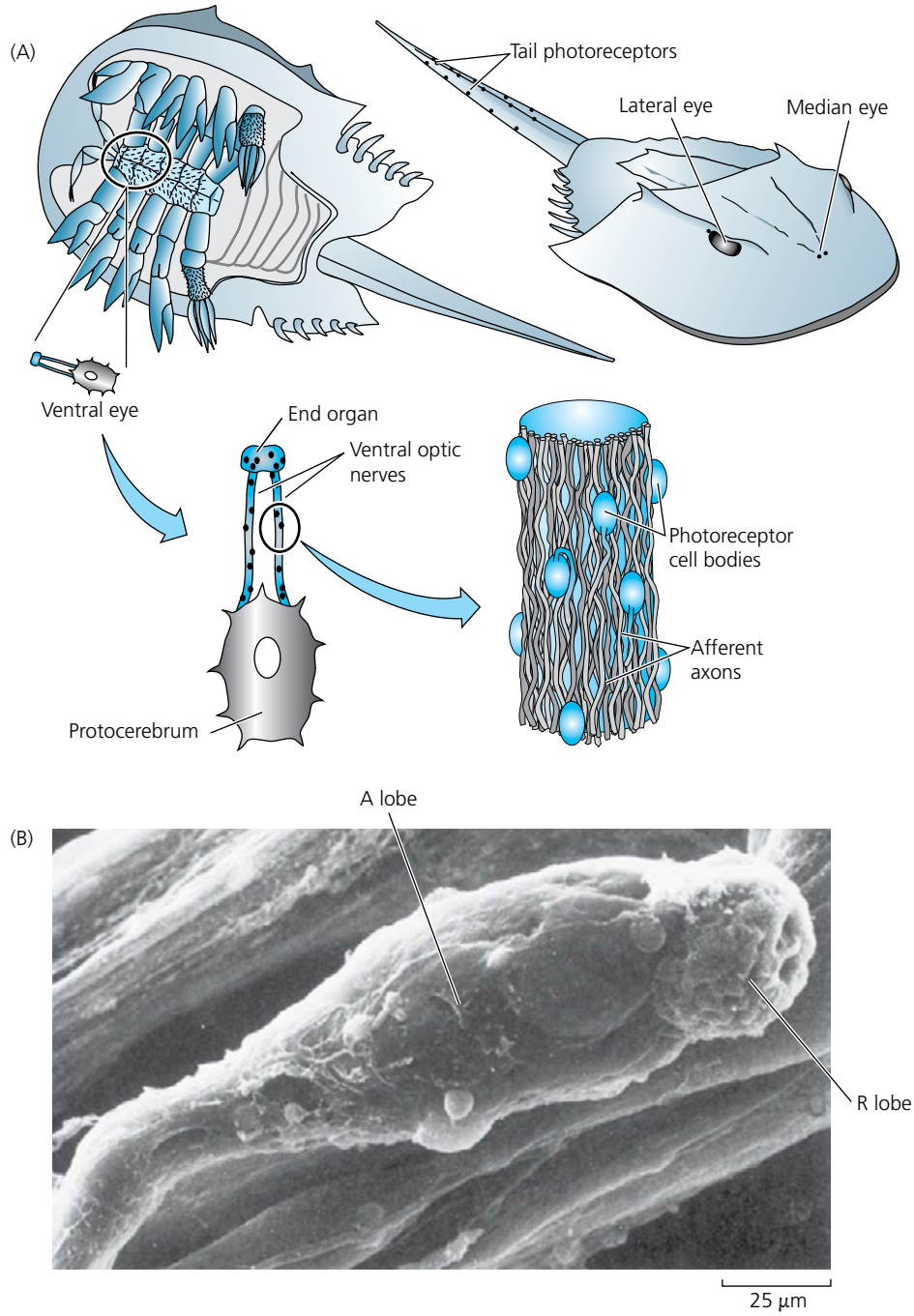

Figure 9.4 Photoreceptors of the horseshoe crab *Limulus*. (A) Photoreceptors are located in many places on the *Limulus* body. Those of the ventral eye are found dispersed along the ventral optic nerve underneath the animal. (B) Ventral photoreceptor denuded of glial cells and connective tissue. (A after Calman and Chamberlain, 1982; Dorlochter and Stieve, 1997; B from Stern et al., 1982.)

cross-sections of the ommatidium show the rhabdomere of one cell or the other but never both (Figure 9.5C). In most of the retina, R7 contains one of two ultraviolet-absorbing photopigments, with peak absorption either at 345 nm or 375 nm, whereas R8 contains one of two visible-absorbing pigments with peak absorption either at 437 nm or 508 nm. Different ommatidia express different pigments, but expression is coordinated so that when R7 expresses the 345-nm pigment, R8 expresses the 440-nm pigment, and similarly for the other two (see Montell, 1999; Minke and Hardie, 2000). The rhabdomeres of each photoreceptor contain of the order of 30,000 microvilli each 1–2 μm in length, which are continuous with the plasma membrane and make up over 90 percent of its area (Figure 2.5). The microvillar membrane contains most of the machinery for transducing light into an electrical signal.

In some insects, the rhabdomeres of individual photoreceptors in an ommatidium are fused to form a single structure called the rhabdome (see Chapter 5 of Cronin et al., 2014). In other species, including flies, the rhabdomeres of the different photoreceptors are physically separate from one another (Figure 9.5C). This separation has the consequence that each rhabdomere is directed toward a slightly different place in space. For R7 and R8, this difference poses no difficulty, because there is only one of each of these receptors per ommatidium. For the R1–R6 cells, however, the difference in orientation poses a problem. These photoreceptors need to combine their signals to increase sensitivity and lower noise, but how do they do this if each cell is pointed in a different direction? The animal solves this problem by combining signals from six photoreceptors—not in the same ommatidium but in different ommatidia, all pointed in a similar direction. In this way, signals are summed from cells detecting light from approximately the same spatial location.

The photoreceptors of *Drosophila* are much smaller than those of *Limulus* and more difficult to study with microelectrodes. *Drosophila* ommatidia can be dissociated by mechanical trituration (Hardie, 1991; Hardie et al., 1991; Ranganathan et al., 1991), and single cells can then be used—with difficulty—for patch-clamp recording. The advantages of this species for the study of phototransduction are nevertheless very great. The *Drosophila* genome is entirely sequenced, much is known about its genetics and development, animals are easy to grow and reproduce rapidly, mutant animals with disrupted phototransduction can be readily isolated, and the range of available techniques for studying genes and proteins—including altering or knocking out particular genomic sequences—is as powerful for *Drosophila* as for any other multicellular animal.

Transduction in arthropods

We begin with *Drosophila* because so much has been learned about arthropod vision from its genetics and molecular biology. Starting in the late 1960s and early 1970s, a large number of mutant *Drosophila* were isolated with abnormal visual behavior or defective photoreceptor light responses (see Pak, 1995; Minke and Hardie, 2000; Hardie and Postma, 2008). This work led to the identification of genes necessary for some aspect of phototransduction. The most important are given in Table 9.1, together with the proposed function of the gene product in the sensory cascade (Pak, 1995; Montell, 1999; Xu et al., 2000).

These studies have demonstrated that the G-protein α subunit responsible for activation in *Drosophila* is a member of the $G\alpha_{q/11}$ family. Mutations in the gene for this protein produce large decreases in light sensitivity (see Figure 2.13C and Scott et al., 1995; Scott and Zuker, 1998). Mutations in another gene called *norpA* produce *no receptor potential*. This gene was subsequently cloned and shown to encode a PLC abundantly expressed in the eye (Bloomquist et al., 1988). Activation of PLC produces a large and rapid decrease in PIP_2 (Hardie et al., 2001), generating the two second messengers IP_3 and diacylglycerol (DAG) (see Figure 4.6). In many cells, IP_3 binds to IP_3 receptors and triggers Ca^{2+} release (Figure 4.8). The genome of *Drosophila* seems to have only a single gene for an IP_3 receptor, and this gene is known to be expressed in the eye. Nevertheless, animals genetically engineered so that expression of this gene has been knocked out still have nearly normal photoreceptor responses (Acharya et al., 1997; Raghu et al., 2000b; but see Kohn et al., 2015; Bollepalli et al., 2017), throwing considerable doubt on the role of IP_3 in *Drosophila* phototransduction.

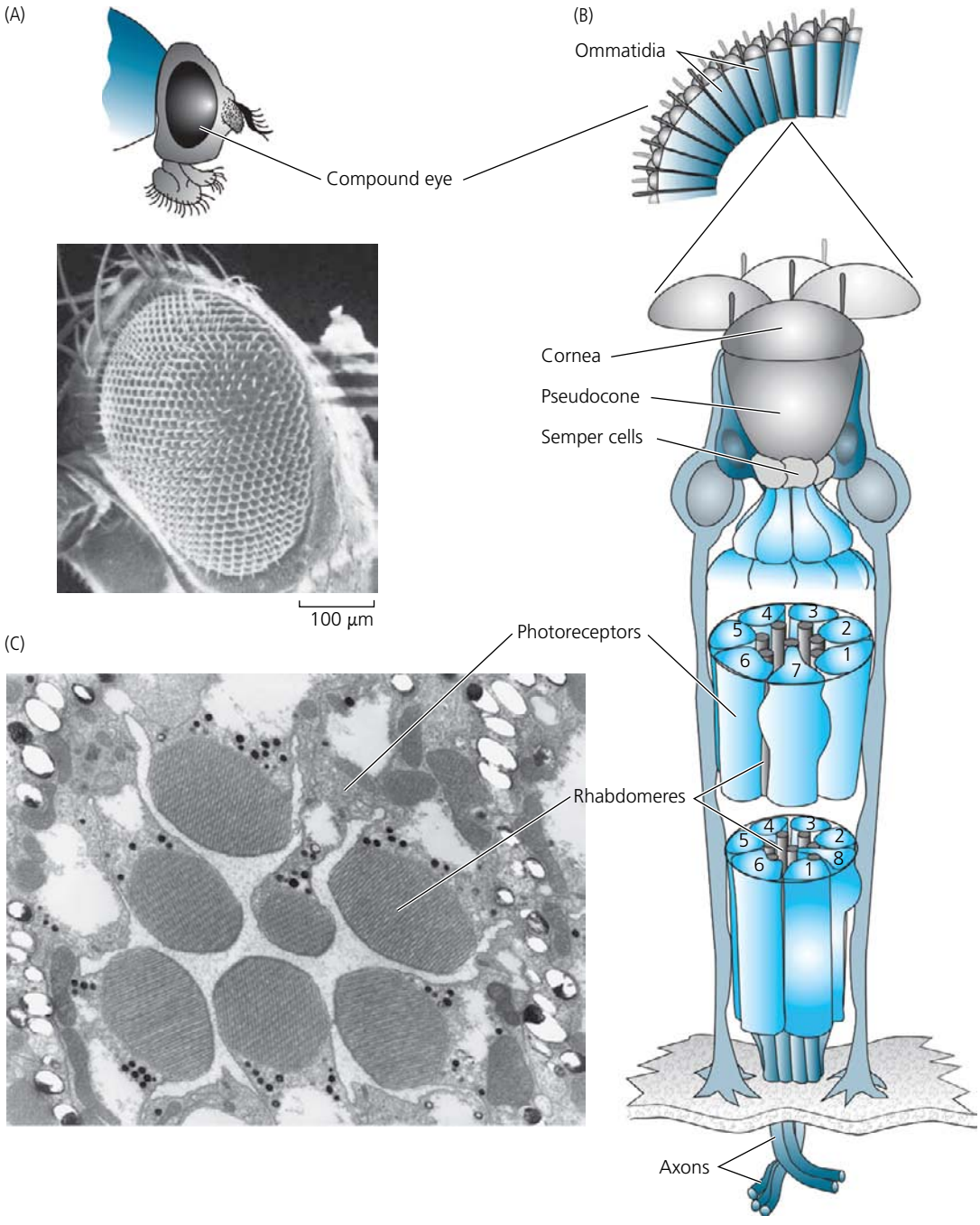

Figure 9.5 The compound eye of the fruit fly *Drosophila*. (A) Diagram and scanning electron micrograph of the compound eye. (B) The ommatidium. (C) Cross-section of the ommatidium. Magnification 11,000×. (A from Hodgkin and Bryant, 1978; B after Carlson et al., 1984; C courtesy of P. Raghu and R. Hardie.)

Table 9.1 Phototransduction proteins of *Drosophila*

Gene	Protein	Function
arrestin 1	arrestin 1	rhodopsin inactivation
arrestin 2	arrestin 2	rhodopsin inactivation
calx	Na^+/Ca^{2+} exchange protein	Ca^{2+} extrusion
cds	CDP-DAG synthase	PIP_2 metabolism
$G\alpha_q$	α subunit of G protein	activation
Gβ, Gγ	β and γ subunits of G protein	activation
inaC	protein kinase C	response turnoff
inaD	PDZ-containing protein	formation of transducisome/signalplex
ninaC	myosin III	response decay
ninaE	rhodopsin of R1–R6	visual pigment
norpA	PLC	activation
rdgA	DAG kinase	PIP_2/DAG metabolism
rdgB	phosphoinositol transfer-protein	PIP_2/DAG metabolism
rdgC	rhodopsin phosphatase	dephosphorylation of rhodopsin
trp	cation channel	light channel
trpl	cation channel	light channel

CDP-DAG, Cytidine diphosphate diacylglycerol.

The other possible second messenger, DAG, serves in many cells to activate protein kinase C (PKC). PKC seems to have some role in transduction, because mutations in a gene for PKC called *ina*C produce photoresponses with abnormal decay and adaptation. However, light responses in flies with eyes lacking PKC still show normal activation, so PKC cannot be essential for the production of the response. No genes have yet been isolated in screens of this sort for any aspect of cyclic-nucleotide metabolism.

These results show that activation in *Drosophila* photoreceptors is produced by binding of $G\alpha_q$•GTP to PLC, which hydrolyzes PIP_2 to generate IP_3 and DAG. What happens next is unclear. Although in *Drosophila* there is no evidence of a role of IP_3 receptors, the messenger of activation may be DAG itself (Raghu et al., 2000a; Estacion et al., 2001; Delgado et al., 2014) or some by-product of DAG metabolism, such as a polyunsaturated fatty acid like arachidonic or linolenic acid (Chyb et al., 1999). An important component of the transduction cascade may be DAG kinase (DGK), the enzyme that phosphorylates and inactivates DAG. This protein is encoded by the *rdgA* gene (Figure 9.6A), and mutations of this gene cause the channels to activate even in darkness (Raghu et al., 2000a). In Figure 9.6B, responses were recorded first from a *norpA* hypomorph, which expresses only a very small amount of the *norpA* protein, which is PLC. Because the photoreceptor has so little of this essential effector enzyme, the light response of the hypomorph gave only a few small, sporadic quantum bumps. If this PLC hypomorph also has a mutation in the *rdgA* gene so that almost no DGK is expressed, the response is augmented by more than a hundred times (Hardie et al., 2002). The simplest interpretation of this observation is that the decrease in the concentration of DGK, the enzyme that inactivates DAG, leads to a buildup of DAG; this in turn augments channel opening. This result would seem to implicate DAG or one of its metabolites in channel gating.

Activation of PLC also results in depletion of PIP_2 and the release of protons, which have been proposed together to gate the opening of the light-dependent channels (Huang et al., 2010). An intriguing possibility is that depletion of PIP_2, which is a significant constituent of the microvillar membrane, reduces the surface area of the membrane and stimulates

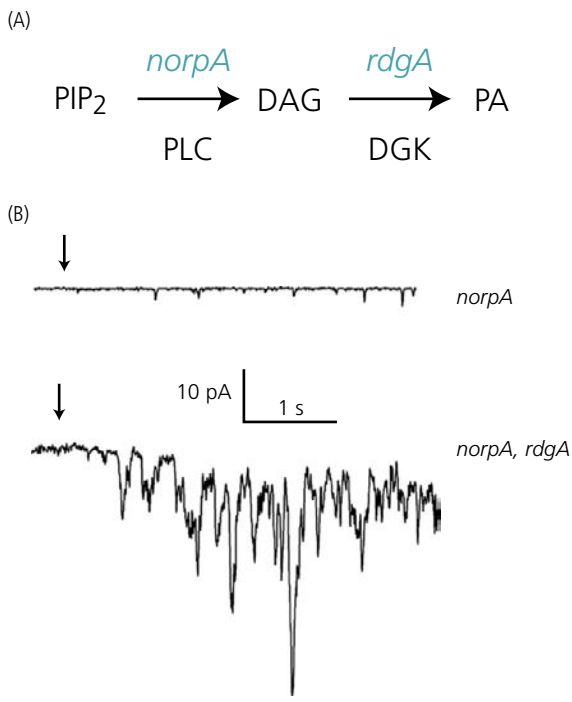

Figure 9.6 Evidence for an excitatory role of DAG. (A) Metabolism of DAG in *Drosophila* photoreceptor. PIP_2, Phosphatidylinositol 4,5-bisphosphate; *norpA*, gene for *Drosophila* PLC (phospholipase C); *rdgA*, gene for DGK (diacylgylcerol kinase); PA, phospatidic acid. (B) Mutations in DGK greatly augment responses in PLC and $G\alpha_q$ hypomorphs. Upper trace, a bright flash in a severe *norpA* hypomorph (*norpAP12*) expressing little PLC elicits no more than a few sporadic quantum bumps 1–2 pA in amplitude. Lower trace, the response to the same intensity is enhanced about one-hundred-fold in the double mutant *norpAp12*/*rdgA1* under-expressing both PLC and DGK. (From Hardie et al., 2002.)

the photoreceptor by mechanosensitive-channel opening (Hardie and Franze, 2012). In the experiment shown in Figure 9.7, an atomic force microscope was used to measure the change in the length of the phnotoreceptors. A 40-µm polystyrene bead was placed on top of the ommatidia in an intact retina, and the position of the bead was measured with a laser and photodiode. Light produced a graded decrease in the length of the rhabdomere (Figure 9.7A), presumably as more and more microvilli contracted. In response to a bright flash, the rhabdomeric length can shorten by as much as 0.5 µm. The decrease in length can precede the change in photoreceptor membrane potential (Figure 9.7B) and occurs over a similar range of light intensities. Moreover, light responses can be produced in photoreceptors lacking native light-dependent channels, if the photoreceptors are supplied with extrinsic mechanosensitive channels. These channels are apparently activated directly by the membrane stretch produced when the microvilli contract.

In contrast to the results from *Drosophila*, there is considerable support in *Limulus* for a direct role of both IP_3 and Ca^{2+} in transduction. The ventral photoreceptors contain abundant receptors for IP_3 (Ukhanov et al., 1998), and if these receptors are blocked, for example with the compound heparin (Frank and Fein, 1991) or with 2-aminoethoxydiphenyl borate (2-APB) (see Wang et al., 2002), the light response is inhibited. Injection of IP_3 into the R lobe of the photoreceptor produces both a release of Ca^{2+} from internal stores and a depolarization like the one produced by light (Figure 9.8A). The increase in Ca^{2+} is rapid and seems to occur even before the opening of the light-dependent channels (Ukhanov and Payne, 1997; Payne and Demas, 2000). The open symbols in Figure 9.8B give the time course of the free-Ca^{2+} concentration measured with a fluorescent dye at four different light intensities, and the lines show the time course of the voltage response.

Other experiments seem, however, to support a role for cyclic nucleotides in producing the *Limulus* light response (Johnson et al., 1986; Bacigalupo et al., 1991; Chen et al., 1999b, 2001a; Garger et al., 2001). Injection of cGMP into the R lobe of a ventral photoreceptor produces a depolarization like that produced by light (Figure 9.9A), and the hydrolysis-resistant cGMP analog 8-Br-cGMP applied to inside-out patches from microvillar membrane gates the opening of channels, which are similar in their properties to channels recorded from on-cell patches stimulated with light (Figure 9.9B). The light-dependent increase in Ca^{2+} (Figure 9.8) has been proposed in *Limulus* to stimulate a guanylyl cyclase (Lisman et al., 2002), producing an increase in cGMP that opens the channels (see also Deckert et al., 1992).

In summary, everyone agrees that light produces the activation of a PLC in arthropod photoreceptors. The PLC then produces IP_3 and DAG. The IP_3 generates a Ca^{2+} increase in *Limulus*, but most of the available evidence indicates that the great majority of the increase in Ca^{2+} in *Drosophila* is produced by influx through channels in the plasma membrane and that IP_3-gated Ca^{2+} release has little if any role

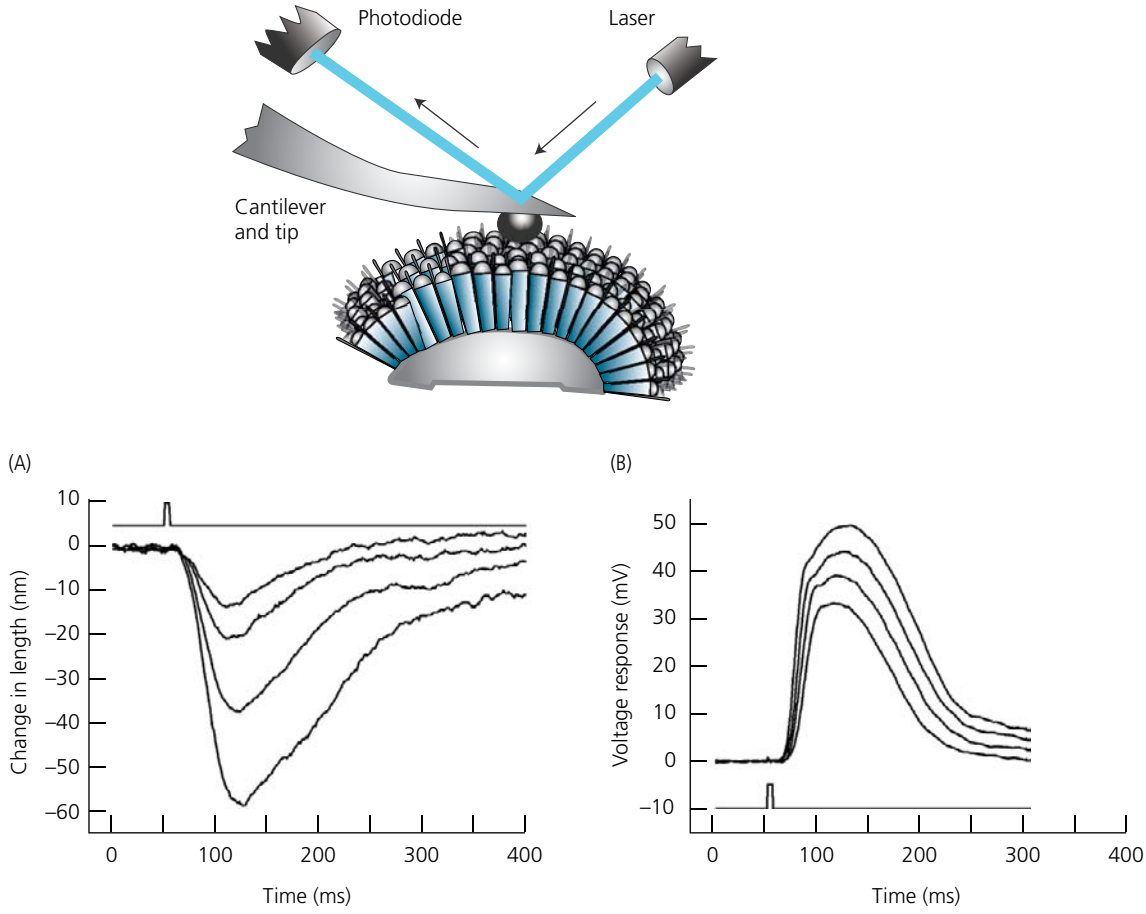

Figure 9.7 Illumination produces a change in length of the rhabdomere. Atomic-force microscopic measurements of the change in length of rhabdomeres in an intact retina. The polystyrene bead of the cantilever of an atomic force microscope was placed directly on top of the distal tips of the ommatidia in an excised *Drosophila* retina, and the change in position of the cantilever was measured with a laser and photodiode. (A) Contractions in a wild-type *Drosophila* retina in response to 5-ms flashes, with intensities from about 200 to 8000 effectively absorbed photons per photoreceptor. Timing of flashes is indicated by uppermost trace. (B) Voltage responses to the same stimuli from a whole-cell patch recording of a single dissociated photoreceptor. Absolute values of voltage are arbitrary. Timing of flashes is indicated by lowermost trace. (Courtesy of R. C. Hardie.)

in transduction. DAG, the other second messenger produced by PLC, typically activates a PKC. There is evidence of a role for PKC in both *Drosophila* (Hardie et al., 1993) and *Limulus* (Dabdoub and Payne, 1999) but no indication that PKC is required for the production of the light response. The channels may be activated by DAG itself or one of its metabolites. Light also depletes the microvillar membrane of PIP_2, which may trigger excitation in combination with proton production and membrane contraction. Some evidence suggests a role for cyclic nucleotides in *Limulus*, but there is no indication that cyclic nucleotides are responsible for channel gating in *Drosophila*.

Photoreceptor channels in arthropods

The first voltage-clamp recordings from *Limulus* (Millecchia and Mauro, 1969a) showed that light produces an increase in conductance primarily to Na^+ with a reversal potential between 10 and 15 mV positive of zero. Light also produces an increase in conductance to Na^+ in *Drosophila*, and there are two molecular forms of light-activated channels with a

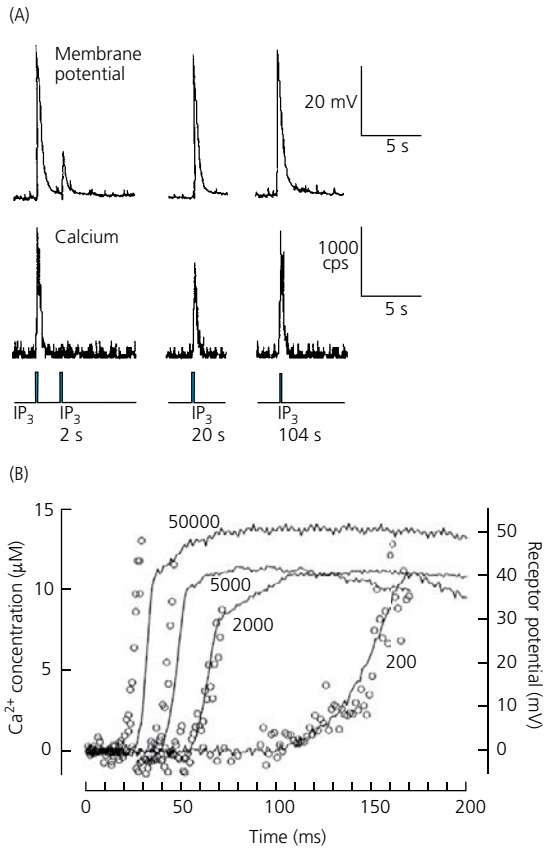

Figure 9.8 Calcium and IP_3 in *Limulus* ventral photoreceptors. (A) Effect of IP_3 injection on membrane potential and free-calcium concentration. Calcium was measured with the luminescent protein aequorin. cps, Counts per second measured by the photomultiplier tube from the luminescence of aequorin. Increase in counts indicates increase in the free-Ca^{2+} concentration. (B) Comparison of the time course of the increase in Ca^{2+} concentration measured with a fluorescent indicator dye (circles) with membrane potential (solid lines) at four different light intensities, given in units of effective photons next to each trace. (A from Payne et al., 1990; B from Payne and Demas, 2000.)

similar structure. The first to be identified and sequenced is encoded by the *trp* gene, which stands for *transient receptor potential*. This gene was the very first TRP-channel gene to be discovered and is the founding member of the large ion channel superfamily now known to be present throughout the animal kingdom, with for example twenty-seven distinct TRP channel genes in humans alone. The reason for the name TRP can be seen in Figure 9.10A (from Hardie et al., 2001). The response on the left is from a voltage-clamped wild-type *Drosophila* photoreceptor to a step of light 5 s in duration. The current is negative or inward as for the voltage-clamp currents of rhabdomeric photoreceptors in the proximal retina of *Pecten* (Figure 9.3A). The amplitude of the current is largest just at the beginning of the response, and the photoreceptor current then rapidly adapts and approaches a steady plateau level. In the *trp* mutant photoreceptors, on the other hand, the response decays back almost to the baseline even in the presence of maintained illumination (Figure 9.10A, right). There is little if any steady plateau response—compare the amplitude of the response near the end of the light stimulus in the two records.

The sequence of the *Drosophila trp* protein (Figure 9.11) shows many similarities to the thermoreceptor TRPV1 (Figure 3.6A) and mechanoreceptor NOMPC (Figure 5.10D). The amino-terminal end of the protein is cytosolic and contains ankyrin repeat units, but there are only four instead of the six in TRPV1 and twenty-nine found in NOMPC. The carboxyl end of the protein is also cytosolic and has several recognizable domains. Among the most interesting for our purposes are a calmodulin-binding domain suggesting possible modulation of the channel by Ca^{2+}-calmodulin, and a region near the carboxyl terminus for binding to a PDZ domain. We saw in Figure 2.5C that the INAD protein of *Drosophila* photoreceptors contains five protein-binding PDZ domains and forms a transducisome or signalplex, which is held in place within the microvillus (Li and Montell, 2000). The three-dimensional structure of the *Drosophila trp* protein has not yet been solved, but it is likely to be tetrameric like other channels of the TRP family (see Figures 3.6D and 5.12A, and Madej and Ziegler, 2018).

A second channel was subsequently discovered, whose gene was called *transient-receptor-potential-like* (*trpl*) (Phillips et al., 1992). The amino acid sequence of the *trpl* protein, though similar to that of *trp* and also containing ankyrin repeats and calmodulin-binding domains, lacks a region for binding to INAD and is not thought to associate with the transducisome. Both the *trp* and *trpl* proteins are localized to the rhabdomeres of the photoreceptors

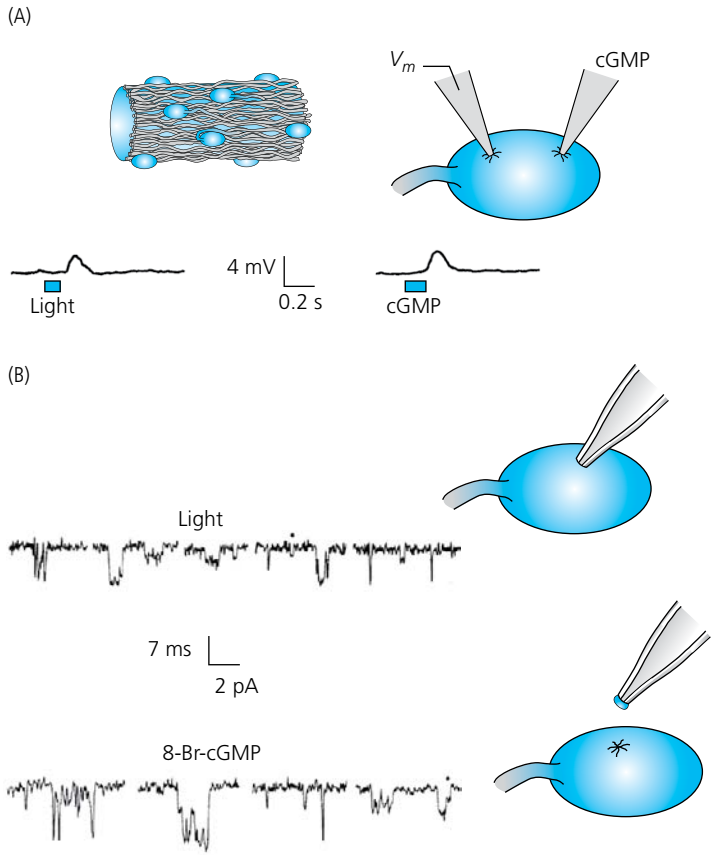

Figure 9.9 Evidence of cGMP-gated channels in ventral photoreceptors of *Limulus*. (A) Membrane potential was measured with an intracellular micropipette, and a second pipette was used to inject cGMP into the cell. Injection of cGMP produced a depolarization like that produced by light. (B) Comparison of light-activated single-channels recorded on-cell (above), and channel openings in an inside-out patch gated by the perfusion of 8-bromo-cGMP (below). (A from Johnson et al., 1986; B from Bacigalupo et al., 1991.)

(Huber et al., 1996; Niemeyer et al., 1996), but the *trp* protein is much more abundant (see Montell, 1999) and dominates the light response (Reuss et al., 1997).

The light responses of flies that are mutant for *trp* or for *trpl* behave differently, indicating that the *trp* and *trpl* channels have different properties (Niemeyer et al., 1996; Reuss et al., 1997). In particular, the light-dependent change in conductance recorded from these two mutant strains has a dramatically different permeability to Ca^{2+}. In the experiment shown in Figure 9.10B, voltage-clamp recordings were made with whole-cell patch pipettes containing a solution of 130 mM Cs^+ and no other permeable cations. This solution was dialyzed into the cell to replace the cations normally present. The external solution contained 10 mM Ca^{2+} and no other permeable cations. The reversal potential of the response was measured much as I described for hair cells in Chapter 3, and it was used to estimate the relative permeability of the channel to Ca^{2+} and Cs^+, that is P_{Ca}/P_{Cs}. As can be seen from the figure, the reversal potential was quite different for *trp* and *trpl* mutant photoreceptors. For wild-type flies, the value of the reversal potential was intermediate between the values for the two mutants. These experiments show that the TRPL channels left in the photoreceptors once the *trp* protein was deleted have a P_{Ca}/P_{Cs}

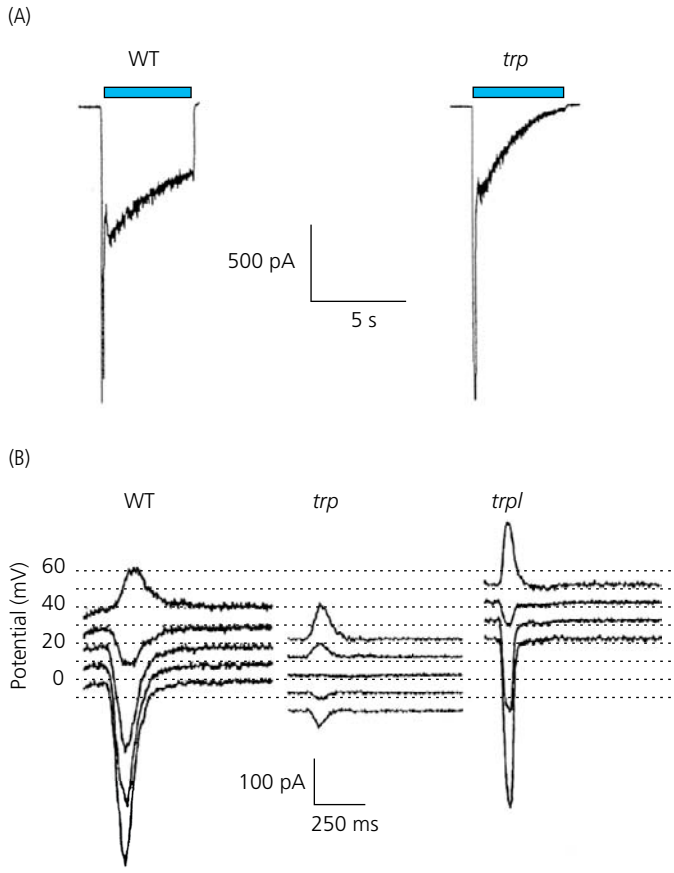

Figure 9.10 The TRP and TRPL channels of *Drosophila*. Currents were recorded from dissociated photoreceptors with whole-cell voltage clamp. (A) Photoreceptors from flies mutant for *trp* have transient light responses. (B) Measurement of the reversal potential of the light response from photoreceptors of three different lines of flies: WT (wild-type), *trp* (flies mutant for the *trp* gene and lacking functional TRP channels), and *trpl* (flies mutant for the *trpl* gene and lacking TRPL channels). Light responses to the same bright flashes were recorded for each photoreceptor at the different holding potentials indicated by the scale to the left. Notice that the reversal potential in *trp* photoreceptors is much less positive than for *trpl* or WT, showing that the TRPL channels left once TRP is removed have a much lower Ca^{2+} permeability than TRP or wild-type channels. (A from Hardie et al., 2001; B from Reuss et al., 1997.)

of only about seven, whereas TRP channels (in *trpl* mutant flies) have a surprisingly large P_{Ca}/P_{Cs} of about fifty-seven (Hardie, 2014). For both mutant strains and wild-type flies, Cs^+ and Na^+ were nearly equally permeant, that is P_{Cs}/P_{Na} was nearly one. TRP channels seem also to be present in the *Limulus* ventral eye (Bandyopadhyay and Payne, 2004), but their properties are likely to be somewhat different from those in *Drosophila*. The *Limulus* light-dependent conductance has considerably less Ca^{2+} permeability (see for example Brown and Mote, 1974).

The role of Ca^{2+} in the regulation of gain and turnoff

In both *Limulus* and *Drosophila*, light produces an increase in intracellular Ca^{2+} either by entry through the TRP channels from the extracellular medium or by IP_3-dependent release from intracellular stores. This light-induced increase in Ca^{2+} concentration may be as large as 100–200 μM and may act on transduction at some early stage. An experiment that supports such an interpretation is shown in

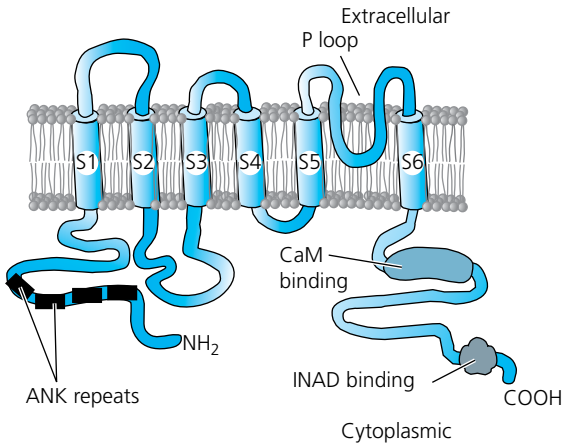

Figure 9.11 Predicted membrane topology of *the Drosophila trp* gene product.

Figure 9.12. This experiment utilized DM-nitrophen, often called caged Ca^{2+}. This molecule has the convenient property that it binds Ca^{2+} with high affinity, literally forming a cage around the calcium ion (Figure 9.12A). Bright ultraviolet light produces a photochemical reaction that causes the cage to fall apart, releasing the Ca^{2+}. In Figure 9.12B, a *Drosophila* photoreceptor was voltage-clamped with a whole-cell patch electrode (Hardie, 1995). Exposure to bright light (blue stimulus marker) produced an inward current with a latency of the order of 10 ms (note the rapid time base of the recording). Then, as the current was beginning to increase, a xenon flash lamp was triggered to give a brief but very intense exposure to ultraviolet light (arrows, Xe). In the first two records without DM-nitrophen, labeled "Controls," the flash lamp produced a brief deflection called the early receptor potential (ERP), caused by a movement of charge within the rhodopsin molecule itself; but there was no effect on the waveform of the light response produced by channel opening. In the third record, the patch pipette contained DM-nitrophen. When the effects of the xenon flash are compared in control photoreceptors and photoreceptors in which DM-nitrophen had been included in the patch pipette, the release of Ca^{2+} can be seen to produce an additional rapid increase in inward current, indicating a large and accelerated amplification of the photoreceptor response.

Ca^{2+} also has a role in response turnoff and light adaptation. In the experiment of Figure 9.12C, the delivery of the xenon flash-lamp exposure was given at a much later time after the stimulating light, and the xenon lamp then caused a rapid *decrease* in current. A Ca^{2+}-dependent desensitization is thought to be primarily responsible for light adaptation, both in *Limulus* (Lisman and Brown, 1975) and in *Drosophila* (Ranganathan et al., 1991; Minke and Hardie, 2000; Gu et al., 2005).

These results indicate that the rise in Ca^{2+} during the light response, produced either by IP_3-dependent release or by Ca^{2+} entry through the TRP channels, initially activates or facilitates the cascade by means of a non-linear boosting of the light response. In a rather short time, however, the increase in Ca^{2+} then accelerates the decay of the response and adapts the photoreceptor to maintained illumination. There are many possible sites of Ca^{2+} regulation of the cascade. When rhodopsin absorbs a photon, rhodopsin is converted to Rh*. The decay of Rh* occurs as for other G-protein receptors (Figure 4.1), by phosphorylation and binding of arrestin (Byk et al., 1993), though in *Drosophila* photoreceptors phosphorylation is not required for arrestin to bind and turn off the cascade (Vinos et al., 1997; Kiselev et al., 2000). Arrestin binding occurs much more slowly in the absence of external Ca^{2+}, and this Ca^{2+} dependence seems to be mediated by calmodulin and a protein called *neither inactivation nor afterpotential protein C* (NINAC), which is a form of myosin (see Table 9.1 and Liu et al., 2008). Moreover, arrestin can be phosphorylated by a kinase that is Ca^{2+} dependent (Matsumoto et al., 1994), and phosphorylation of arrestin may affect its association with rhodopsin (Alloway and Dolph, 1999). Dephosphorylation of rhodopsin is produced by the rdgC protein (Table 9.1), which is Ca^{2+} dependent. TRP channels have calmodulin binding sites and are inactivated by the binding of Ca^{2+}-calmodulin (Scott et al., 1997).

PKC, whose activity is boosted by an increase in Ca^{2+} concentration, also seems to have an important role in turnoff and light adaptation in *Drosophila* (Hardie et al., 1993). One of these roles appears to be the rapid down-regulation of PLC activity in response to Ca^{2+} influx through the TRP channels (Hardie et al., 2001). When there is little or no Ca^{2+}

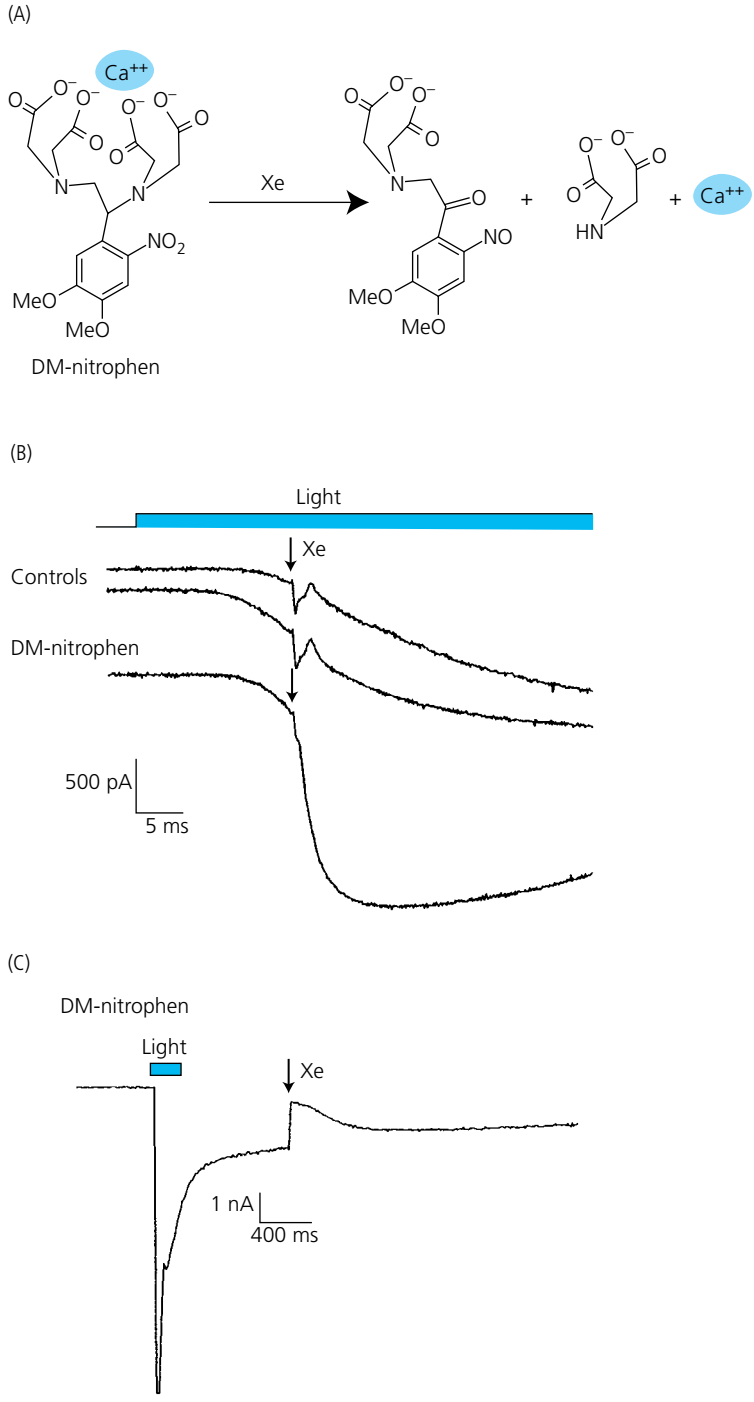

Figure 9.12 Effect of Ca^{2+} release on *Drosophila* photoreceptor light response. Whole-cell patch recording was used to load dissociated photoreceptors with DM-nitrophen (caged Ca^{2+}), from which the Ca^{2+} was released with brief illumination from a xenon flash lamp (Xe). The patch pipette was also used to record light responses. (A) Mechanism of Ca^{2+} release from DM-nitrophen. (B) Release of Ca^{2+} just after the beginning of the light stimulation augments the photoreceptor response. Light stimulus is indicated by blue bar. Records labeled "Controls" lacked DM-nitrophen. Small response to the xenon flash was produced by a conformational change of rhodopsin in response to the very bright illumination of the flash lamp. Record below with DM-nitrophen produced a rapid amplification of the response. (C) Release at later times produced an abrupt decrease in the response. (B and C from Hardie, 1995.)

entry, as for example in *trp* mutants lacking Ca^{2+}-permeant TRP channels, PIP_2 hydrolysis by PLC continues unabated until there is almost no PIP_2 left in the microvillus. This collapse of PIP_2 concentration is probably the cause of the aberrant waveform of the *trp* mutant photoresponse (Figure 9.10A).

All of these effects may participate to some degree in the modulation of the cascade and in light adaptation, though there is still no consensus which of these mechanisms is most important or exactly what Ca^{2+} does. We return to this subject later for vertebrates, where Ca^{2+} also plays an important role as a second messenger in light adaptation.

Vertebrate rods and cones

There are two kinds of vertebrate photoreceptors, rods and the cones (Figure 9.13). Both 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. The area of sensory membrane is greatly increased by numerous invaginations, which in rods detach from the plasma membrane as disks but in cones remain accessible to the extracellular solution as membrane lamellae. The repeat distance between disks or lamellae is about 30 nm and is rather uniform from species to species. The number of disks or lamellae is therefore mostly a function of the length of the outer segment: typical values are 1100 rod disks and 750 cone lamellae for the amphibian *Necturus* (Brown et al., 1963), and 1200 lamellae for a monkey cone (Dowling, 1965). The packing density of rhodopsin in the disk seems to be determined by the concentration necessary to maximize the probability of light capture. In a mammalian rod 25 μm long, something like two-thirds of the incident light will be absorbed at the wavelength of peak sensitivity of the photopigment.

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 9.14). The very edge of the disk forms a specialized structure called a rim, which is known to contain proteins not found in the rest of the outer segment. Molecules called peripherin and rom-1 are localized to the rim and seem to have an important role in the formation of the disks (see

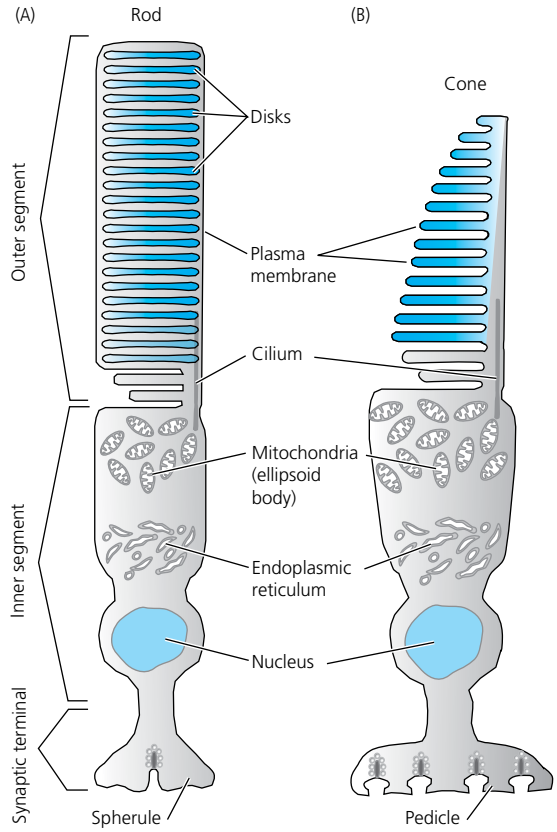

Figure 9.13 Vertebrate rods and cones. Principal structural features of vertebrate photoreceptors. (A) Rod. The outer segment is composed of disks detached from external plasma membrane. (B) Cone. The outer segment has membrane infoldings or lamellae instead of disks.

Molday, 1998; see also Burgoyne et al., 2015; Ding et al., 2015; Volland et al., 2015). This part of the disk also contains the ABCR/Rim protein, a transporter (or flippase) that moves retinal and lipid across the disk membrane from the inside surface of the membrane to the cytoplasmic surface (see Molday et al., 2009).

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*, 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 ER and nucleus lie below the ellipsoid body, and at the proximal end of the cell there is a presynaptic terminal.

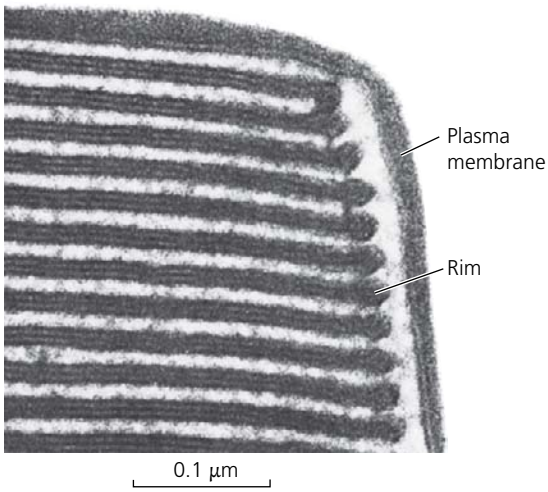

Figure 9.14 Low power electron micrograph of rod outer segment showing disk rim. Note fibrous protein between adjacent disks and between rim and plasma membrane. (Courtesy of Walter Schröder.)

Photoreceptors are secondary receptor cells lacking axons or voltage-gated Na^+ channels, and they do not in general produce Na^+-dependent action potentials (but see Kawai et al., 2001; Ohkuma et al., 2007). The change in membrane potential produced by light is communicated at specialized synapses onto second-order horizontal and bipolar cells. As for invertebrate photoreceptors (Figure 2.12A), the presynaptic terminals contain dense bodies that in rods and cones are called synaptic ribbons and resemble the presynaptic structures of electroreceptors (Figure 2.12B) and hair cells (Figure 6.5C).

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 9.15A). When the ribbons are followed through many serial sections, their shape can be reconstructed (Figure 9.15B), and they can be seen to occupy much of the synaptic ending and bind hundreds of synaptic vesicles. The major component of the ribbon is a protein

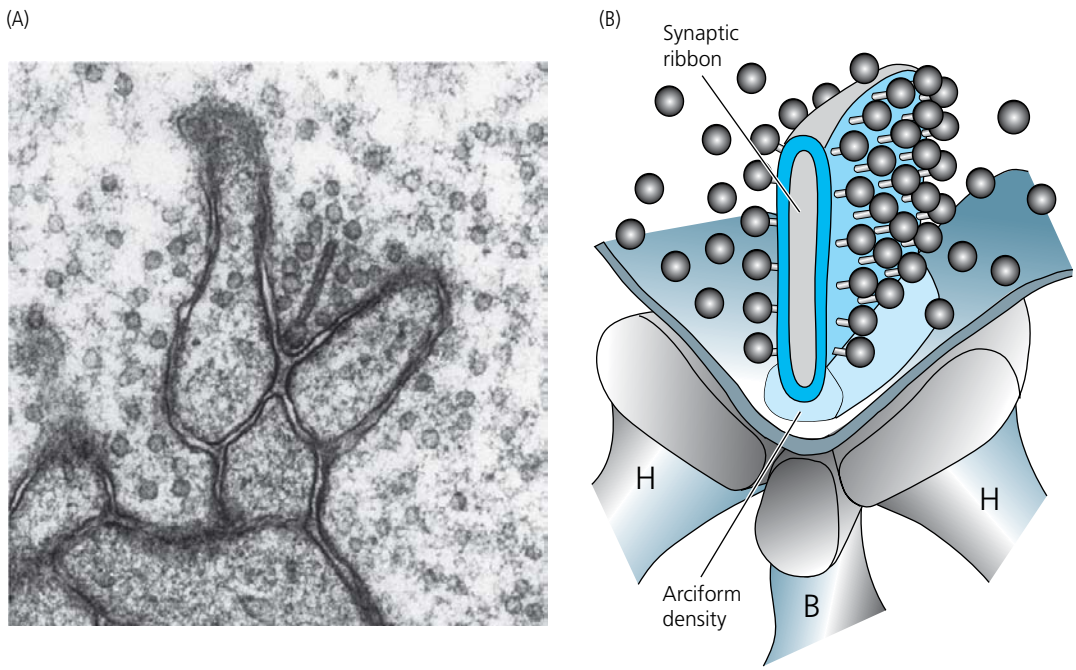

Figure 9.15 Photoreceptor synapse. (A) Electron micrograph of a synapse of a primate cone. Magnification 70,000×. (B) Schematic drawing of a photoreceptor synapse. H, Horizontal-cell process; B, bipolar-cell process. (A courtesy of S. J. Schein.)

called ribeye, which is associated with many other proteins including piccolo and bassoon (see Zanazzi and Matthews, 2009). These proteins together facilitate the movement of vesicles to their release sites just adjacent to another specialized structure, called the arciform density. Vesicle release is Ca^{2+} dependent and mediated by $Ca_V1.4$ voltage-gated Ca^{2+} channels. Because both rods and cones are depolarized in darkness and hyperpolarize to light, the release of synaptic transmitter is continuous in darkness and decreased by illumination (Dowling and Ripps, 1973; Cervetto and Piccolino, 1974). The synaptic transmitter is glutamate (see for example Ishida and Fain, 1981; Copenhagen and Jahr, 1989).

Transduction in vertebrate photoreceptors

The mechanism of activation in a vertebrate photoreceptor is now fairly clear (Figure 9.16A). The formation of Rh* produces a change in the conformation of the parts of the rhodopsin molecule exposed to the cytoplasm, primarily a shift of the sixth transmembrane domain outward toward the cytoplasmic surface of the lipid bilayer, and a smaller, similar movement of the fifth transmembrane domain (Figure 4.3B). These movements open up 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 transducin α subunit (Tα). GDP then falls off this binding site, and GTP binds in its place. Tα•GTP separates from the transducin β and γ subunits and comes off the disk membrane, diffusing within the cytoplasm of the photoreceptor between the disks of rods or membrane lamellae of cones (Kuhn et al., 1981).

Tα•GTP then binds to an effector enzyme, which for vertebrate photoreceptors is a cyclic nucleotide phosphodiesterase (PDE6). Rod PDE6 is a tetramer with α and β catalytic subunits and two inhibitory γ subunits (Figure 4.5B). Cone PDE6 is similar, but the two catalytic subunits (α′) are identical and different in sequence from those of the rod. The inhibitory γ subunits are also distinct in cones. In the inactive rod or cone enzyme, the γ subunits prevent the catalytic subunits from hydrolyzing cGMP by blocking access to the catalytic regions of the enzyme. The conformation of the γ subunits changes when Tα-GTP is bound, exposing the catalytic regions of PDE6 and greatly increasing the activity of the enzyme. Each catalytic subunit also contains a high-affinity, non-catalytic binding site for cGMP, and binding of cGMP to these sites can affect the nature of the interaction of the PDE6 inhibitory γ subunit with the α and β catalytic subunits (D'Amours and Cote, 1999). The affinity of cGMP for these non-catalytic sites is, however, fairly high, with the result that cGMP probably comes on and off too slowly to make much of a contribution to the photoreceptor light response (Calvert et al., 1998).

For every thousand rhodopsin molecules in the disk membrane of a rod, there are about a hundred transducins and something like ten PDE6 molecules (Figure 9.16B). As we saw in Chapter 2, rhodopsin can diffuse within the surface of the disk or cone lamella, and PDE6 and the inactive transducin heterotrimer are also attached to the membrane and membrane diffusible. Attachment to the membrane augments the chance of collision of these molecules—in effect, the disk or lamellar membrane acts as a catalyst. As a result, a single Rh* during its lifetime can collide randomly with many transducin molecules and produce many molecules of Tα-GTP, perhaps ten to fifteen in a mouse rod but several hundred in the much larger rods of amphibians (Leskov et al., 2000; Krispel et al., 2006; Reingruber et al., 2013;Yue et al., 2019).

Although the sensory cascade of vertebrate photoreceptors relies upon random collisions of membrane proteins instead of a highly organized transducisome/signalplex as in arthropod photoreceptors, transduction in rods and cones is surprisingly fast and efficient. Single-photon voltage responses in rods are of the order of 1 mV (Fain, 1975; Schneeweis and Schnapf, 1995)—smaller than for arthropod photoreceptors, but sufficiently large to produce reliable detection. The minimum latency of the photoreceptor to a bright light flash is only 7 ms for both rods and cones (Cobbs and Pugh, 1987; Hestrin and Korenbrot, 1990), not much different from that for *Drosophila* photoreceptors (see Figure 9.12B). These are the fastest G-protein, second-messenger cascades known to science.

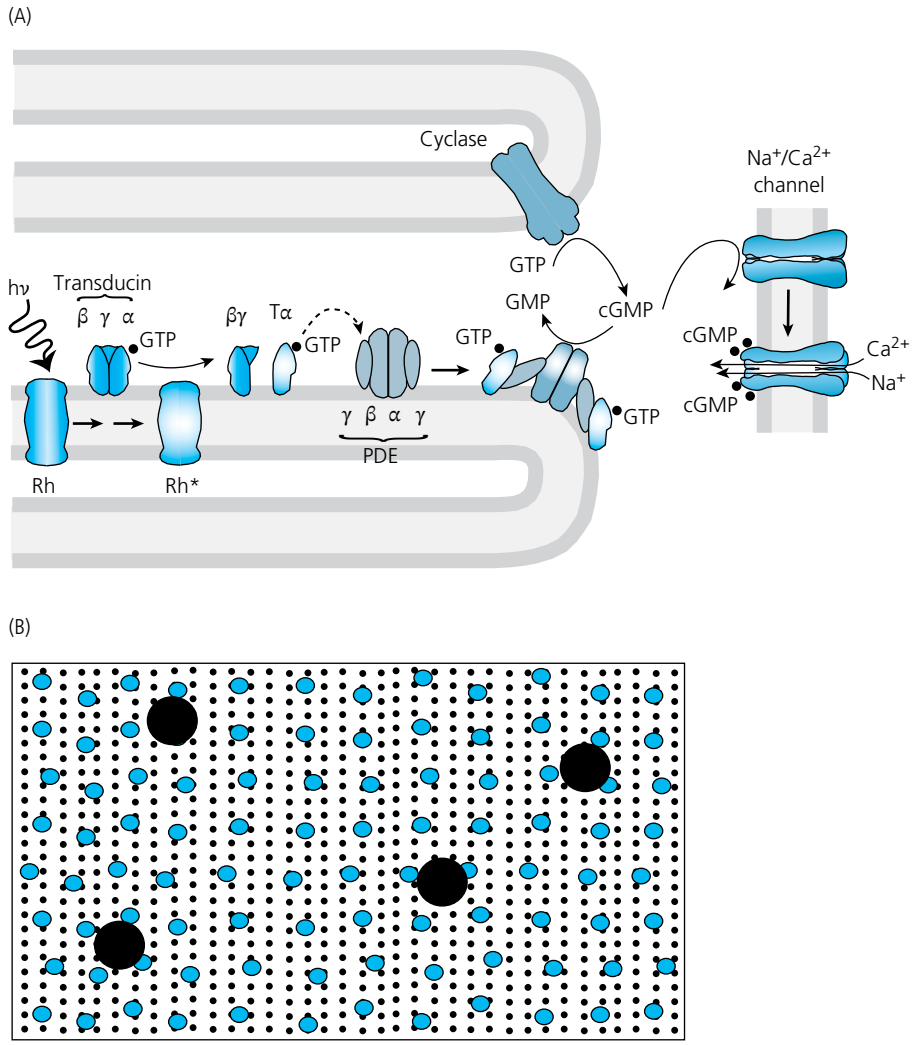

Figure 9.16 Vertebrate phototransduction. (A) Transduction cascade. (B) Relative proportions of rod transduction proteins. The small black dots represent rhodopsin molecules, the blue dots represent transducin, and the large black dots represent PDE6 molecules. Only about a third of the PDE6 molecules have been illustrated for clarity. The distribution of molecules would be much less regular in an actual disk membrane because all of these proteins can diffuse rather freely in the lipid of the disk. A disk membrane of this dimension would have adjacent to it an average of one free cGMP molecule. (A after Pugh and Lamb, 1993; B after Bownds and Arshavsky, 1995; Fain, 1999.)

Ion channels of rods and cones

Figure 9.17 gives the principal types of ion channels and transporters in a vertebrate photoreceptor. 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 heterotetramers, consisting of α subunits called CNGA1 for rods and CNGA3 for cones, and β subunits called CNGB1 for rods and CNGB3 for cones. Both rod and cone channels have a stoichiometry of $\alpha_3\beta$ (Weitz et al., 2002; Zheng et al., 2002; Zhong et al., 2002). All of the channel subunits have different though related amino-acid sequences, with six transmembrane domains, a P region forming the channel pore, and a cytoplasmic carboxyl terminus with a

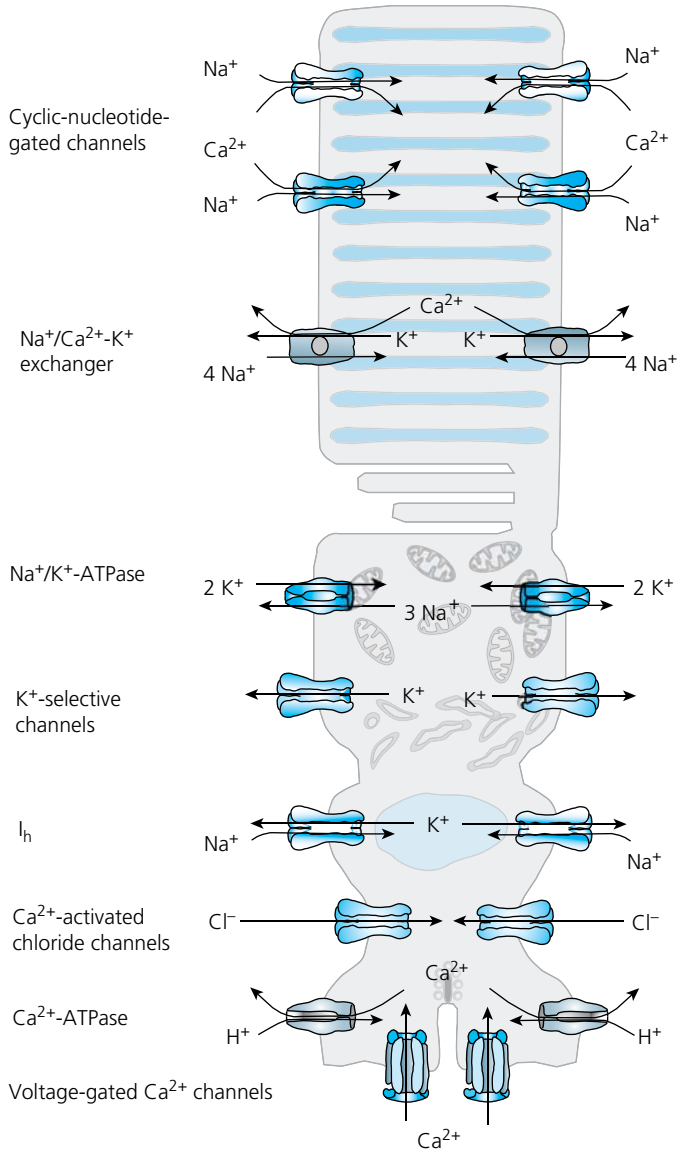

Figure 9.17 Channels and transporters in the membrane of a vertebrate rod. Major proteins responsible for ion conduction and transport are different in the inner and outer segment. See text.

binding site for cyclic nucleotide (see Figures 4.10B and 4.11). In rods but not cones, the β subunit has a large cytoplasmic amino-terminal region called the glutamic-acid-rich protein (GARP), which can also be secreted into the cytoplasm as a separate, soluble protein. GARP may play some role in protein–protein interactions in the outer segment (Korschen et al., 1999) or in channel gating (Michalakis et al., 2011). Channels in both rods and cones are quite selective for cGMP over cAMP, unlike channels in olfactory receptors which bind both nucleotides nearly equally; but rod channels bind even cGMP with less affinity than olfactory channels bind either nucleotide.

The cGMP-gated channels in rods and cones, like those in the lizard parietal eye (see Chapter 4), are rather non-specifically permeable to monovalent

cations and actually more permeable to Ca^{2+} than to Na^+. Since, however, the Na^+ concentration is so much higher than the Ca^{2+} concentration in the extracellular medium, only of the order of 10–15 percent of the current entering the cyclic-nucleotide-gated channels in a rod is carried by Ca^{2+}. Cones have channels different from those in rods, and nearly twice as much of the current or about 20–30 percent can be carried by Ca^{2+} (Perry and McNaughton, 1991; Ohyama et al., 2000). As we shall see, this Ca^{2+} influx has an important role in the physiology of the light response.

As in olfactory receptor cells, the entering Ca^{2+} is removed by a very active transport molecule, which 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 stoichiometry has the result that four charges are moved inward and three charges outward for each cycle of the transporter, so that, like the Na^+/K^+ ATPase, Na^+/K^+-Ca^{2+} exchange is electrogenic. The inward current carried by the transporter can actually be recorded (see Figure 9.22), and this observation provided 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 at least in rods, 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 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 voltage-gated channels called I_h (or HCN channels) permeable to both Na^+ and K^+ and activated by hyperpolarization (Fain et al., 1978; Hestrin, 1987; Wollmuth and Hille, 1992). There are also Ca^{2+}-activated Cl^- currents especially prominent in cones (Barnes and Hille, 1989; Jeon et al., 2013), which may help stabilize the membrane potential during synaptic transmission (Lalonde et al., 2008). Finally, at the synaptic terminal there are voltage-gated ($Ca_V1.4$) Ca^{2+} channels near the release sites, which regulate the exocytosis of the vesicles. The Ca^{2+} entering the rod or cone at the synaptic terminal is removed primarily by a Ca^{2+} ATPase rather than by Na^+/K^+-Ca^{2+} exchange (Krizaj and Copenhagen, 1998; Morgans et al., 1998).

The photocurrent

In darkness, the cyclic-nucleotide-gated channels in both rods and cones are gated open by the resting concentration of cGMP in the outer segment. Because these channels are selectively permeable to cations, and in particular to Na^+ and Ca^{2+}, the light-dependent conductance has a reversal potential similar to that of hair cells (see Figure 3.14), somewhat positive of 0 mV. In darkness the resting membrane potential of a rod or cone is about –35 mV, with the consequence that there is a standing current through the channels in darkness which we can calculate from Eq. (3.7):

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

The conductance g is positive (the channels are open), and $(V_m - E_{rev})$ is approximately (–35–0) or about –35 mV. The current i_m will therefore be negative, or inward, from the extracellular space into the outer segment. This standing inward current in darkness was first discovered by Penn and Hagins (1969) and was given the appropriate name *dark current*.

The large resting conductance of the cell to Na^+ produced by the cyclic-nucleotide-gated channels is responsible for the rather positive value of the resting membrane potential in darkness, which, as we have said, is of the order of –35 mV. This value is 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, which is near zero. Light decreases the probability of opening of the cyclic-nucleotide-gated channels and decreases the Na^+ permeability. The decrease in Na^+ permeability causes the membrane potential of the rod to hyperpolarize—just the opposite of the effect of the increase in Na^+ permeability which occurs during the firing of an action potential.

Figure 9.18A 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 (from Baylor and Nunn, 1986). 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 9.18A). Hyperpolarization activates the I_h channels, and since these channels are also rather permeable to Na^+ (Wollmuth and Hille, 1992), the I_h current has a reversal potential near the dark resting membrane potential of the photoreceptor. As the I_h channels activate during the light response, they cause the membrane potential rapidly to depolarize 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 of the salamander rod 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. These channels are mostly localized to the outer segment, and they seem to be the only functioning channels in this part of the cell. Suction-electrode recordings of light responses are shown in Figure 9.18B from the same cell for which voltage responses are given (Figure 9.18A). They show that a sustained dark current of about –35 pA flowing into the outer segment is decreased when the rod is illuminated with brief flashes. Bright light closes all of the cyclic-nucleotide-gated channels and reduces the current entering the outer segment to zero (Baylor et al., 1979b).

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

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

and since during voltage clamp $V_m = V_c$ and for the cyclic-nucleotide-gated channels E_{rev} is near zero, $\Delta i \sim \Delta g\,(V_m)$. As a result, changes in the value of membrane voltage during the light response like those shown in Figure 9.18A would be expected to influence the value of the current. This possibility was examined by Baylor and Nunn (1986), who recorded outer segment currents with a suction electrode from salamander rods that were simultaneously voltage clamped (Figure 9.18C). The upper traces show the total membrane current 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. Because 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 currents is smaller than that of the voltage-clamp currents, 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 had been brought into the pipette. What is remarkable, however, is that the relative amplitude and time course of the suction-electrode currents in Figure 9.18C are quite similar to those of the currents recorded in Figure 9.18B, where no voltage clamp was used.

The reason for this correspondence is that the change in conductance Δg in Eq. (3.8) turns out to be voltage dependent. The voltage dependence isn't very large, but it is about the same magnitude and opposite in polarity to the change in the driving force, $(V_m - E_{rev})$. As a result, the photoreceptor current $\Delta g(V_m - E_{rev})$ shows very little dependence on voltage as V_m changes during the light response, because the change in V_m is nearly compensated by the voltage-dependent change in Δg. This has the happy consequence that suction-electrode recording, which is much easier than voltage clamping, gives a fairly faithful representation of the waveform of the light response of a salamander rod. Whether this circumstance is also true for cones or for photoreceptors in other species remains an open question.

Suction-electrode recordings have been made from the photoreceptors of many vertebrates, even from

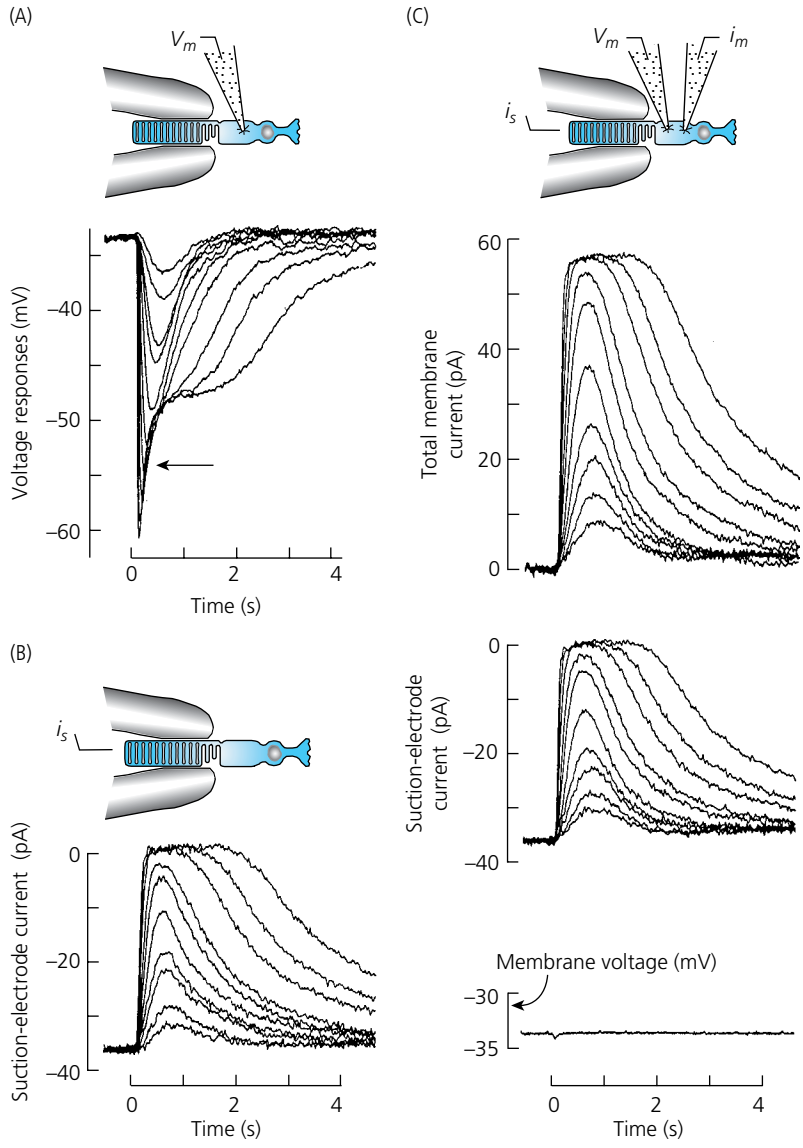

Figure 9.18 Electrical recording from a vertebrate rod. The responses of a salamander rod to a series of brief flashes given at t=0 of increasing intensity obtained by (A) intracellular recording, (B) suction-electrode recording, and (C) voltage clamp. Arrow in (A) indicates rapid repolarization of response produced by activation of voltage-gated I_h (HCN) channels. In (C), the upper records show total membrane current measured with the voltage-clamp circuit, and the lower records show suction-electrode currents measured from the same voltage-clamped rod from the outer segment. Lowermost trace shows that there was no change in the voltage of the rod during the voltage-clamp measurements. (After Fain, 1999 and 2014, with data published by permission from Baylor and Nunn, 1986.)

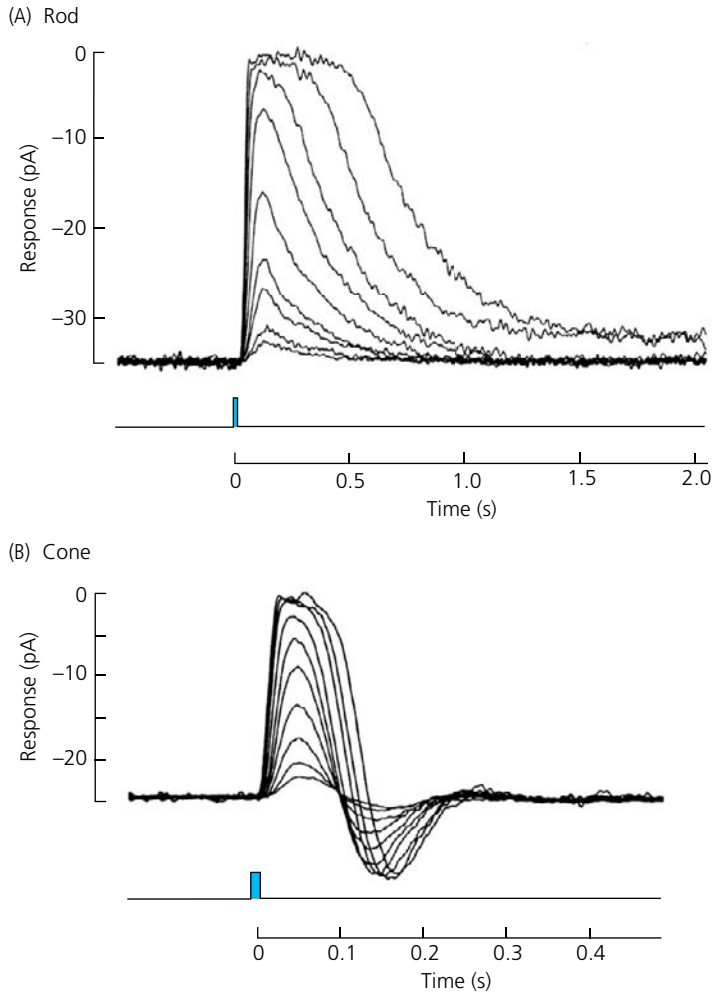

Figure 9.19 Suction-electrode recordings from photoreceptors of monkey (*Macaca fascicularis*). Responses of (A) rod and (B) long-wavelength-sensitive (L) cone to a series of flashes of increasing intensity. Note difference in time scale. (A from Baylor et al., 1984; B from Baylor et al., 1987.)

the very small rods and cones of mammals. Figure 9.19 illustrates the responses of monkey rods (Baylor et al., 1984) and cones (Baylor et al., 1987), each to a series of brief flashes of increasing intensity. Rods in mammals are typically about one-hundred times more sensitive to light than cones. Notice also the difference in time scale: responses of cones reach peak amplitude and decay much more rapidly than do those of rods.

Why are rods so much more sensitive? One possibility is the difference in anatomy—rods have detached, closed disks, whereas cones have open membrane lamellae. This difference in anatomy seems, however, not to contribute to the difference in sensitivity. The evidence comes from lamprey, which (with hagfish) are cyclostomes and the last remaining representatives of vertebrates without jaws. Lamprey rods and cones have an identical anatomy; that is, both rods and cones have membrane lamellae without disks. They nevertheless respond very much like the rods and cones of other vertebrates. Lamprey rods are about seventy times more sensitive than lamprey cones, and lamprey rods have single-photon responses as large as those

in mammals (Morshedian and Fain, 2015; Asteriti et al., 2015).

The difference in sensitivity between the two kinds of photoreceptors may rather result from molecular differences in the cascade. Rods and cones contain different isoforms of most of the proteins involved in sensory transduction (see Ingram et al., 2016), including the photopigment, transducin, PDE6, the cyclic-nucleotide-gated channel subunits, and the Na^+/K^+-Ca^{2+} transporter. Even when the proteins are the same, they can be expressed at very different levels (Cowan et al., 1998; Zhang et al., 2003a). Cone channels are more permeable to Ca^{2+} and can extrude Ca^{2+} more quickly by Na^+/K^+-Ca^{2+} exchange (Sampath et al., 1999).

Many attempts have been made to investigate the effects of each of these molecular differences, often by exploiting the tools of molecular biology to replace the rod protein with the cone protein and measure changes in photoreceptor sensitivity and response waveform (summarized in Ingram et al., 2016). No one change seems to be responsible for more than a factor of two or three of the difference in sensitivity. Because rods emerged after cones (Nickle and Robinson, 2007; Shichida and Matsuyama, 2009), it is likely that the rods evolved from cones by a series of small changes in many of the transduction proteins until the sensitivity of the rod was sufficient to produce single-photon responses enough above the noise of transduction to be detectable. All of these changes seem to have happened very early, before the lamprey (and the rest of the cyclostomes) separated from the other vertebrates in the late Cambrian. Darwin wondered how an organ as complicated as the human eye could possibly have evolved, and he speculated that eons of time must have been required. In truth, an eye like our own was present very early in vertebrate evolution. The physiology of vertebrate photoreceptors has remained nearly unaltered for 500 million years (Morshedian and Fain, 2017).

Shutting down the light response

Activation of the sensory cascade must be followed by response decay. The faster decay can occur, the more rapidly the cell can detect another stimulus, and the more accurately the visual system can signal change and motion. All the steps in transduction must be returned to their initial condition: Rh* must be inactivated, Tα must come off the inhibitory subunits of the PDE6 and recombine with Tβγ, the cyclic-nucleotide concentration must be restored to its dark level, and the channels must re-open. The steps in decay are highly orchestrated and can be modulated, at least in part, by changes in Ca^{2+} concentration.

The inactivation of Rh* occurs as for other G-protein-coupled receptors (see Figure 4.1). 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. Phosphorylation (Bownds et al., 1972; Kuhn and Dreyer, 1972) and arrestin binding (Kuhn, 1978) of G-protein receptors were first discovered for vertebrate rods and have been extensively characterized. The carboxyl terminus of rhodopsin contains six or seven serine and threonine groups (depending on the species), which can all be phosphorylated (Wilden and Kuhn, 1982). Rh* inactivation seems to proceed by phosphorylation of at least three of these residues (Vishnivetskiy et al., 2007), with the phosphorylation of serine and threonine residues having somewhat different effects (Azevedo et al., 2015). If rhodopsin phosphorylation is prevented either by genetically altering rhodopsin to remove its carboxyl tail (Chen et al., 1995b; 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 (Figure 9.20A, *Rh truncation*).

Rods have two molecular forms of arrestin, which are both splice variants of the same gene and have different affinities for rhodopsin (Palczewski et al., 1994; Burns et al., 2006). In mice for which the arrestin gene has been disrupted (–/–, Figure 9.20A), 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. The reason for this difference seems to be that phosphorylation even without arrestin binding can produce some inhibition of transducin binding (Wilden et al., 1986). The phosphorylation of rhodopsin and binding of arrestin happen rather quickly,

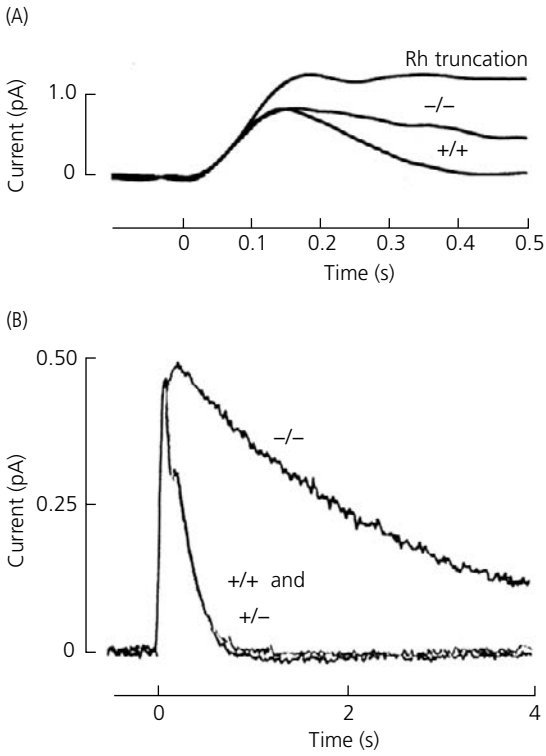

Figure 9.20 Components of rod response decay. (A) Average single-photon response of a rod recorded with a suction-electrode in wild-type mouse (+/+), in a mouse in which the gene for arrestin had been disrupted (–/–), and in a mouse in which the gene for rhodopsin had been altered so that the C-terminus of the molecule had been truncated, removing all of the sites of protein phosphorylation (Rh truncation). (B) Same as for part (A) but from mouse lacking photoreceptor RGS9 protein (–/–), a wild-type mouse (+/+), and a mouse heterozygous for the *rgs9* gene (+/–). (A from Xu et al., 1997; B from Chen et al., 2000.)

for a mouse rod probably with a time constant of no more than about 40 ms (Krispel et al., 2006; Chen et al., 2010a).

As for other heterotrimeric G proteins (see Chapter 4 and Figure 4.4), the inactivation of Tα-GTP and restoration of the Tαβγ complex requires the hydrolysis of GTP to GDP on the Tα guanosine nucleotide binding site. Although transducin by itself can hydrolyze GTP, the rate is rather slow. It is, however, greatly accelerated by GTPase-activating proteins (GAPs), and in particular by the protein RGS9 (He et al., 1998), which is abundant in rods and found at an even higher concentration in cones (Cowan et al., 1998; Zhang et al., 2003a). RGS9 is present in the outer segment in a tight *GAP complex* together with a G-protein β subunit called Gβ5L (Makino et al., 1999; He et al., 2000), which is not the same as the β subunit of transducin, and the R9AP binding protein, which attaches the GAP complex to the disk or lamellar membrane (Hu and Wensel, 2002). The rate of turnoff is further accelerated by the PDE6γ subunit (Arshavsky and Bownds, 1992; Tsang et al., 1998), though PDE6γ seems to have little effect on its own and requires RGS9 (Chen et al., 2000). In mice in which the gene for the RGS9 protein has been disrupted (Chen et al., 2000), the recovery of the light response is again greatly retarded (Figure 9.20B).

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 (Figure 4.2). They are present in the outer segment as homodimers and are integrated into the disk or lamellar membrane (Yang and Garbers, 1997). Like other membrane-bound guanylyl cyclases, these proteins have an "extracellular" ligand-binding domain, which has no known ligand and is located in rods inside the disk (Figure 9.21A). This part of the protein is then 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 the 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 Na^+/K^+-Ca^{2+} exchanger. When the photoreceptor is illuminated, the channels close, which decreases the entry of Ca^{2+} into the outer segment. The exchanger seems not to be directly affected by light (Nakatani and Yau, 1988a; 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. In effect, the closing of the channels by light causes a decrease in the Ca^{2+} concentration (Figure 9.22), from a dark level in a salamander rod of about 400–600 nM to as low as 5–10 nM when the channels are all closed (see Fain et al., 2001); and from about 250 nM to less than 25 nM in a mouse rod (Woodruff et al., 2002).

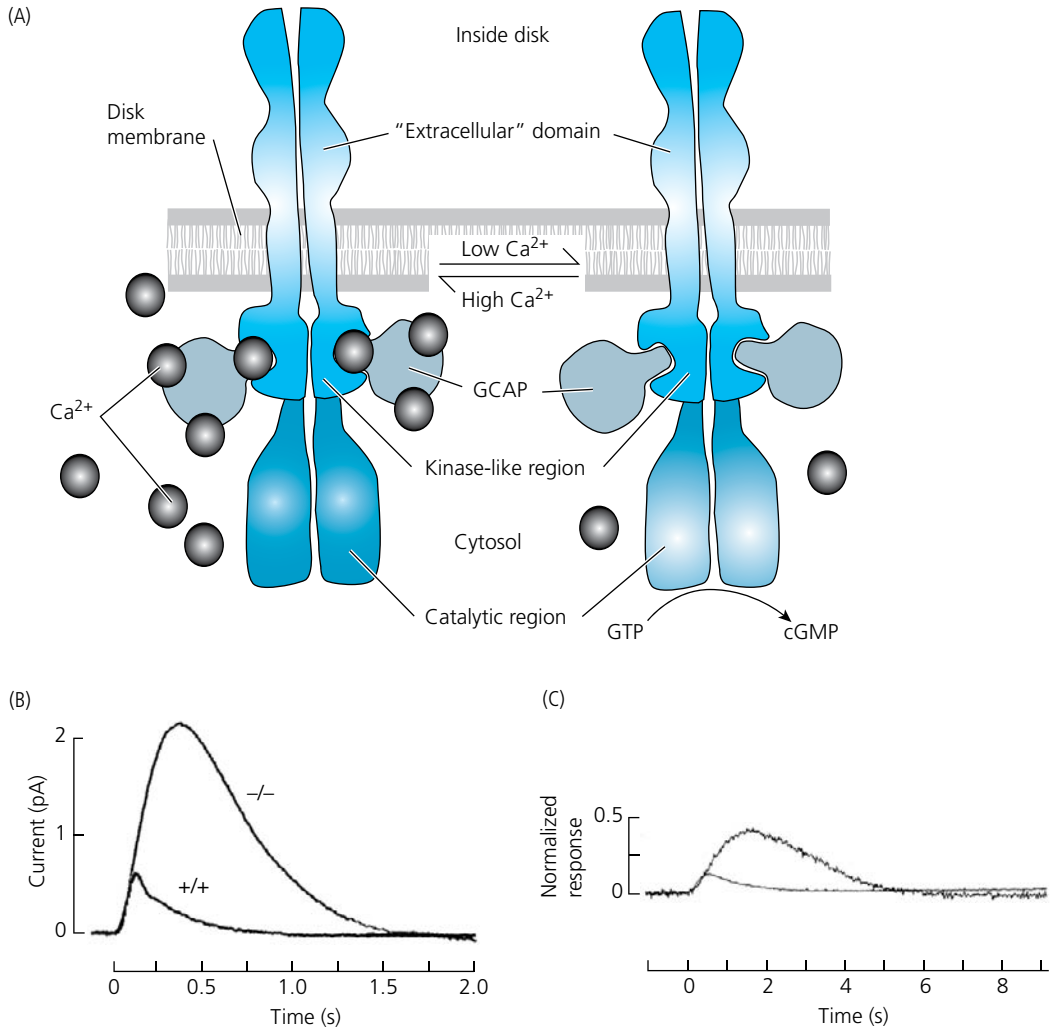

Figure 9.21 Role of Ca^{2+}-dependent regulation of guanylyl cyclase in response decay. (A) Schematic diagram of membrane-bound guanylyl cyclase and GCAPs in rod disk membrane. (B) Average single-photon response of a rod recorded with a suction-electrode from a wild-type mouse rod (+/+) and from a rod for which both GCAP genes had been disrupted (–/–). Note much larger amplitude and slower decay after GCAP deletion. (C) Small-amplitude responses normalized to peak response amplitude for a salamander rod in Ringer solution (smaller response) and in 0-Ca^{2+}/0-Na^{+} solution (larger response), which prevented Ca^{2+} entry and exit. (A after Polans et al., 1996; B after Mendez et al., 2001; C from Fain et al., 1989.)

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 (GCAPs) (see Dizhoor et al., 2010). 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 9.21A). 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, which re-opens the channels. If the genes for both GCAPs are disrupted (Mendez et al., 2001), the initial

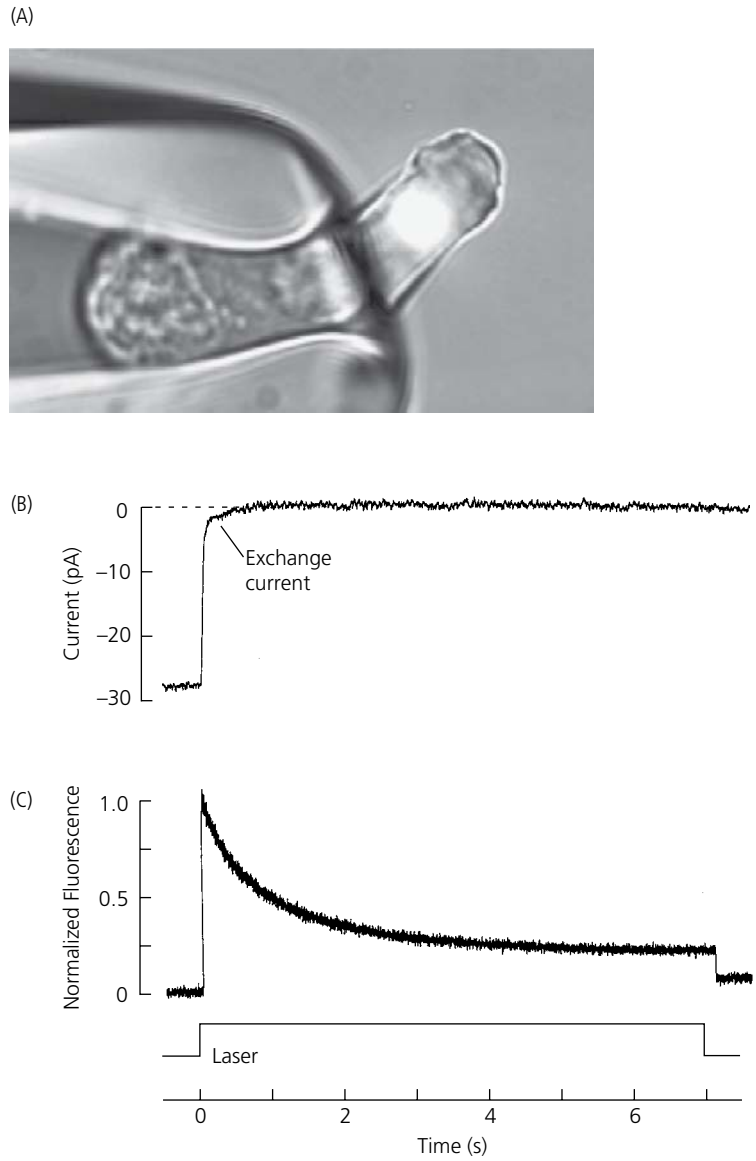

Figure 9.22 Light produces a decrease in photoreceptor intracellular Ca^{2+} concentration. (A) Isolated salamander rod loaded with the fluorescent Ca^{2+} indicator dye fluo-3 was held with its inner segment in a suction pipette so that its outer segment could be illuminated with visible light from an argon laser. (B) Turning on of the laser produced a rapid decline in suction-electrode current due to the closing of the channels. The more slowly declining component labeled "Exchange current" is the inward current produced by electrogenic Na^+/Ca^{2+}-K^+ exchange, which declined as Ca^{2+} decreased and reached a new steady-state concentration. (C) From the same rod, the time course of Ca^{2+} decrease measured from the fluorescence of the fluo-3 indicator dye. Fluorescence has been normalized to its peak value in darkness. (From Sampath et al., 1998.)

phase of the photocurrent is unaffected, but the channels continue to be closed for a longer time and re-open much more slowly (Figure 9.21B).

Virtually the same effect can be produced by preventing the light-dependent change in outer segment Ca^{2+} concentration. This can be done at least for a few seconds by rapidly perfusing the outer segments of rods or cones with a medium lacking both Ca^{2+} and Na^+ (Matthews et al., 1988). The removal of Ca^{2+} stops Ca^{2+} influx, whereas substituting Na^+ with

another ion like Li^+ or guanidinium$^+$ stops the efflux of Ca^{2+} by the exchanger, since the exchanger requires extracellular Na^+ to function. The photoreceptors still have responses to light in this solution, because 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 rod in this solution (Figure 9.21C) produces a response that is prolonged in much the same way as disruption of the GCAP genes. Similar effects have been seen in cones (Nakatani and Yau, 1988b; Matthews et al., 1990). 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 (Burns et al., 2002).

Which of the steps of recovery is the slowest? That is, which step is the one that limits the rate of recovery of the light response? One possible approach might be to knock out each of the genes responsible for the various mechanisms of decay one by one. We could then see which of them alters the response waveform. The results in Figures 9.20 and 9.21 show, however, that this approach is unhelpful. When we reduce the rate of any of the steps that turn off the transduction cascade, we slow that step and make it the one that limits the recovery rate. The light response is prolonged whether we retard Rh* phosphorylation or the binding of arrestin (Figure 9.20A) or the rate of Tα•GTP hydrolysis (Figure 9.20B) or the activity of the guanylyl cyclase (Figure 9.21B). None of these results indicates which recovery step is rate limiting in a wild-type rod.

A better approach is to *increase* the rate of the steps of recovery. Suppose for example that in a normal rod the rate of Tα•GTP hydrolysis is very rapid, but phosphorylation of Rh* and binding of arrestin is much slower and limits the rate of return of the light response. If we increase the expression of the GAP molecules and make Tα•GTP hydrolysis even faster, we will not speed the rate of recovery. The response will still return only as Rh* is phosphorylated and arrestin binds. Increasing GAP expression and speeding Tα•GTP hydrolysis would only accelerate response recovery if Tα•GTP hydrolysis were the rate-limiting step. It should therefore be possible to determine the slowest step in recovery by systematically increasing the expression of the relevant genes.

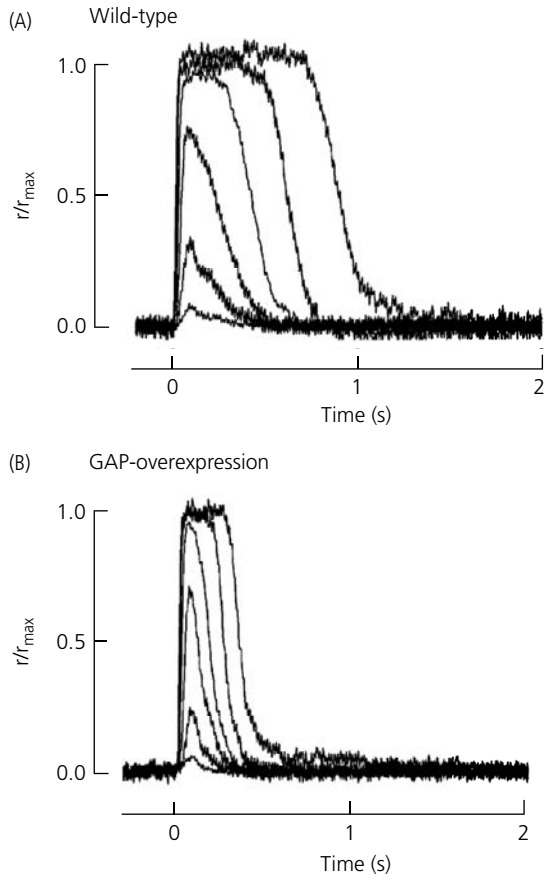

Figure 9.23 Effect of overexpression of GAP proteins on rod response decay. Superimposed responses from mouse rods to a series of flashes of increasing light intensity; the flashes were 10 ms in duration and were given at $t = 0$. Responses have been normalized to the peak amplitude of the response to the brightest intensity (r/r_{max}). (A) Wild-type rod. (B) Rod in mouse for which GAP-complex proteins had been overexpressed by four-fold. (From Krispel et al., 2006.)

Figure 9.23 illustrates an experiment of this kind (from Krispel et al., 2006). The expression of the R9AP binding protein was increased by a factor of four. Because the expression of the three GAP-complex genes is linked, overexpression of R9AP also increases expression of RGS9 and Gβ5L, and by approximately the same amount. Increasing GAP expression accelerates the decay of the response

(Figure 9.23B). In mice with variable expression of GAP genes, the greater the GAP expression, the greater the acceleration of response decay (Krispel et al., 2006; Chen et al., 2010a). The simplest explanation is that increasing GAP concentration in the outer segment accelerates the binding of the GAP complex to Tα•GTP and increases the rate at which the PDE is turned off. These experiments are representative of several studies that indicate that extinction of activated PDE6 by hydrolysis of Tα•GTP is rate limiting for recovery of the rod response (see also Sagoo and Lagnado, 1997; Tsang et al., 2006). The resynthesis of cGMP by the cyclase and binding of cyclic nucleotide to the channels are both so rapid that the electrical response effectively tracks the decline of PDE6 activity. In a mammalian rod, extinction of Rh* is also rapid and never rate-limiting under physiological conditions (Burns, 2010).

Light adaptation

Vertebrate photoreceptors adapt in the presence of steady light. Constant stimulation decreases sensitivity and resets the operating range of a photoreceptor, much as maintained hair bundle deflection does for a hair cell (Figure 6.11). This phenomenon can be seen in Figure 9.24, a suction-electrode recording from a salamander rod. In Figure 9.24A, the rod in darkness was stimulated with brief flashes at intensities that increased systematically by about a factor of four. The peak amplitude of these responses became larger as the light intensity increased (as in Figures 9.18 and 9.19) and trace out a response-intensity curve, giving peak amplitude of the response as a function of flash intensity. In Figure 9.24B and C, 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 reached a steady plateau level. Flashes superimposed on this background produced a further closing of the channels but with decreased sensitivity, such that the whole operating range of the photoreceptor was shifted to higher light intensities.

The decrease in sensitivity is perhaps clearer in Figure 9.24D. Here, small-amplitude responses of a rod to brief flashes have been superimposed. Because the sensitivity of the rod changed in the presence of the background light, the flashes in darkness and in the different backgrounds were not of the same brightness. The response amplitudes in each case have therefore been divided by the number of photons in the flashes and plotted in units of sensitivity. The largest response was recorded without a background light, and the others are for backgrounds of progressively increasing background intensity. As the background increased, sensitivity declined, and the waveform of the response was also altered. At each of the progressively brighter background intensities, the responses rose along approximately the same initial curve but began to decline at an increasingly earlier time. These recordings illustrate that one of the principal mechanisms 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, because the sensitivity of the whole of the 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 demonstrating a role of Ca^{2+} is to use the approach of Figure 9.21C, perfusing the outer segment with a solution lacking both Ca^{2+} and Na^{+}, to eliminate or greatly reduce Ca^{2+} influx and efflux and minimize changes in outer segment Ca^{2+} concentration. Under these conditions, adaptation seems entirely eliminated (Matthews et al., 1988; Nakatani and Yau, 1988b). A large body of experimental work indicates that changes in Ca^{2+} are an important 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 clear, but one very important role of Ca^{2+} is the modulation of the activity of guanylyl cyclase (Figure 9.21). In the presence of a steady background light, there is a steady increase in the rate of hydrolysis of cGMP by the PDE6. This steady PDE6 activity decreases the cGMP, closes the channels, and decreases the intracellular free-Ca^{2+} concentration (Figure 9.22). As Ca^{2+} falls, the cyclase activity increases until the synthesis of cGMP equals its rate of hydrolysis. This increase in cyclase activity

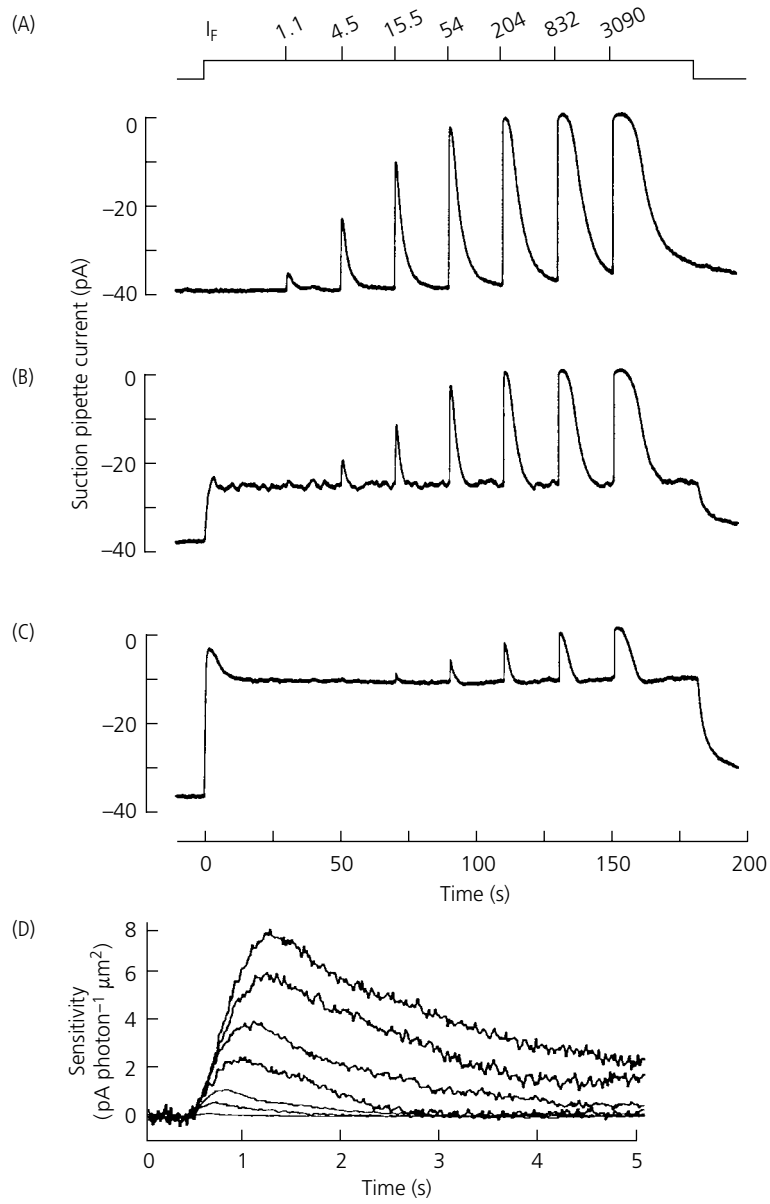

Figure 9.24 Light adaptation in a vertebrate rod. (A–C) Suction-electrode recording of responses of the same salamander rod to flashes given in darkness and in the presence of two steady background lights. I_F gives the number of photons in the flash in units of photons per micrometer squared (μm^{-2}). Background intensities were 1.7 (B) and 37.2 (C) photons μm^{-2} s^{-1}. (D) Suction-electrode recordings of responses of a different 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 number of photons in the flash (in units of photons per micrometers squared). (A–C from Matthews, 1990; D from Fain, 1993.)

causes a fraction of the channels to re-open, preventing the light from saturating the rod and allowing the receptor to continue to respond even in the presence of maintained illumination.

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 about their contributions. The rate of rhodopsin phosphorylation by rhodopsin kinase has been shown to be altered by a small-molecular-weight Ca^{2+} binding protein (Kawamura, 1993; Chen et al., 1995a), usually referred to as recoverin. Although recoverin can modulate the rate of rhodopsin decay under physiological conditions (Chen et al., 2010a), the magnitude of this effect is rather small. The principal role of this protein seems rather to be the regulation of PDE6 (Fain, 2011), which adjusts the integration time and PDE6 spontaneous activity in background light (Morshedian et al., 2018). 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 or no contribution to adaptation (Chen et al., 2010b), but the one for the cone channel may 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 re-isomerized to its 11-*cis* form before rhodopsin can again be re-activated by photon absorption. In microvillar photoreceptors, all-*trans* retinal remains covalently attached to the opsin, forming a thermally stable metarhodopsin which can also absorb light. In *Drosophila* the rhodopsin of the R1–6 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 ultraviolet-sensitive pigments also have metarhodopsins absorbing in the visible, at wavelengths between 460 and 520 nm. When arthropod metarhodopsin absorbs a photon, no light response is produced, but all-*trans* retinal is converted back to 11-*cis* (see for example Richard and Lisman, 1992; Liu et al., 2008). Most of pigment regeneration occurs by this energy-efficient mechanism, but some of the phosphorylated metarhodopsin is endocytosed and degraded (see for example Satoh and Ready, 2005). Arthropods accordingly also have a slower enzymatic pathway in retinal pigment cells to regenerate the 11-*cis* chromophore (Wang et al., 2010).

For the ciliary pigments of rods and cones, the bleaching of retinal from 11-*cis* to all-*trans* causes the chromophore to be released from the opsin, and most of the chromophore must then migrate to a different cell type to be regenerated. The mechanisms of pigment conversion are somewhat different for the two kinds of photoreceptors. For rods, most of the all-*trans* retinal is converted to 11-*cis* retinal by an enzymatic pathway (Figure 9.25 and see Fain et al., 2001; Lamb and Pugh, 2004). The all-*trans* retinal comes off the opsin protein and is reduced to all-*trans* retinol within the rod by a retinol dehydrogenase. All-*trans* retinol then leaves the photoreceptor and is carried through the extracellular space, perhaps in part by binding to interphotoreceptor retinol binding protein (IRBP). The all-*trans* retinol is then conveyed into an adjacent layer of cells called the retinal pigment epithelium (RPE). The RPE contains an enzyme called RPE65 which re-isomerizes the chromophore (Jin et al., 2005) and which is part of a complex of proteins converting all-*trans* retinol to 11-*cis* retinal. The 11-*cis* retinal is transported back to the photoreceptor, where it recombines with opsin and regenerates rhodopsin.

For cones, 11-*cis* retinal can also be produced enzymatically in the RPE, but much of the chromophore is re-isomerized by an alternative mechanism. We have long known that a second pathway must exist, because cone pigment can be regenerated in an isolated retina in the absence of the RPE (Goldstein, 1970; Hood and Hock, 1973). An important advance in our understanding was the demonstration that at

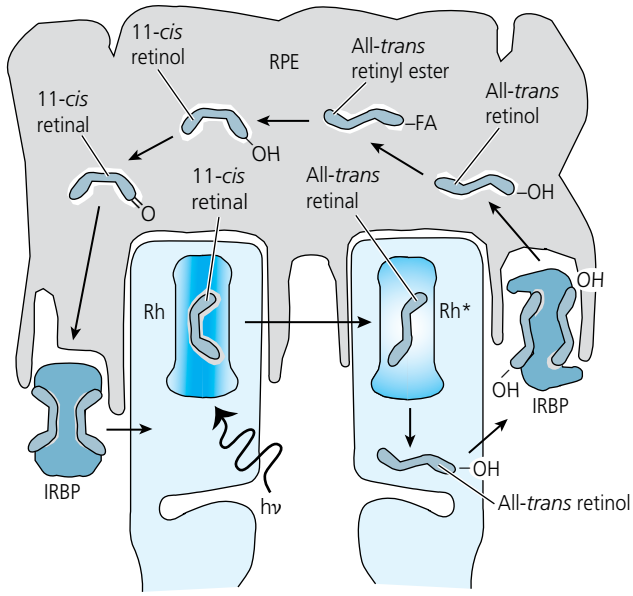

Figure 9.25 Principal enzymes and transport proteins responsible for regeneration of rhodopsin in a vertebrate rod and retinal pigment epithelial cell. RPE, Retinal pigment epithelium; FA, fatty acid; Rh, rhodopsin; IRBP, interphotoreceptor retinol binding protein; hν, light. (After Bok, 1993; Fain et al., 1996.)

least some of this regeneration can occur within the retinal Müller glial cells (Wang et al., 2009; Wang and Kefalov, 2009; see Wang and Kefalov, 2011). The Müller-cell pathway produces 11-*cis* retinol rather than 11-*cis* retinal (Mata et al., 2005), and this difference is important because cones can utilize 11-*cis* retinol but rods can't (Jones et al., 1989). As a consequence, the 11-*cis* chromophore made by the Müller cells can be preferentially utilized by cones to speed the rate of their recovery.

Recent evidence indicates that there is a protein in the Müller cells (and also in the RPE) which may be responsible for re-isomerizing at least part of the cone chromophore (Morshedian et al., 2019). This protein is called *retinal G-protein-coupled receptor* (RGR) opsin (Chen et al., 2001b). It is a member of the opsin family and is similar in structure to rhodopsin, again with a lysine in the seventh α helix; but it binds all-*trans* retinal instead of 11-*cis* retinal. Absorption of a photon then isomerizes all-*trans* retinal back to 11-*cis* retinal, which is subsequently converted to 11-*cis* retinol and conveyed to the cones. RGR opsin can act effectively as a photoisomerase, much like metarhodopsin in arthropods.

Such a mechanism of photoconversion would be particularly appropriate for cones, which must continue to respond even in continuous bright light. Although it had long been thought that insects and vertebrates have quite different pathways for photopigment regeneration, both may use light together with enzymes in order to supply sufficient chromophore to their photoreceptors.

Cone pigment regeneration can be quite rapid, whereas enzymatic regeneration of rod pigment after bright light exposure is comparatively 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 markedly depressed. This is the reason why, when we turn on the lights in the bathroom in the middle of the night to get an aspirin or a drink of water, we can barely find our way back to bed once the light is turned off. Part of the reason sensitivity is decreased is that there is less pigment to absorb photons, since a fraction of the pigment has been bleached and has lost its chromophore. 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, indicating that 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, their suggestion would mean that some component of bleached pigment can stimulate the transduction cascade much like light, producing an activation of the PDE6, 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. The question is, which intermediate is most important?

The answer seems to depend upon 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 converted 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 to be mostly produced by opsin itself, which stimulates the cascade, though with low probability (Cornwall and Fain, 1994). Opsin continues to activate the cascade and depress sensitivity until all of the photopigment has been regenerated.

Intrinsically photosensitive retinal ganglion cells

We have been accustomed to thinking that rods and cones are the only photoreceptors in the vertebrate retina capable of converting light into an electrical signal. We now know better: a small fraction of ganglion cells can do this trick too (Do and Yau, 2010; Schmidt et al., 2011; Hughes et al., 2012; Lucas, 2013). Ganglion cells receive signals from other retinal cells (see Figure 1.2B), and they send their axons into the optic nerve, which carries visual information from the retina into the CNS. A few of these cells can also respond to light directly, and this population is especially important in non-image-forming visual tasks, such as pupillary light constriction and the setting of the circadian clock.

These light-sensitive cells are usually referred to as *intrinsically photosensitive retinal ganglion cells* (*ipRGCs*). Their light responses were first described by David Berson's laboratory (Berson et al., 2002), who labeled ganglion cells projecting to the superchiasmatic nucleus of the hypothalamus, a center in the brain known to house the master circadian pacemaker. When Berson and colleagues recorded from these cells (Figure 9.26A), they discovered responses to light even when all of the inputs from other retinal cells had been eliminated with synaptic blockers. At about the same time, Hattar and colleagues (2002) showed that these same cells express a visual pigment called melanopsin or Opn4, initially isolated from *Xenopus* melanophores (see Lucas, 2013). Melanopsin is similar in structure to other members of the opsin family but is more like the microvillar pigment of *Drosophila* and other invertebrates than the ciliary pigments of rods and cones. Melanopsin has a stable metarhodopsin intermediate, capable of regenerating the chromophore with light (Matsuyama et al., 2012). That is, absorption of one photon can isomerize 11-*cis* retinal to all-*trans* retinal and trigger the transduction cascade, ultimately depolarizing the membrane potential and generating action potentials (Figure 9.26A). Another photon can then reisomerize all-*trans* to 11-*cis* and regenerate the visual pigment. Other mechanisms may also be capable of regenerating the chromophore by an alternative pathway that does not require illumination (Walker et al., 2008; Zhao et al., 2016).

The first kind of ipRGC to be identified is now called M1; it is the easiest to study because it has the highest melanopsin concentration, and it is also the most sensitive with the largest intrinsic light response. As a result, we know most about its mechanism of transduction, which resembles that of microvillar photoreceptors like those of *Drosophila* (Graham et al., 2008; Xue et al., 2011; Jiang et al., 2018). Melanopsin in an M1 ipRGC activates a G protein whose α subunit is a member of the $G\alpha_{q/11}$ family, and this G protein in turn stimulates a PLC like the *norpA* protein of *Drosophila.* The rest of the cascade is unclear but ultimately results in the opening of TRP channels, again much like in the fruit fly. The mechanism of channel gating seems not to involve Ca^{2+} release

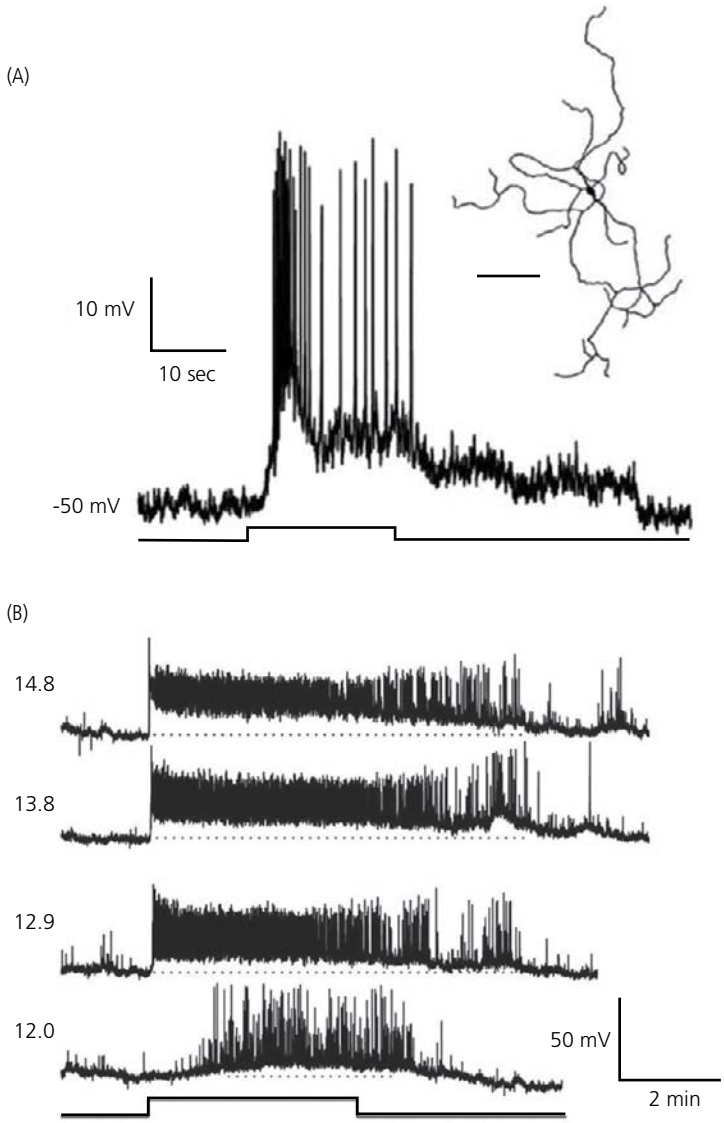

Figure 9.26 Responses of intrinsically photosensitive retinal ganglion cells. (A) Light response of an intrinsically photosensitive retinal ganglion cell (ipRGC) in the rat, recorded with patch clamp in an isolated retina in the presence of 2 mM $CoCl_2$, which blocks all synaptic input onto the ganglion cell. Timing of light stimulus is indicated by lowermost trace. Cell was identified by retrograde-labeling from the suprachiasmatic nucleus. Inset shows camera-lucida drawing of the recorded cell, scale bar 100 μm. (B) Voltage responses recorded in another cell to 4-minute exposures of increasing brightness showing maintained firing even to prolonged stimulation. The number to the left of each trace gives the light intensity in $\log_{10}$ photons per second per centimeter squared at a wavelength of 500 nm (blue-green). (From Berson et al., 2002.)

or IP_3. There are an additional five classes of melanopsin-expressing cells called M2–M6 (see for example Ecker et al., 2010; Schmidt et al., 2014; Quattrochi et al., 2018), each with rather different properties. There is increasing evidence that these cells do not transduce like M1s, but can use a variety of mechanisms, apparently including cyclic nucleotides (Jiang et al., 2018; see also Sonoda et al., 2018).

Even the best-responding of the ipRGCs are orders of magnitude less sensitive than rods and cones. Although the single-photon response to an excited melanopsin can be as large as that of a rod,

ganglion cells have no outer segments with closely spaced disks or membrane lamellae, and the concentration of pigment is as much as 10^4 times lower than in a rod or cone (Do and Yau, 2010). The probability of absorption of a photon is therefore considerably lower. Although much more light is required to stimulate the ipRGCs, these cells can nevertheless produce maintained responses to continuous light spanning intensities from moonlight to full daylight (Figure 9.26B). At dimmer intensities, ipRGCs receive synaptic input from other retinal cells which can produce depolarization and action potentials. Together, the combined synaptic and intrinsic responses of the ipRGCs can cover the whole of the operating range of vision (Hattar et al., 2003; Guler et al., 2008). These cells therefore comprise an ideal system for adjusting the diameter of the pupil and setting the circadian clock according to the brightness of ambient illumination. They also seem to contribute to other visual tasks, including a primitive form of pattern vision (see for example Ecker et al., 2010; Stabio et al., 2018). Moreover, bright light makes us more cheerful, and the lack of light during the long winter months can trigger seasonal affective disorder (SAD). The ipRGCs may have a role in these phenomena as well (see Lazzerini Ospri et al., 2017).

Summary

Photoreceptors respond to the part of the electromagnetic spectrum we call light. They do this with a visual pigment, consisting of a chromophore covalently attached to a protein called opsin. From archaeobacteria to man, 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 bacteria, 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, which breaks the salt bridge, triggering a change in conformation of rhodopsin to an active form that initiates the sensory cascade.

In bacteria, 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 activates a heterotrimeric 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 producing the hyperpolarizations of vertebrate rods and cones.

In both *Limulus* and *Drosophila*, the photopigment is contained within numerous microvilli, collectively referred to as a rhabdomere, which greatly increase the surface area of the plasma membrane. Rh* activates a trimeric G protein with a $G\alpha_q$ subunit to produce $G\alpha_q$•GTP. The $G\alpha_q$•GTP then stimulates a PLC, generating the two second messengers IP_3 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 in *Drosophila* are the founding members of the *trp* family of proteins, of which two different forms are expressed: TRP and TRPL. In *Limulus*, on the other hand, the channels have been proposed to be similar to those in vertebrates, gated by cyclic nucleotides. Light produces a large increase in intracellular Ca^{2+}, in *Drosophila* mostly the result of Ca^{2+} entering the cell through the light-dependent channels, and in *Limulus* from IP_3-gated Ca^{2+} release. In both species, the increase in Ca^{2+} plays an important role in activation and modulation of the transduction cascade. Several different components of transduction are affected by Ca^{2+}, but it is not yet known exactly how the gain and sensitivity are regulated.

In the rods and cones of vertebrates, transduction occurs in a part of the cell called the outer segment, which contains the photopigment, transduction enzymes, and channels necessary for producing the light response. Rh* excites a G protein called transducin, which is a member of the α_i/α_o family and is coupled to PDE6. The Tα-GTP binds to the PDE6 γ inhibitory subunits, relieving inhibition and

stimulating the PDE6 to hydrolyze cGMP. Rod and cone outer segments contain cation-permeable channels gated by cyclic nucleotides, which are open in darkness and allow a large influx of both Na^+ and Ca^{2+}. The decrease in cGMP concentration caused by PDE6 activation leads to closing of the channels, reduction in ion influx, and hyperpolarization of the membrane potential. The transduction cascade is turned off by the quenching of Rh*, produced by C-terminal phosphorylation of serines and threonines by rhodopsin kinase, followed by the binding of arrestin. The Tα-GTP is quenched when GTP is hydrolyzed to GDP, and the rate of this reaction is greatly accelerated by the GAP-complex proteins RGS9, Gβ5L, and R9AP together with the inhibitory γ subunit of PDE6. The decay of PDE6 activity is the rate-limiting step of inactivation, at least for mammalian rods.

The Ca^{2+} coming into the rod through the cyclic-nucleotide-gated channels in darkness is extruded by a Na^+/K^+-Ca^{2+} transporter, which exploits the energy of both the Na^+ and K^+ ion concentration gradients. Light closes the cyclic-nucleotide-gated channels, and continued extrusion by the transporter produces a decrease in intracellular Ca^{2+} concentration. The change in Ca^{2+} is thought to have several effects on the transduction cascade, but the most important seems to be the modulation of the rate of the guanylyl cyclase via small-molecular-weight binding proteins called GCAPs. As the Ca^{2+} concentration decreases in the light, Ca^{2+} comes off the GCAP proteins and cyclase activity is increased. The increase in cyclase in turn increases the cGMP concentration and re-opens a fraction of the channels, speeding the return of the photoreceptor current back toward its dark level. Ca^{2+} also plays an important role in light adaptation. If changes in outer segment Ca^{2+} concentration are prevented, adaptation is significantly impaired. The Ca^{2+} regulation of cyclase makes an important contribution to adaptation by preventing saturation of the photoreceptor response, but Ca^{2+} is also thought to regulate the cascade by additional mechanisms that have not as yet been clarified.

After bright light exposure, photoreceptors slowly recover sensitivity. The retinal isomerized by light from 11-*cis* to all-*trans* must be re-isomerized back to 11-*cis*. For microvillar photoreceptors, including those of arthropods, the chromophore remains attached to the protein and is re-isomerized by light. In ciliary rods and cones, the all-*trans* retinal is released from the photopigment, converted to all-*trans* retinol, and transported to an adjacent cell layer, either the retinal pigment epithelium or Müller glial cells. There the all-*trans* retinol is reconverted either enzymatically or by light exposure to an 11-*cis* isomer, which can diffuse or be transported back to the photoreceptor to recombine with opsin and regenerate the rod or cone visual pigment. During this process, the sensitivity of the photoreceptor is depressed as if it were illuminated by an equivalent background light. In molecular terms, this equivalent background is produced by low-level stimulation of the transduction cascade by intermediates of bleaching including opsin itself, which can activate the cascade, though with much less effectiveness than Rh*.

In addition to the photoreceptors, the retina of vertebrates contains a small fraction of ganglion cells that express a visual pigment and can respond directly to light. These cells, called intrinsically photosensitive retinal ganglion cells (ipRGCs), contain melanopsin, a visual pigment similar in structure to arthropod rhodopsin. Although much less sensitive than either rods or cones, the ipRGCs can respond to light with maintained firing of action potentials. The ipRGCs also receive synaptic input from other retina cells, and these signals, together with the intrinsic photosensitivity, enable the ipRGCs to respond to illumination over an extended range. They project to a variety of targets in the CNS to help control many tasks not requiring image formation, including the control of pupillary diameter and circadian rhythm. They may in addition have a limited role in visual detection and the modification of affect or mood.