Retina: Neuroanatomy and Physiology 22

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Contents

Brief History 675
Retina- Development, Structure, and Function 678
    Evolutionary Background 678
    Basic Ontogenetic Mechanisms of Retinogenesis 688
    Structural Organization of the Mammalian Retina 695
    Rods and Cones as the Main Light Sensors in the Retina 701
    The Optical Properties of the Tissue Are Important in the Inverted Retina 705
    Vertebrate Photoreceptors Are Inactivated by Light 710
    The Recovery of Light Sensitivity Is Slower in Rods than in Cones 713
    The Type of Opsin Defines the Spectral Sensitivity of Photopigments 714
    The Yellow Macula Acts as a Sunglass 717
    Retinal Neurons Mediate Processing of Visual Information Before It Is Transmitted to the Optic Nerve 717
    RPE Cells Are the Major Photoreceptor-Supporting Cells 729
    Retinal Glial Cells Mediate Neuronal Nutrition, Waste Management, and Inflammation 732
Outlook 741
Glossary 742
References 744

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Abstract

The sensory retina of vertebrates, a highly specialized extension of the brain, is a thin (~0.25 mm thick in the human eye), multilayered, photosensitive tissue coating the inner back of the eyeball (Fig. 1). The retina is responsible for (1) photoreception and transduction of light energy into neuronal activity and (2) initial stages of visual processing and integration according to the environmental light conditions. The visual information is then transferred through the optic nerve to the brain.

Keywords

Amacrine cells • Bistratified cells • Blood-borne macrophages • Blue opsin gene mutation • Cholinergic amacrine cells • Color process • Color vision • Color-coded ganglion cells • Cone • Cone pathway • Cytogenesis • Dichromates • dLGN • Dopamine • Electroretinogram • Emmetropization • Fovea • Fovea centralis • Glutamate receptors • Helmholtz's hypothesis • Henle's fiber layer • Interneurons • Interplexiform cell • Invertebrate photoreceptors • Inverted retina • Light • Light sensitivity • Macroglial cells • Mammalian retina • Melatonin • Metarhodopsin • Microglial cells • Microphthalmia • Müller cells • Müller glial cells • Neuroretina • Neurulation • OFF-center cells • ON-center cells • Opponent process theory • Opsin • Opsin photopigments • Perikarya of cones • Photoreceptor cells • Photoreceptors • Photosensitive ganglion cells • Phototransduction • Phototransduction process • Pluripotent retinal progenitor cells • Red-green blindness • Retina • Retinal ganglion cells • Retinal neurons • Retinogenesis • Rod • Rod bipolar cells • Rods and cones • RPE cells • Transducin • Trichromatic theory • Vertebrate photopigments, bleaching of • Vertebrate retina • Visual cortex • Visual information • Water fluxes • Yellow macula

Abbreviations

AQP	Aquaporin
ATP	Adenosine 5′-triphosphate
cGMP	Cyclic guanosine 5′-monophosphate
CRALBP	Cellular retinaldehyde-binding protein
dLGN	Dorsal lateral geniculate body
EAAT	Excitatory amino acid transporter
GABA	γ-aminobutyric acid
GAT	GABA transporter
GCL	Ganglion cell layer
GFAP	Glial fibrillary acidic protein
ILM	Inner limiting membrane
INL	Inner nuclear layer
IPL	Inner plexiform layer
Kir	Inwardly rectifying potassium
NFL	Nerve fiber layer
ONL	Outer nuclear layer

OPL	Outer plexiform layer
PRS	Photoreceptor segment
RPE	Retinal pigment epithelium
TH	Tyrosine hydroxylase
TRP	Transient receptor potential

Brief History

Since Kepler described the light projection onto the retina in the early seventeenth century, a steadily increasing number of scientists devoted their research to the structure and function of the retina. Nevertheless, in the first half of the nineteenth century, it was still unknown which elements of the mammalian retina might be the "light sensors." By 1830, Purkinje had demonstrated that the blood vessel pattern of the inner retina became visible when an observer looks through a pinhole. By exploiting this phenomenon, H. Müller (1854) established a position for the light detectors, placing them behind the vessels in the outer retina, at the rod and cone layer (Fig. 1). In 1866, M. Schulze published his theory on the *dual nature of the photoreceptor cells* and their function. It had already been shown at that time that the fovea centralis of the human retina contains exclusively cones and that rods are mixed with cones outside the fovea; as the fovea is blind at night, Schultze concluded that cones are responsible for photopic (i.e., color) vision at daylight, whereas rods mediate scotopic or night vision. Using Golgi's newly discovered silver staining method, Ramón y Cajal described in 1893 (and in his 1906 Nobel lecture) "the connections of the visual fibers and the cells of the retina" with beautiful precision

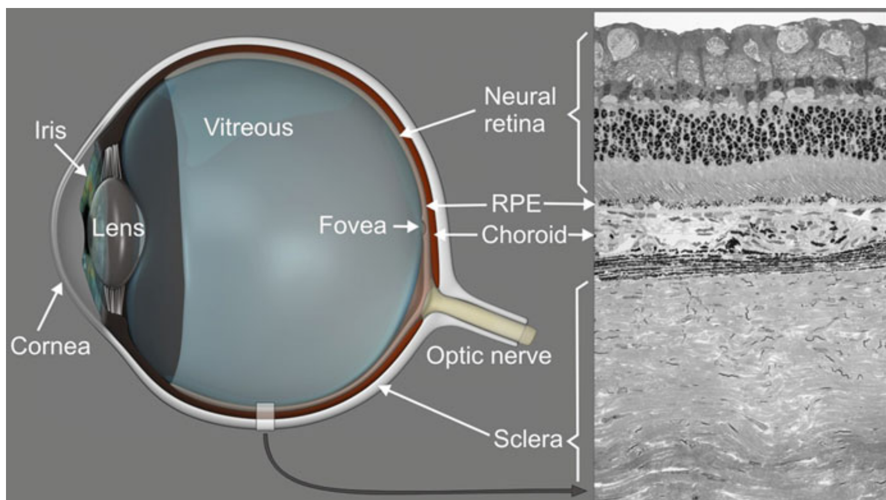

Fig. 1 Section through the back wall of the eye (*right*). *RPE* retinal pigment epithelium

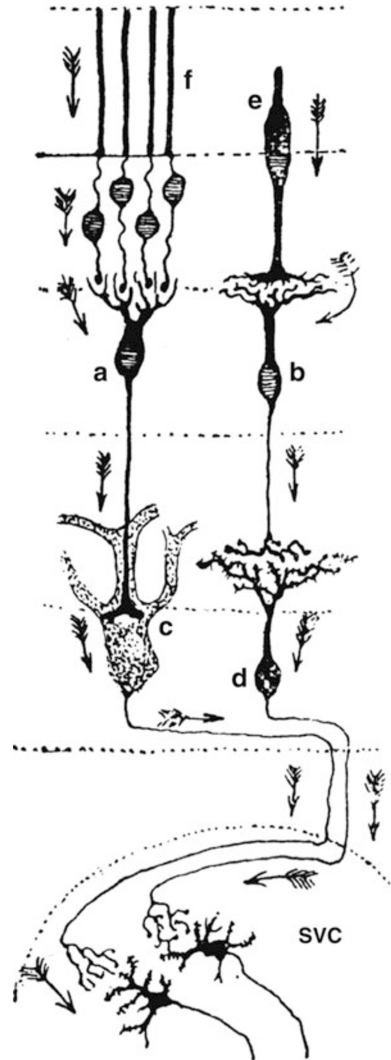

Fig. 2 Signal flow through the vertebrate retina according to Ramón y Cajal. *a* rod bipolar cell, *b* cone bipolar cell, *c* large ganglion cell, *d* small ganglion cell, *e* cone, *f* rod, *SVC* subcortical visual center

and clarity. Knowing that "vision propagates from the outer toward the inner retina," Cajal generated a diagram of signal flow from the photoreceptors through the retina to the brain (Fig. 2). Cajal regarded retinal organization as a particularly strong support for his ideas about the *functional polarity of neurons*, with the dendrites receiving signals and axons being the outputs. In 1851, Müller also described the "radial fibers" of the retina which later became known as *Müller (glial) cells*. Thus, at the end of the nineteenth century, the basic cellular constituents of the vertebrate retina were known, and reasonable concepts about their functions and interactions ("circuits") had been developed. Helmholtz' invention of the *ophthalmoscope* in 1851 even allowed to view the retina in the intact eye of human patients.

Whereas this progress in structural knowledge was fostered by new optical (particularly microscopic) techniques, functional studies at this time were mainly based upon psychophysical experiments. These led to the development of two complementary theories of *color vision*, the *trichromatic theory* and the *opponent process theory*. The trichromatic theory (meaning that color vision is a result of three different photoreceptors) was proposed by Th. Young (1802). Later, H. von Helmholtz (1852) further developed Young's ideas using color-matching experiments which showed that people with normal vision needed three wavelengths to create the normal range of colors. E. Hering proposed the opponent process theory in 1874. It proposes that the visual system interprets color in an antagonistic way, red versus green, blue versus yellow, and black versus white. Now we know that both theories are correct and describe different stages in visual information processing, but there were almost 100 years to wait before objective methods of sensory physiology became available to prove these theories.

An electrical light response of the retina was first recorded in 1865 by Holmgren. This complex response, the *electroretinogram*, is caused by spatially and temporally overlapping electric activities of several different cell types. Thus, while it still constitutes a useful diagnostic tool for ophthalmologists, it did not provide clear insights into retinal cell physiology. Retinal electrophysiology contributed to our knowledge about retinal cell function basically after microphysiological methods were developed. H.K. Hartline (1938; Nobel prize 1967) performed recordings from single fibers of the frog optic nerve. He described the functional dichotomy of *ganglion cell receptive fields* into ON- and OFF-types and found that lateral inhibition contributes to the detection of contour and contrast. He introduced the term "receptive field" as "the region of the retina which must be illuminated in order to obtain a response in any given fiber." He found ON-cells, ON/OFF-cells, and OFF-cells. S.W. Kuffler (1953) discovered the concentric center-surround organization of ganglion cells in the mammalian retina. R. Granit (Nobel prize 1967) further developed recording techniques for neurons of the mammalian retina. H.B. Barlow (1953), J.Y. Lettvin et al. (1959), and later Barlow and coworkers (1964) recorded frog retinal ganglion cells with more complex receptive fields, such as cells that selectively responded to moving stimuli.

Whereas these studies were based on the analysis of the action potential-firing rates of retinal ganglion cells, it turned out that most retinal nerve cells do not generate action potentials. G. Svaetichin (1953) recorded slow potential changes ("S-potentials") from cells which he first thought were glial cells but which actually were horizontal cells. It was T. Tomita who first recorded light responses from vertebrate photoreceptor cells; he found that these cells are inactivated (hyperpolarized) rather than activated (depolarized) by light. He also discovered (1965, 1968) that in the fish retina, three different types of cone photoreceptors each respond to different wavelengths of light.

This observation supported Helmholtz's hypothesis on the existence of different light-absorbing molecules in distinct types of cone photoreceptor cells. Spectral absorption measurements from single, isolated cone cells also demonstrated the presence of three different *photopigments* in human cones (Wald and Brown 1965). One of the light-absorbing molecules, the photopigment of the rods

(*rhodopsin*), had already been discovered 1877 by Kühne. G. Wald (Nobel prize 1967), and his group pioneered our understanding of the molecules responsible for the first steps of vision. He determined that the protein rhodopsin in the rod cells consists of two molecular parts, an opsin and retinal. The molecular/genetic identity of the different cone pigments was revealed more recently (beginning in the late 1980s, by Nathan and others). Noteworthily, it has been shown that not only the renewal of the bleached photopigments but also the renewal of the photoreceptor outer segments involves an interaction between photoreceptor and glial cells; in the case of the rods, the recycling of rod outer segment material via the pigment epithelium had been discovered by R. Young during the mid-1960s.

Of course, this section (just like every short historical excurse) certainly misses a lot of important discoveries and may wrongly suggest that the above-mentioned steps in knowledge were or are complete and ultimate. Of course, the latter is not true. For instance, although all basic retinal cell types had been described at the end of the nineteenth century, still their (ultra)structure and their involvement into functional retinal circuits are subject of sophisticated studies. Moreover, old dogmas continue to be challenged by new findings. To mention an example, it has been generally accepted until very recently that photoreceptors are the only light-sensitive elements in the vertebrate retina. This was despite it been shown already in 1923 that rod and cone photoreceptors cannot account for the spectral sensitivity of the pupillary light reflex and that in 1980 W.W. Morgan and C.W. Kamp reported the persistence of light-regulated changes of the dopamine levels in rat retinas even after complete degeneration of rods and cones. This problem was solved not before 1991 when R.G. Foster and colleagues demonstrated the presence of *intrinsically photosensitive retinal ganglion cells*, expressing a particular own photopigment. The discovery of these cells caused as much surprise as initial scepticism within the scientific community. Likewise, in 2004, Mouritsen and colleagues showed that the retina of migratory birds expresses cryptochromes and may be involved in the magnetic sense of the animals. Thus, this article definitely is aimed at stimulating its readers to search for new discoveries in the retina although it has been intensely studied over the last centuries.

Retina- Development, Structure, and Function

Evolutionary Background

Basically, this chapter deals with the retina in vertebrate eyes (and even focuses upon the mammalian retina). Nevertheless, some evolutionary considerations appear essential to understand its structure and function.

Light Perception in Animals Goes Back to Common Origins

Light perception is already found in some protozoa such as in flagellates where the cell membrane contains a light-sensitive “dot.” When many metazoan phyla were generated during the “Cambrian explosion” some 530 millions of years ago, this important function became widely distributed and further evolved. Still, it was (and

is) based upon the same type of light-sensitive molecule, the *opsin photopigments*, which are thus homologous for all animals. The photopigment rhodopsin appears to have been present in a common metazoan ancestor. Likewise, certain genes controlling eye development (such as the “eye master gene,” *Pax6*) had very early origins and are shared by vertebrates and invertebrates.

However, the further evolution and specialization of animals led to a great structural and functional variety of photoreceptors, retinas, and eyes. There emerged two basic types of photoreceptor cells, the *ciliar type* (as the rods and cones in vertebrate eyes) and the *rhabdomeric type* (as photoreceptors in many invertebrates/protostomes) (Fig. 3). In ciliar photoreceptors, invaginations of the cilium

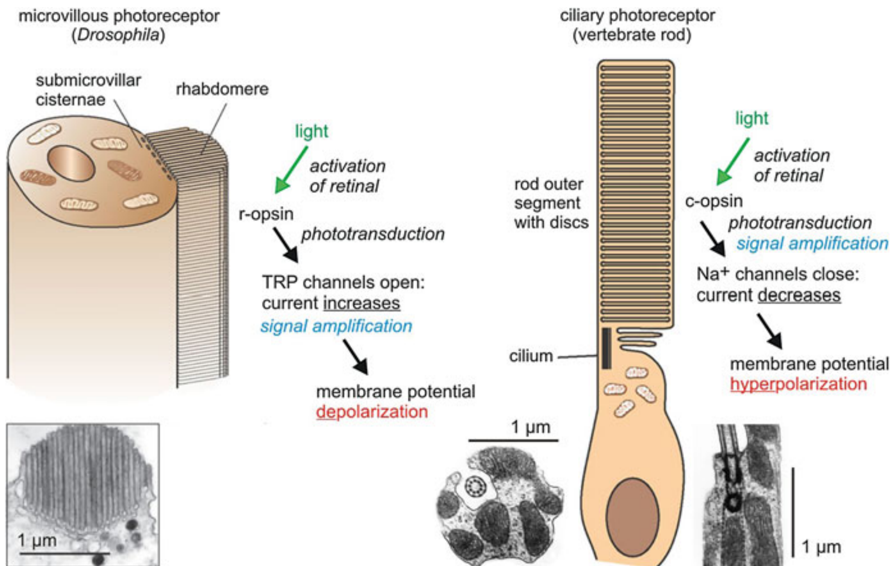

Fig. 3 Rhabdomeric (*left*) and ciliar-type (*right*) photoreceptor cells. Both receptor types serve to maximize the absorption of light by forming a cylindrical light-guiding structure with a high density of photopigment-containing membranes. *Left*: In *Drosophila* (as in most invertebrate photoreceptors), the photoreceptive membrane is organized into tightly packed, tubular microvilli (rhabdomere). Submicrovillar cisternae have an important role in phosphoinositide turnover and represent smooth endoplasmic reticulum Ca^{2+} stores endowed with inositol 1,4,5-triphosphate (IP_3) receptors. Activation of the photopigment after absorption of a photon by retinal causes activation of a G_q protein. The G_q protein-mediated activation of the phospholipase C signaling cascade involves the formation of diacylglycerol and IP_3 from phosphoinositides (PIP_2). This leads to opening of Ca^{2+}- and Na^{+}-permeable transient receptor potential (*TRP*) channels in the plasma membrane and the release of Ca^{2+} from IP_3-sensitive endoplasmic reticulum Ca^{2+} stores. Both events cause a depolarization of the cell membrane. The *image below* shows electron micrograph of one rhabdomere (Courtesy of A. Polyanovsky). *Right*: Vertebrate rod outer segments contain stacks of membranous disks. The outer segments are connected to the inner segments by a narrow cilium. Activation of the photopigment after absorption of a photon by retinal results in activation of transducin and phosphodiesterase, reduction of cGMP level, and closure of Na^{+}-permeable membrane channels which leads to hyperpolarization of the cell. The *images below* display cross (*left*) and length sections (*right*) though photoreceptor segments showing the cilium (In part adapted from Hardie and Raghu 2001)

membrane contain the photopigments, while in rhabdomeric photoreceptors, the photopigments are embedded in the membranes of microvilli. The two types of photoreceptors use different phototransduction cascades which cause opposite changes of the membrane potential. Apparently, all photoreceptor cell types of vertebrates (section "Evolutionary Background") can be traced back to an ancestral ciliar-type photoreceptor. By contrast, photosensitive ganglion cells of the vertebrate retina probably evolved from ancient rhabdomeric photoreceptor precursor cells because they behave as nonclassical photoreceptors responding to light by triggering neurochemical events similar to those of the invertebrate phototransduction cascade (and because they share the expression of many genes with the rhabdomeric photoreceptors). During ontogenetic development, photosensitive ganglion cells mature very early, before the formation of rods and the maturation of cones.

The Vertebrate Retina Is a Part of the Brain

To understand the complex makeup of the vertebrate retina, it is essential to keep in mind that vertebrates belong to the deuterostomian animals and that our ancestors must be searched among the relatives of recent starfish. If the nervous system of the starfish is used as a model of the origin of our CNS, it is noteworthy that it is not only embedded in the "skin" epithelium but is an *epithelial nervous system* by itself, it forms the outer surface of the body. The sensory processes of the receptor cells extend into the maritime environment of the animal as the source of the (hitherto unknown) stimuli to be monitored.

In the further course of evolution, the epithelial nervous system was maintained as such, but was enrolled into a tube and moved down under the surface of the body now formed by the skin and subepidermal layers. Similar events occur during our embryogenesis when the (originally superficial) neural plate is enrolled and "enveloped" in a process called *neurulation* (Fig. 4). Inevitably, this mechanism is accompanied by an inside-out turn of the polarized epithelium, i.e., the sensory cells which had faced the environment at the surface of the body now extend their sensory processes into the lumen (i.e., the inner surface) of the *neural tube*. As this lumen becomes closed against the outside world and filled by a substitute of the seawater (the cerebrospinal fluid),

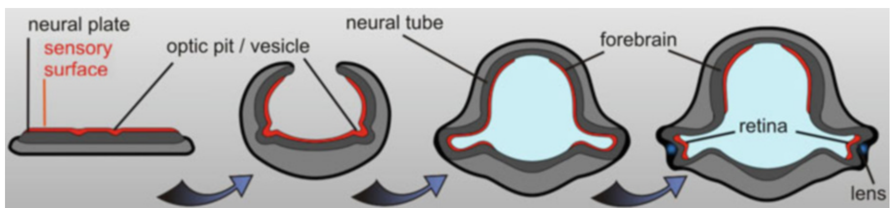

Fig. 4 Embryonic development of the retina by evagination of the eye anlage (optic pit/vesicle) from the neural plate/neural tube (from *left* to *right*). Later on, the *outer wall* of the optic vesicle becomes invaginated by the developing lens. This part differentiates into the neural retina whereas the *inner wall* develops to the retinal pigment epithelium; the stalk is transformed into the optic nerve. Initially, the "sensory surface" (*red*) constitutes the outer face of the neural plate (and the embryo), but it is translocated to the inner surface by the invagination of the neural tube

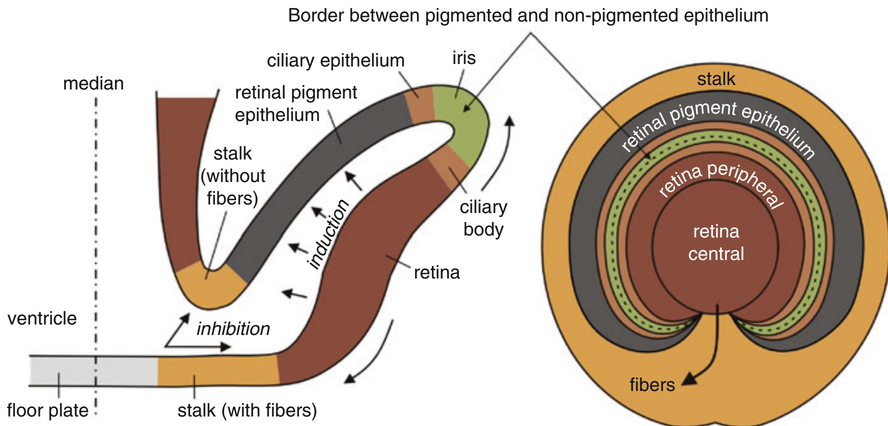

Fig. 5 Fate of the early eye anlagen. *Left*: Frontal section through the optic cup in the region of the optic fissure. The midline neuroepithelium may release inhibitory signals that determine the optic stalk (but prevent the formation of a retina). The same or other signals may act as chemoattractants for the first optic fibers (outgrowing from the first postmitotic ganglion cells, located close to the stalk). Other signals are released by the (future) neuroretina; they determine the RPE (but prevent the differentiation of a neuroretina in the outer wall of the optic cup) and, later also, the tissue at the border between the neuroretina and RPE. *Right*: Projection of the future tissue specifications onto the ocular field of the neural plate. Note that after evagination of the optic vesicle, the stalk region remains narrow while the distal regions undergo further growth

these receptor cells lose their original function as environmental receptors. This means that these cells had to be functionally replaced by "novel" receptor types and sense organs at the surface of the animals (which probably was the evolutionary driving force for the emergence of the vertebrate peripheral nervous system). As an exception, the light-sensitive part of the neural plate/tube (i.e., the prospective retina with the photoreceptor cells) was *evaginated* from the neural tissue and shifted toward the surface of the body (Fig. 4). There, by interacting with local tissue components, it becomes encapsulated into the developing *eye*. The connection with the nonevaginating parts of the brain is maintained as the optic nerve.

During this development, the original *optic vesicle* is transformed into an *optic cup* by another invagination (Figs. 4 and 5). This invagination process provides a double wall of the optic cup; the inner sheet differentiates as the neural retina and the outer one develops into the retinal pigment epithelium. As another consequence, the sensory retina becomes *inverted* which means that the photoreceptor cells are turned away from (rather than toward) the incoming light.

Light Sensitivity in Vertebrates Is Not Restricted to the Retina of the (Lateral) Eyes

Unfortunately, the origin and early evolution of the vertebrate retina cannot be studied on recent relatives of our ancestors; the lancelets, as the "most advanced" chordates, have no retina, but the most primitive jawless hagfish already possess a

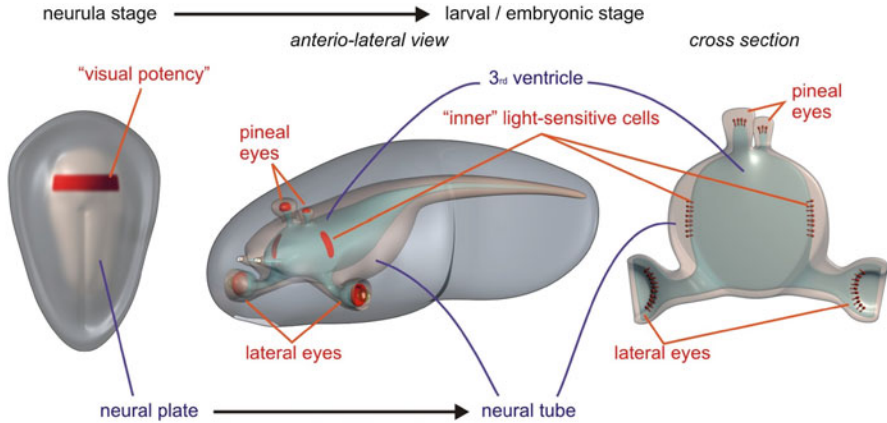

Fig. 6 Occurrence of light-sensitive cells in the vertebrate CNS. The neural plate contains a transversal stripe of tissue with "visual potency" (*left side*). This stripe is involved in the evagination of both the lateral eyes and the one or two pineal eyes, as well as in the formation of the lateral wall of the third ventricle. Consequently, all these regions may contain light-sensitive cells (*red*). Schematic vertebrate larva

well-developed retina almost indistinguishable from that of advanced vertebrates including mammals. Likely, visual information has been gathered by primitive ciliar-type receptor cells in the anterior part of the neural tube ("brain") of early vertebrate ancestors, the same as in the recent lancelet. It appears to have been a distinct "innovative step" in (pre-)vertebrate evolution when a transverse stripe near the anterior end of the neural plate (in the anlage of the diencephalon) was determined for a "visual fate or potency" by a novel combination of homeobox genes, including Pax6 as a "master gene" in early eye development (Fig. 6). This area gives rise to light-sensitive neurons not only in the "main" *lateral eyes* (corresponding to our eyes) but also in one or two *dorsal eyes* (pineal and/or parietal) and in the *wall of the third ventricle* (as well as to visually specialized areas of the midbrain). Still in recent fish, amphibians, and reptilians, all these visual sensory organs can be found (Fig. 6). Embryonic birds have a pineal "retina" which is transformed into a neurosecretory organ during later developmental stages; such a pineal retina fails to occur in mammals. The parietal eye of adult lizards is endowed with a lens and contains a noninverted, rather simple retina; the photoreceptors are very similar to those in the lateral eyes. Its optical resolution is not sufficient for image analysis, but it can be used to trigger flight reactions (when a predatory bird causes a shadow against the bright sky) and to control the diurnal rhythm of the animals.

Vertebrate Cones and Rods Differ in Their Development, Function, and Neuronal Circuits

Although both rods and cones are ciliar-type photoreceptor cells, coexisting in most adult vertebrate retinas, there are good reasons to assume that *rods developed later in evolutionary history than cones* and from different precursor cells. It is possible that

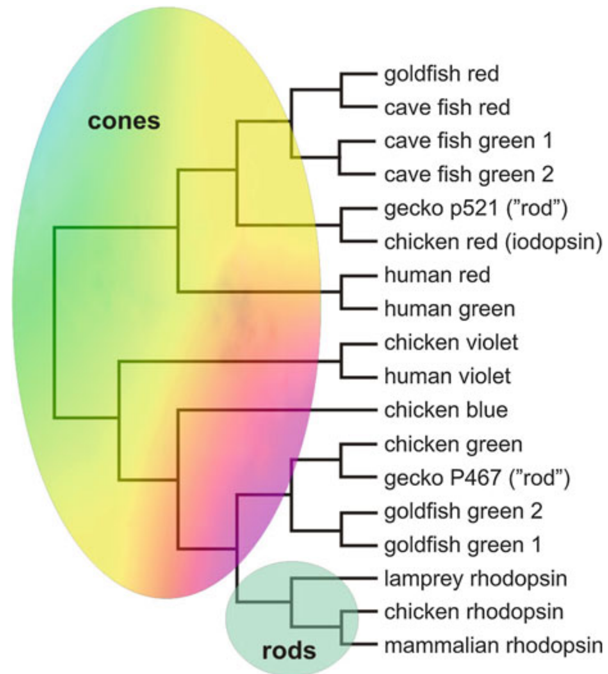

Fig. 7 Evolution of rod and cone opsins in vertebrates

rods originated from modified bipolar cells rather than from (cone-like) photoreceptor cells. Molecular genetics analyses have shown that the cone photopigments are phylogenetically older than the rod photopigment(s) of recent vertebrates (Fig. 7). Furthermore, the cone outer segments display a less complex ultrastructure than those of the rods and are significantly less light sensitive. Another argument is that cones are generated early in ontogenesis, from early precursor cells, whereas rods are generated much later, from late progenitors (Fig. 8). Particularly in the mammalian retina, the rod pathway is "superimposed" onto the (preexisting) cone output pathway (Fig. 9). Finally, it has been argued that the early ancestral animals probably were diurnal and mainly inhabited the coastal waters where enough daylight is available to use photoreceptor cells with average light sensitivity. It was only later when some animals occupied new ecological niches such as the deep sea or a nocturnal lifestyle that it became important to possess photoreceptors with very high light sensitivity.

This does not mean that the cones are "primitive," or less useful, photoreceptors. Rather, we use to think of our cones as of the more important type of receptors, as they provide the input for high visual acuity and color vision. This is because the *photopic circuits* of our retina are constituted by cones (as input providers), and rather many specific interneurons and ganglion cells involved in distinct tasks of information processing (contrast, shape, color, etc). In retinas or retinal areas extremely specialized for photopic vision such as the fovea centralis of the primate retina, each cone even is endowed with its "private" circuit, consisting of several

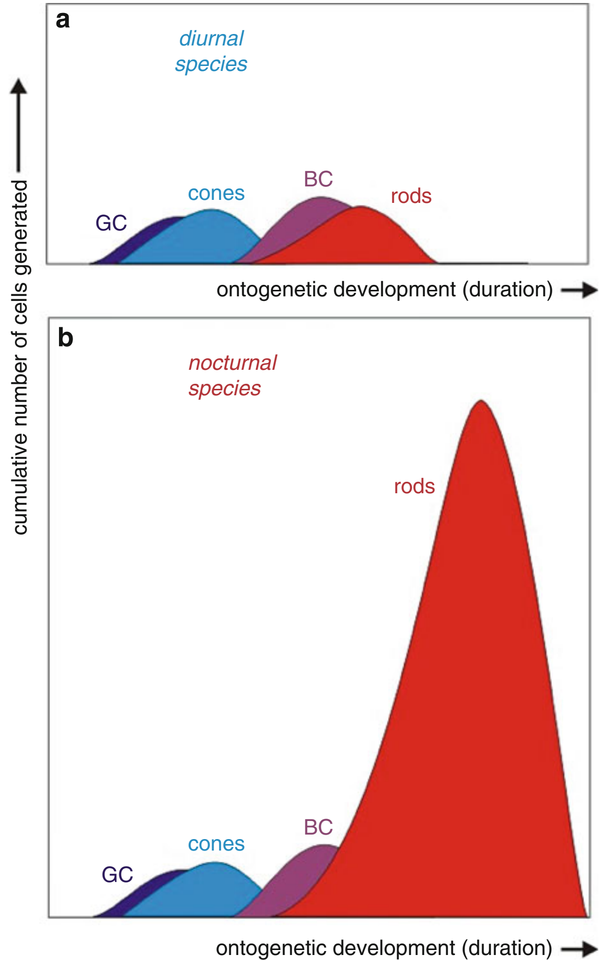

Fig. 8 The development of a nocturnal mammalian retina from a diurnal retina was achieved by prolongation of the proliferation phase of the late progenitor cells. In diurnal animals (**a**), the durations of the proliferation phases of early and late progenitor cells are rather short. This results in a small number of neurons per retinal column and a cone-to-rod ratio of 1 or more. In nocturnal animals (**b**), the proliferation of the late progenitor cells occurs over an elongated time period which results in an enhanced number of rods per retinal column. *BC* bipolar cells, *GC* ganglion cells (Modified from Finlay 2008)

interneurons and two to three ganglion cells (Fig. 10), which is reflected by comparatively thick inner retinal layers where these neurons and their processes and synapses are located. On the opposite, many (up to $>$1,000) rods converge onto one ganglion cell in the *scotopic circuits* (Fig. 10). In the scotopically specialized retinas of nocturnal mammals and deep-sea fish, the outer nuclear layer is very thick because as many rods as possible are placed close together in order to enhance the chances of photon absorption and, thus, light sensitivity. This goes along with rather thin inner retinal layers because, due to the convergence, the interneurons and ganglion cells are relatively sparse.

Cone-based *color vision* seems to be an evolutionary old retinal function. Several different types of cone photopigments emerged long before the first rod opsin (Fig. 7). The visible range of cones might differ due to variations in the vitamin A-derived chromophores and in the type of opsin and because of further structural adaptations of

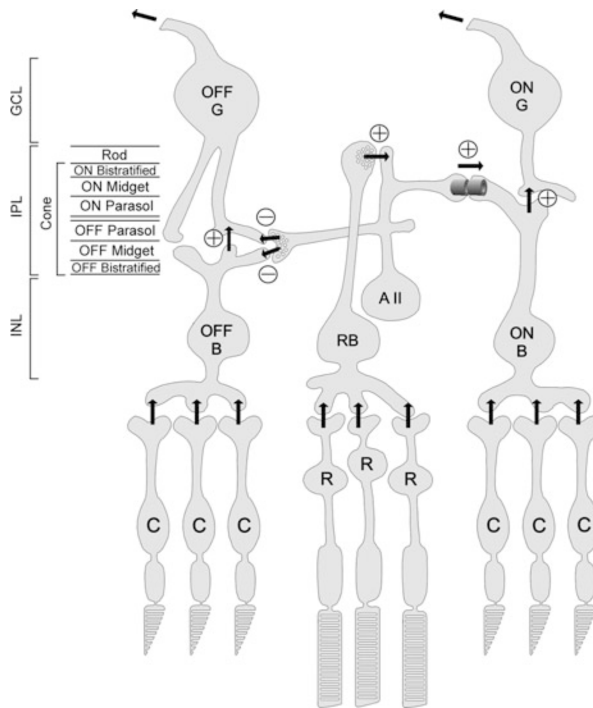

Fig. 9 Signal flow through the rod (scotopic) pathway in the mammalian retina. The rod pathway is "superimposed" onto the cone (photopic) pathway by the AII amacrine cell. Many rods (*R*) converge onto one rod bipolar cell (*RB*) which makes conventional chemical synapses with an AII amacrine cell in the inner plexiform layer (*IPL*). This bistratified cell makes gap junctions with ON-cone bipolar cells (*ON-B*) which provide excitatory contacts onto ON-ganglion cells (*ON-G*). Conventional chemical synapses between AII and OFF-cone bipolar cells (*OFF-B*) and OFF-ganglion cells (*OFF-G*) inhibit the information output from the OFF-channel. The IPL has three major functional subdivisions, the ON-sublamina, the OFF-sublamina, and the "rod" sublamina which is mainly occupied by rod bipolar cell terminals. Synapses between OFF-midget bipolar and ganglion cells are located closer to the inner nuclear layer (*INL*). Synapses between ON-midget bipolar and ganglion cells are located closer to the ganglion cell layer (*GCL*). Axon terminals of diffuse (parasol) bipolar cells are found in a more central position within the IPL. The two dendritic planes of the small bistratified ganglion cells stratify even further toward the outer and inner edges of IPL. The relative proportions of the different subdivisions of the IPL are species dependent. For example, the thickness of the "rod" sublamina critically depends on whether a retina is rod-dominated as in the cat or cone-dominated as in the tree shrew (compare Fig. 10d, e). +, excitatory; −, inhibitory synapses

cone outer segments. Color perception depends on evolutionary factors, especially recognition of food sources. Many fish retinas are tri- or even tetrachromatic (i.e., possess three or four different cone photopigments with different absorption spectra). Reptiles and birds typically possess photopically specialized retinas with four different cone types/photopigments; some are pentachromatic (e.g., pigeons). In many cases, the fine-tuning of different spectral sensitivities is even increased by placing a colored

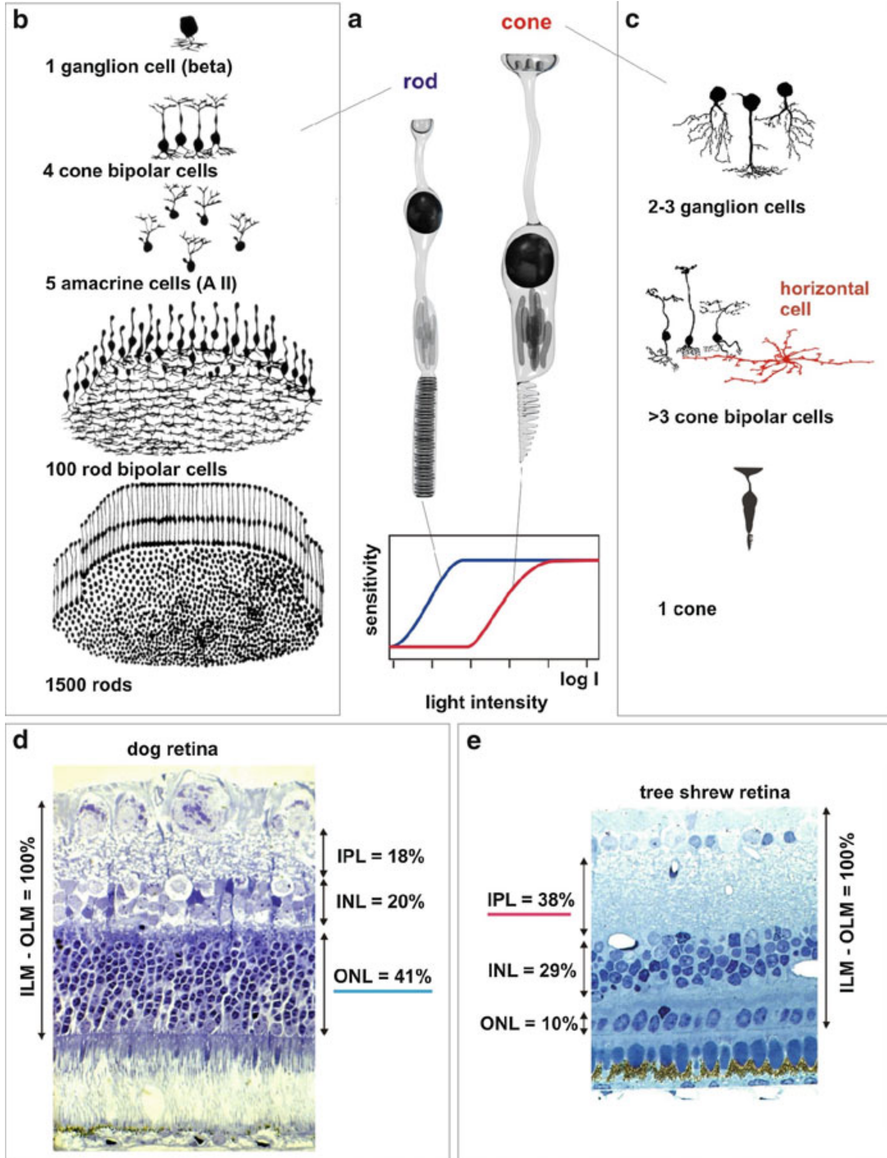

Fig. 10 Rod-driven (scotopic) and cone-driven (photopic) circuits in the mammalian retina. (**a**) Rods and cones differ in their cellular structure as well as in their physiological properties; e.g., the threshold of cone light sensitivity is about two orders of magnitude higher (i.e., at higher light intensities) than that of rods (*inset* diagram). (**b**, **c**) Rods and cones also differ in the neuronal circuits to which they deliver their information. (**b**) The typical mammalian scotopic pathway is characterized by an enormous signal convergence via rod-specific bipolar and amacrine cells; eventually, the summarized signals from >1,000 rods are "superimposed" onto the bipolar-to-ganglion cell synapses of the photopic circuit(s) of a few local cones. (**c**) At the other extreme, the

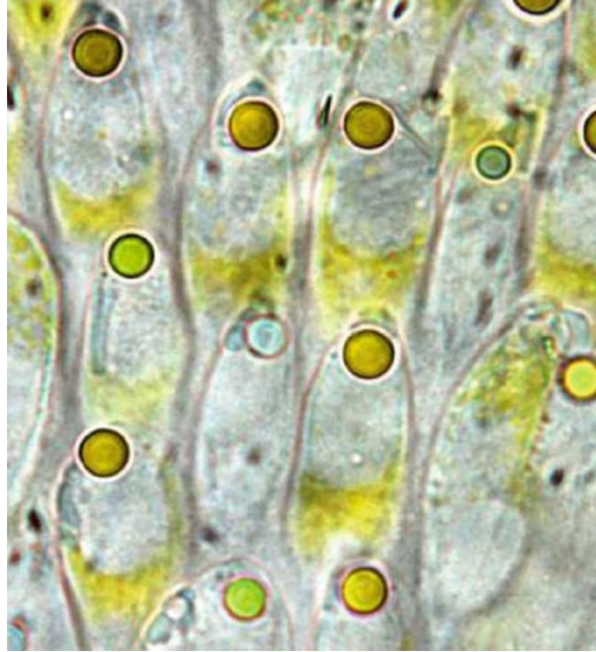

Fig. 11 Colored oil droplets within cones of a tropical fish alter the spectral sensitivity of the cell. This increases the number of color gradations perceived by the animal

oil droplet in front of the cone outer segments (Fig. 11). Rather than further increasing the repertoire of color-specific cone types, the early mammals greatly reduced the expression of cone pigments. Most mammals are still *dichromates* (Fig. 12b); they possess only two types of cones, a green-sensitive major type (used for shape and contrast detection) and a minority of blue- or ultraviolet (UV)-sensitive cones (used for color information). There are many modifications of this basic pattern; for instance, some rodents almost exclusively have blue-sensitive cones in their inferior retina (viewing the sky) but mostly green-sensitive cones in the median and superior retina

Fig. 10 (continued) foveal photopic pathway in primates provides each cone with up to three "individual," parallel bipolar and ganglion cells (for the ON and OFF "channels" and perhaps for color processing). In addition to this divergence, horizontal (and amacrine) cells provide contrast enhancement. Outside the fovea in the primate retina and in other mammalian retinas, such "private" pathways for individual cones are missing; rather, a convergence of the signals from a few cones onto a "set" of ganglion cells (ON, OFF, and others) can be observed, but still the spatial resolution of the photopic circuits remains better than that of the scotopic pathway by orders of magnitude. (**d**, **e**) The different "wiring" of rods and cones is reflected by differences in the structure of the retina of nocturnal and diurnal mammals, respectively. (**d**) The scotopically specialized retinas of nocturnal mammals are characterized by a thick ONL and a relatively thin INL and IPL. (**e**) By contrast, in the photopically specialized retinas of diurnal animals, the thickness of the INL (and of the IPL) exceeds that of the ONL which, in the extreme case of the tree shrew, consists of a single stack of large cone somata, almost without intermingled rods. Toluidine blue-stained semithin sections; courtesy of Leo Peichl (Frankfurt/M.)

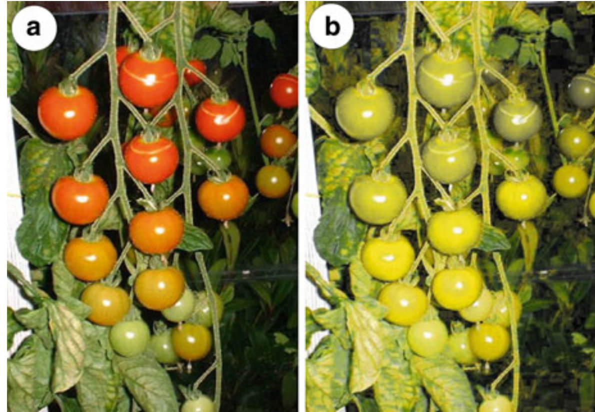

Fig. 12 In trichromatic primates (**a**), mutations in the genes of red or green opsin result in red–green blindness, and the subjects are dichromats (**b**)

(viewing the close environment of the animals). Very counterintuitively, blue-sensitive cones are missing in all aquatic mammals studied so far. It is believed that the first mammals, which were comparatively small and constituted an easy prey of the coexisting dinosaurs, avoided the dangerous confrontation and went into a nocturnal lifestyle for which color vision was not relevant. It was much later (35 millions of years ago) during the development of the ancestors to humans and other Old World primates that a splice mutation of the gene encoding the green-sensitive cone photopigment generated a long wavelength ("red")-sensitive photopigment. In *Callithrix*, considered as close relatives of the ancestors among which the mutation occurred, all females possess three different cone pigments, whereas among the males, all different degrees of anomalous color vision occur (only two cone photopigments; deviant "red" photopigment, full trichromacy). So basically, the Old World primates and humans are *trichromats* (Fig. 12a), but still up to 10 % of human males display some degree of red-green recognition deficit (Fig. 12b). The presence of red and green opsins has advantages in the recognition of food sources (e.g., to detect red fruits among green leaves; Fig. 12a) and in the social communication (e.g., blushing as emotional and sociosexual signal). For the latter reason, trichromat primates tend to be barefaced or bare rumped.

Basic Ontogenetic Mechanisms of Retinogenesis

After neural induction and genetic determination of the future retina within the neural plate (Figs. 4, 5, and 6), there occurs a specification of the retinal topography in respect to dorsal versus ventral, nasal versus temporal, and center versus periphery. Thereafter, the species-specific size and functional specification of the future retina are differently regulated in several consecutive steps.

The Number of Cell Cycles of Retinal Progenitor Cells Matters

The identical replication of *pluripotent retinal progenitor cells* causes an increase in the area of the future retinal tissue. As the duration of the cell cycles does not differ

greatly between the species (at least, among homeothermic animals), it is mainly the number of consecutive cell cycles that determines the size of the future retina and eye. Roughly, this correlates with the duration of gestation and, thus, with body weight and size of the species in eutherian mammals. However, there is no constant relation between retinal area/eye size and body size even among closely related primates. Obviously, the *number of the cell cycles* of these pluripotent progenitor cells is under genetic control, generating the striking differences between the extremely small (but otherwise well-organized) eye and retina of the mole and the large eye and retina of the giraffe, for example. Cell cycle mutants in zebrafish display small eyes (*microphthalmia*); the proto-oncogene N-myc has been shown to the involved in the control of retinal progenitor cell proliferation and eye size in the murine retina.

Once the future retina is determined in size, (the majority of) the proliferating cells restrict their potency to become so-called *early retinal progenitor cells*. After commitment, one of the daughter cells becomes postmitotic and starts differentiation into one of a distinct set of "early-born" *retinal neuron types*, including ganglion cells, horizontal cells, cones, and certain amacrine cells (Figs. 8 and 13). The other daughter cell retains the properties of an early progenitor cell and continues to proliferate. This asymmetric division of the early progenitor cells lasts for a distinct period which (1) appears to be similar throughout the variety of mammals and (2) ends by a distinct break in marsupials and in large mammals with long gestation periods, whereas in small laboratory rodents, the first proliferation phase overlaps considerably with the subsequent proliferation of "late retinal progenitors."

These *late retinal progenitor cells* generate a different set of retinal cells, mainly *rod photoreceptor cells, bipolar cells*, a subset of amacrine cells, and *Müller glial cells* (Figs. 8 and 13). Clonal analysis has shown that the progeny of late progenitor cells often contains one (and never more than one) Müller cell and that their last division may generate one Müller cell and one rod or one bipolar cell. In contrast to the proliferation of the early progenitors which appears to be rather constant in duration, the late progenitor cells may undergo a variable number of cell divisions in different mammals. This appears to be a major mechanism to control the scotopic specialization of retinas in nocturnal mammals (Fig. 8); the more rods are required to guarantee high light sensitivity, the more rounds of division are performed by the late progenitor cells. (As a peculiarity, the proliferation of the late progenitors appears to be largely suppressed in the region of the future primate fovea, such that no rods are generated there.) Quantitative data fit well with the assumption that the two phases of retinal cytogenesis are differentially regulated, with an early constant phase, generating a "uniform" set of (photopic) neurons, and a late variable phase, generating the necessary bipolar (and amacrine) cells plus rod photoreceptors (the number of which later roughly doubles with every *additional round of cell divisions*) and one Müller cell. After a few of these late divisions, the *number of rods* dominates the number of retinal neurons (e.g., more than 80 % of retinal cells are rods in *nocturnal* rodents).

Retinal Layers and Mosaics Are Formed Early After Cytogenesis

All retinal cells are born at the ventricular surface of the neural retina (i.e., close to the retinal pigment epithelium). Ganglion cells, which are the first cell type born,

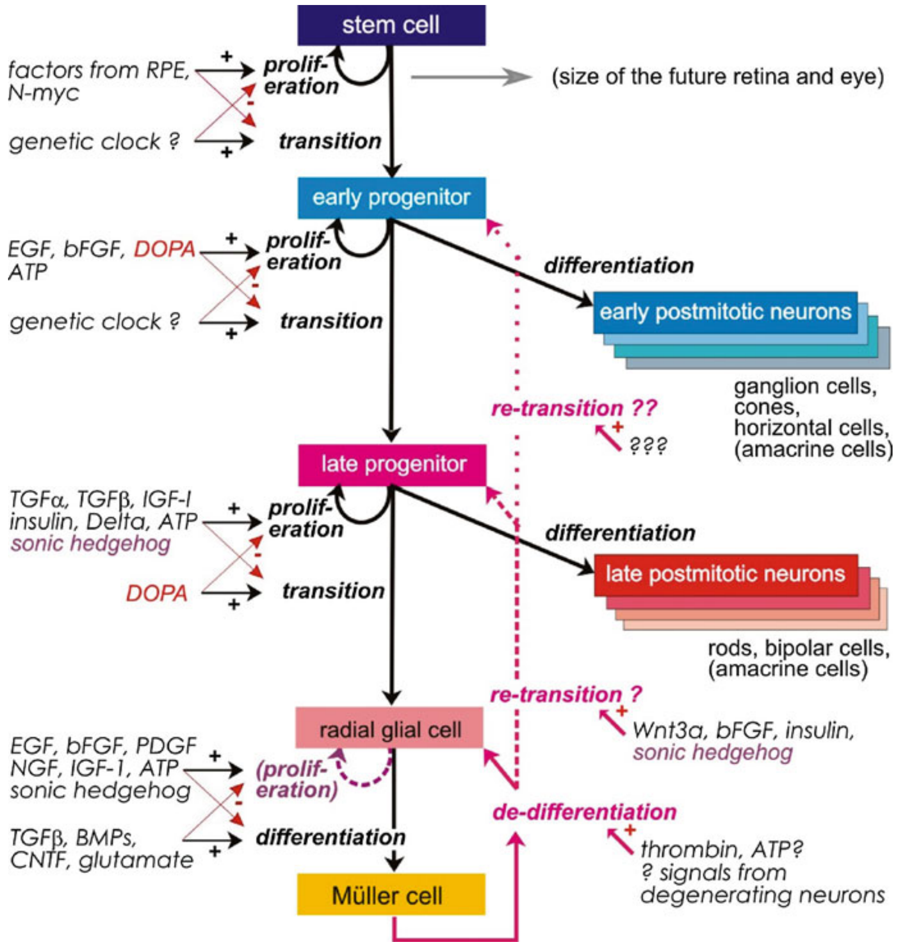

Fig. 13 Schematic diagram of cytogenesis in the mammalian retina. After a phase of identical replication of retinal stem cells, a stepwise transition occurs to early and then to late progenitor cells and, finally, to radial glial cells. This development is accompanied by distinct restrictions/changes of potency (*thick black arrows*). The duration of each proliferation phase (i.e., the number of cell divisions) until transition to the next step is tightly controlled, in a species-specific manner, by a variety of factors (some of the known factors are given at the *left side*). This allows a subsequent determination of (1) the size of the future retina and eye (stem cells: very variable), (2) the contribution of primary photopic cells (early progenitors: not very variable), and (3) the addition of complementary bipolar and amacrine cells (not very variable) and rod photoreceptor cells (very variable) by the late progenitors. There appear to be some key regulators that modulate the relation between early and late neuron production (e.g., DOPA and sonic hedgehog). One of the daughter cells of the late progenitor cells becomes a radial glial cell which normally differentiates as a Müller cell without further proliferation. Under pathological conditions (*thick red arrows*), however, Müller cells may dedifferentiate into radial glial cells and undergo proliferation. Under experimental conditions, even a retransition to late progenitors has been induced, whereas a retransition into early progenitors was not yet achieved in mammals

start their differentiation by extension of a radial process toward the inner basal lamina and by the movement of their nucleus within this process up to an inner position close to their final destination. Eventually, they withdraw their external (ventricular) cell process and start to grow an axon along the end feet of the progenitor cells toward the future optic nerve. As the retinal neuroepithelium is rather thin during this early developmental stage and merely consists of parallel tubular cells spanning the epithelium, the young ganglion cells do not need any specific aid or guidance for this translocation from outer to inner retina. Early-born horizontal cells translocate their somata in a similar way, but over a shorter distance. The cones, as another type of early-generated cells, just reside at the very ventricular surface where they were born and do not need to migrate at all.

The later-born bipolar and amacrine cells are confronted with another situation; now, the retinal neuroepithelium has become thicker and is crowded with many proliferating as well as early-differentiating cells which later grow processes not in parallel to the radial "palisades." In order to reach their final destination, the (future) inner nuclear layer, they may need a "climbing guidance." Very probably, they use their "sibling" radial glial (Müller) cell as a climbing pole. As such "sibling groups" of young neurons migrate together along the same radial glial/progenitor cell process, their leading and training processes will touch each other frequently and may later form synapses among each other with high probability. This may support the formation of functional units by such *columnar arrangements of cells*.

Once all cell types arrived in their respective layers of destination, they form synapses with the appropriate partner cells, and the *two synaptic* (*plexiform*) *layers* form between the *three nuclear layers* (Figs. 14 and 15). This process contributes to the formation of retinal mosaics. The term "retinal mosaics" describes the regular arrangements of the populations of retinal cell types (Fig. 16). The members of distinct types of ganglion cells, for instance, are regularly spaced such that every retinal area is "covered" by the dendritic tree of (at least) one of the cells; the so-called coverage factor may vary for individual cell types from close to 1 up to >4 (Fig. 16). The early-generated neurons (i.e., cones, ganglion cells, and horizontal and amacrine cells) are probably spaced via contact or near-distance inhibition of the generation and/or differentiation of the same cellular (sub-)type. In some sense, the Müller cells and their accompanying late-generated neurons also form a mosaic; this is explained by the above-described processes of cell generation and migration. Although not generated at the same time and not spaced by the same mechanism, densely arranged early-born neurons such as the cones basically display the same arrangement as the columns of late-generated cells; it has been shown that there is roughly one cone per Müller cell throughout the mammalian retinal diversity.

Late Shaping Processes Occur During Retina Expansion and Foveation

Once cell proliferation ceases, and the retinal layers and mosaics are formed, the shaping of the retinal tissue continues. As a main mechanism, there occurs a considerable *expansion of the retinal surface area*, together with growth of the eyeball, *after* cessation of cell generation (Fig. 17a). As no new cells are generated, and as cell

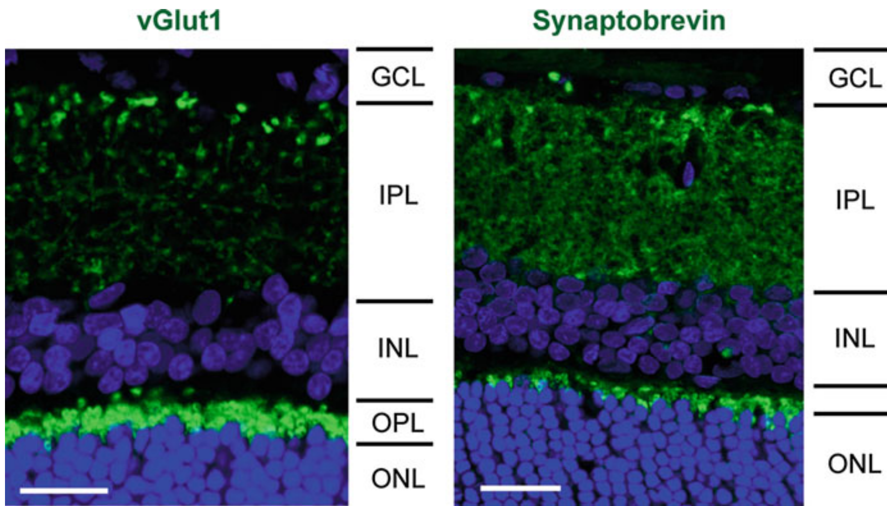

Fig. 14 The retina contains two synaptic layers, the inner (*IPL*) and outer plexiform layers (*OPL*). Retinal slices of the rat were stained against the glutamatergic synapse marker, vesicular glutamate transporter-1 (vGlut1), and synaptobrevin, respectively (*green*). Cell nuclei were labeled with Hoechst 33258 (*blue*). vGluts mediate the glutamate loading of secretory vesicles. *GCL* ganglion cell layer, *INL* inner nuclear layer, *ONL* outer nuclear layer. Bars, 20 μm

death is negligible during later postnatal development, constant populations of retinal cells become redistributed within a larger surface area; this means that cellular densities must decrease (up to a factor of more than four, depending on the species-specific degree of eye growth; Fig. 17b). Noteworthily, the degree of retinal expansion is *not uniform* across the retinal topography. Retinal areas which are responsible for high-acuity vision in the mature retina (area centralis, visual streak) expand less than the retinal periphery such that the neonatal high cell densities are reduced significantly less than in the periphery. The basic mechanism of this postnatal retina expansion has been described as a continuous series of stretching and "after-grow" processes according to the so-called balloon model. The driving force is the intraocular pressure; the basic resistance is provided by the sclera. Local differences in retinal stretching correspond to local differences in retinal stiffness.

It is noteworthy that local growth of the sclera and the eyeball is under control of retinal circuits. This is necessary in order to achieve and/or maintain visual acuity in a process called *emmetropization*. If the optic apparatus of the growing eye fails to project a sharp image onto the retina, certain amacrine cells trigger a (hitherto widely unknown) signaling cascade toward the underlying sclera which stimulates the expansion of the globe; in mammals this seems to involve a decrease of the mechanical resistance of the sclera against the inner ocular pressure, whereas in the chicken, active growth processes in the sclera seem to dominate. Excessive eye elongation due to this mechanism is called "form deprivation-induced axial myopia."

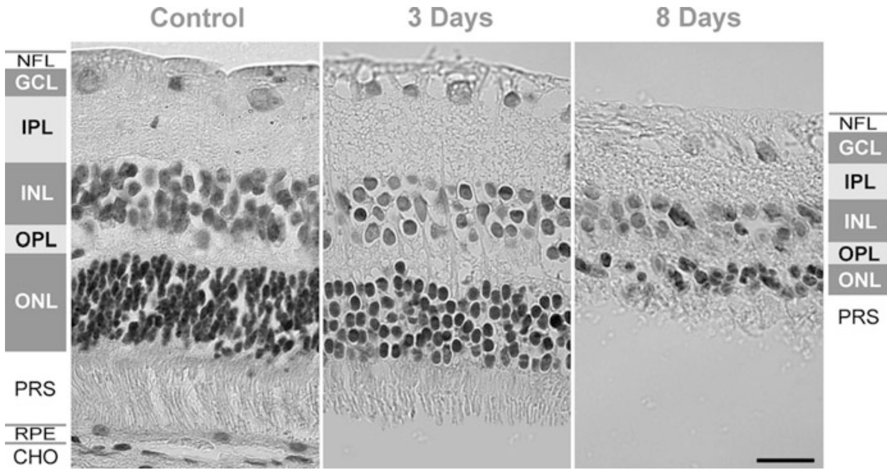

Fig. 15 The neuroretina is a well-layered structure consisting of three layers with cell somata which contain the cell nuclei (outer [*ONL*] and inner nuclear layers [*INL*], and ganglion cell layer [*GCL*]) and two layers which contain predominantly neuronal synapses (outer [*OPL*] and inner plexiform layers [*IPL*]). The images display slices of the ventral central retina of the rabbit obtained from a control eye (*left*) and from eyes after 3 (*middle*) and 8 days (*right*) reperfusion following a 1-h retinal ischemia. Transient ischemia of the nonvascularized areas of the rabbit retina results in time-dependent degeneration of photoreceptor cells and second-order neurons, as indicated by the decreases in the thickness of the ONL and INL, respectively. The absence of the retinal pigment epithelium (*RPE*) and the choroidea (*CHO*) after retinal ischemia is a result of the exudative detachment of the neuroretina from the RPE which is associated with subretinal edema. Subretinal edema develops due to opening of the outer blood-retinal barrier normally constituted by the RPE, as well as by the regulatory volume decrease of degenerating photoreceptor cells (which is mediated by an efflux of ions and water from the cells). *NFL* nerve fiber layer, *PRS* photoreceptor segments. Bar, 20 μm

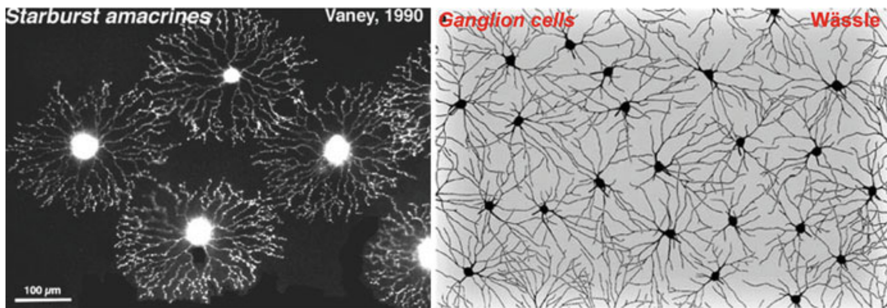

Fig. 16 The populations of different retinal cell types, e.g., starburst amacrines (*left*) and ganglion cells (*right*), are regularly arranged. The dendritic fields of the ganglion cells overlap (*right*), i.e., the "coverage factor" is >1

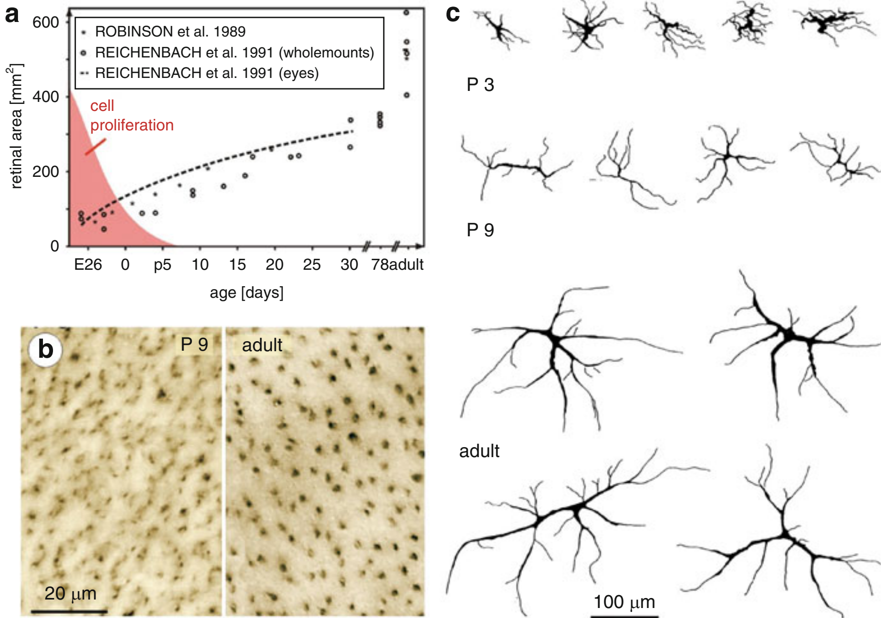

Fig. 17 “Active” and “passive” growth of the rabbit retina. “Active” growth is achieved by cell proliferation, whereas “passive” growth is caused by mechanical tissue expansion due to the intraocular pressure after the cessation of cell proliferation. (**a**) The surface area of the retina increases fourfold even after cessation of cell proliferation which means that the existing cells are redistributed within a larger area. (**b**) As Müller cells do not proliferate in the healthy retina, they constitute a constant population which is redistributed (i.e., spatially separated) in the expanding retina. Accordingly, their spatial density decreases. The images display cross sections through Müller cell stem processes in retinal wholemounts which were immunostained against vimentin. (**c**) Much of the postnatal cell process elongation of horizontally wired interneurons occurs parallel to the local expansion of the retinal area which allows the cells to maintain their synaptic contacts to other cells (e.g., photoreceptor cells) which become spatially separated by the tissue expansion. The images display camera lucida drawings of horizontal cells in Golgi-stained retinal wholemount preparations. The basic dendrite pattern and synaptic contacts of the cells are established shortly after postnatal day (P) 3. Thereafter, the overlap factor of the dendritic fields of the cells remains constant because the length of their dendrites keeps space with the local tissue expansion. Because the developmental tissue expansion is larger in the peripheral retina than in the central retina, the horizontal cells in the peripheral retina of adult animals have greater dendritic fields than the horizontal cells in the central retina

As a consequence of differential retinal stretching, the stretched retina becomes thinner. This is no simple mechanical effect like in a stretched rubber band; rather, the cells become rearranged because there is more space now for them, and the “crowding” is relieved. Accordingly, the number of stacks in the nuclear layers is reduced, and these nuclear layers become thinner. By contrast, the thickness of the plexiform layers remains unchanged or even increases (particularly, in the central retinal areas) due to further elaboration of the synaptic circuits and increasing diameters of the cellular processes. As another consequence of retinal stretching,

the mosaics of many of the wide-field become “stretched” (Fig. 17c). “Active” growth of their dendrites occurs during a fast, short “burst” during the perinatal period (e.g., in the rabbit retina the A-type horizontal cells between the third and ninth postnatal day; Fig. 17c). Thereafter, the basic synaptic contacts to their appropriate partner cells are established, and further *expansion of the dendritic fields* occurs together with the locally expanding retinal tissue area by the same factor of areal expansion, probably to maintain these established synaptic contacts.

To understand the formation of the *fovea centralis* in the primate retina (Fig. 18) is a particular challenge. The region of the future fovea appears to be determined from the beginning of retinogenesis by the local expression of domain-specifying genes. It is characterized as an area of elevated ganglion cell density and a rod-free area from the beginning. However, the characteristic shape of the fovea develops late, long after determination and after cessation of cell proliferation (i.e., after birth in humans). The two main events are a *centripetal movement of the perifoveal photoreceptor cells* (causing a high cone photoreceptor density) and a *centrifugal translocation of the inner retinal layers* (accompanied by the formation of the pit or “fovea”) which together result in a “Z-shaping” of the local Müller cell processes, and of the cone axons forming *Henle’s fiber layer*. The mechanics of foveation are poorly understood and will thus not be discussed here.

Structural Organization of the Mammalian Retina

The Retina Is Composed of Two Main Layers

The inner part of the retina (which borders on the vitreous cavity) is the neural retina (or neuroretina) which contains, among others, the photoreceptors and neuronal cells, and the outer part is constituted by the retinal pigment epithelium (RPE) which is a cellular monolayer (Figs. 1, 15, and 19). The interface between these structures is the subretinal space; it contains a fluid compartment (the remainder of the optic ventricle) and the interphotoreceptor matrix.

The inner surface of the neural retina is covered by a basal lamina (“inner limiting membrane,” ILM) which has contact to the fluid in the vitreous chamber. The outer surface of the RPE abuts Bruch’s membrane, a multilayer basement membrane (Fig. 20). This membrane overlies the choriocapillaris which supplies the RPE and photoreceptors with oxygen and nutrients. The choriocapillaris is the inner part of the choroidea which finally borders on the sclera, the outer capsule of the eye (Fig. 1). The “outer limiting membrane” (OLM) is a thin, eosinophilic structure made by intercellular junctions between Müller glial cells and photoreceptor cells (Fig. 21).

The neuroretina has a well-organized structure with seven main layers (Figs. 15 and 19). Three layers contain the perikarya of cells (outer nuclear layer, ONL; inner nuclear layer, INL; ganglion cell layer, GCL), two layers contain cellular processes and the neuronal synapses (the outer plexiform layer, OPL, which is primarily composed of ribbon synapses, and the inner plexiform layer, IPL, which contains largely conventional synapses; Fig. 14), one layer contains the axons of ganglion

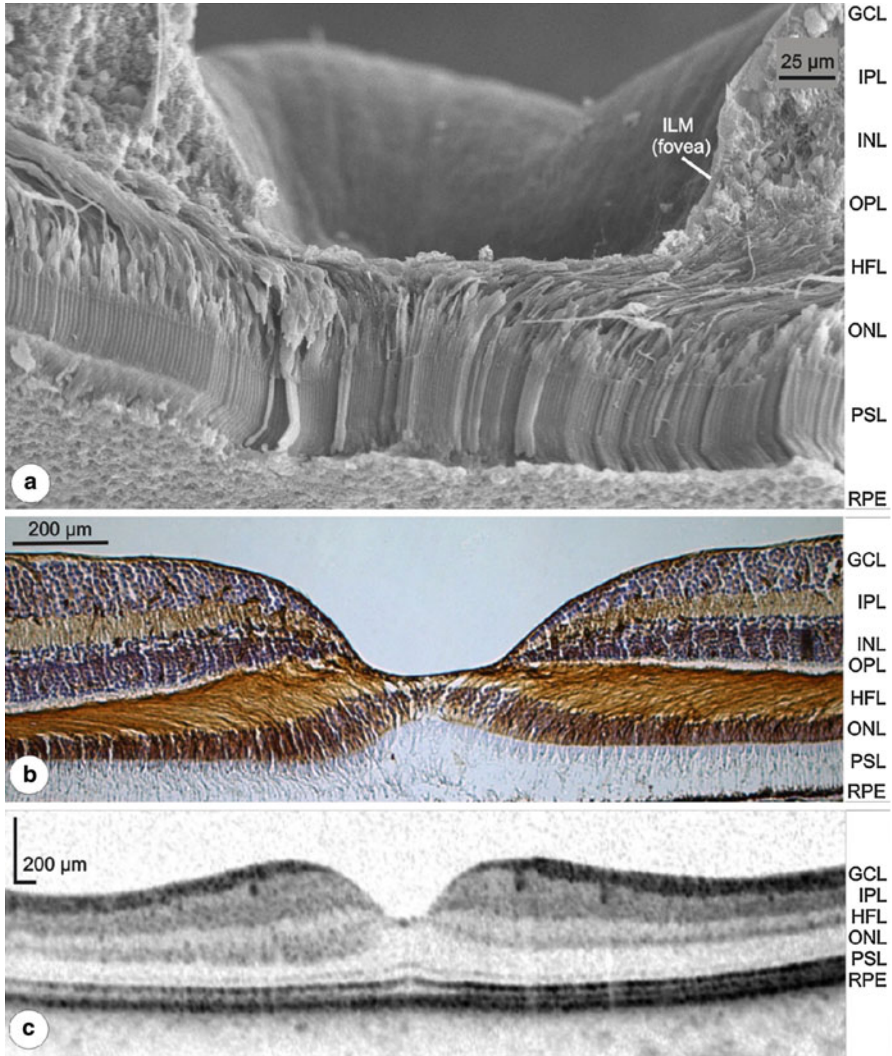

Fig. 18 Shape and cellular organization of the primate fovea. (**a**) Scanning electron microphotograph of the fovea of a macaque monkey. The extremely high packing density of cone inner and outer segments in the photoreceptor segment layer (*PSL*), the complex centrifugal course of their axons crowded in the Henle fiber layer (*HFL*), and the absence of the ganglion cell (*GCL*), inner plexiform (*IPL*), inner nuclear (*INL*), and outer plexiform layers (*OPL*) are well illustrated. The outer processes of Müller glial cells run among the Henle fibers but cannot be reliably identified. (**b**) When the Müller cell processes are visualized by vimentin immunohistochemistry (counterstaining of cell nuclei by H-E; siamang retina), they can be traced along their path from the outer margin of the outer nuclear layer (*ONL*) up to their end feet; it becomes obvious that they run in parallel to the Henle fibers. (**c**) Optical coherence tomographic (*OCT*) scan of a human retina in situ. The main retinal layers including the retinal pigment epithelium (*RPE*) can be identified, and the shape of the foveal pit is clearly depicted

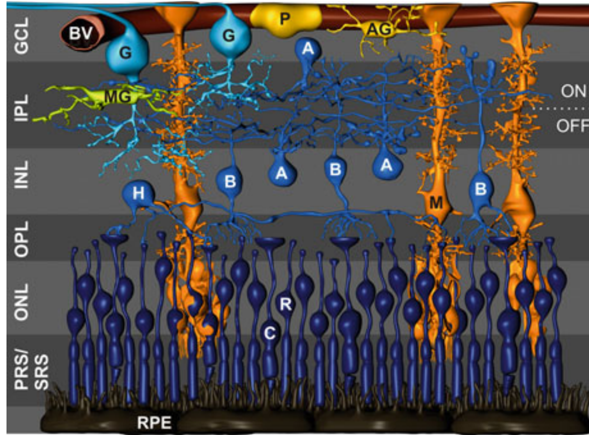

Fig. 19 Layering and cellular constituents of a "typical" (vascularized) mammalian retina. The subretinal space (*SRS*), as the remainder of the optic ventricle, lies in between the retinal pigment epithelium (*RPE*) and the neuroretina proper. The neuroretina basically consists of three layers packed with cell somata, the outer (*ONL*) and inner nuclear layers (*INL*) and the ganglion cell layer (*GCL*), and two synaptic layers, the outer (*OPL*) and inner plexiform layers (*IPL*). At the inner retinal surface (overlaying the *GCL*), a distinct layer of retinal ganglion cell axons (the nerve fiber layer) is found only close to the optic nerve head; elsewhere, the inner retinal surface is formed by the end feet of Müller cells, only crossed by small bundles of axons. The ONL contains the somata of photoreceptor cells, rods (*R*), and cones (*C*). Generally, the nuclei of cones are larger than those of rods and lie at the outermost border of the ONL (i.e., close to the SRS). The INL contains the somata of interneurons, including each of several subtypes of bipolar (*B*), horizontal (*H*), and amacrine cells (*A*), as well as the somata of Müller glial cells (*M*). The somata of retinal ganglion cells (*G*) and displaced amacrine cells form the GCL. In addition to Müller glial cells, astroglial (*AG*) and microglial cells (*MG*) are found in the inner retina. Intraretinal blood vessels (*BV*) are located at the inner surface of the retina and in the GCL as well as, in dependence on the species, in one or two additional deeper layers of the vascularized retinas. The larger blood vessels are equipped with pericytes (*P*). The IPL can be subdivided into several sublayers. An important subdivision reflects the fact that the synapses propagating the "light-on" signals occupy the inner part of the INL (*ON* sublayer), whereas the "light-off" signals are mediated by synapses in its outer part (*OFF* sublayer)

cells on their course toward the optic nerve head (nerve fiber layer, NFL), and the outermost layer is formed by the photoreceptor segments (PRS). The neuroretina can be subdivided into an inner and outer part. The inner half includes the NFL, GCL, IPL, and INL, and the outer part consists of the OPL, ONL, and PRS. The IPL can be further divided into several sublayers according to the location of specific synapses; for instance, the "light ON" information is processed in the inner part of the IPL, the "light OFF" information in its outer part (Fig. 9).

The Neuroretina Contains Several Different Types of Neurons and Glial Cells

The neural retina is composed of over 50 different cell (sub-)types, mostly representing neurons (including photoreceptor cells) and glial cells. A third

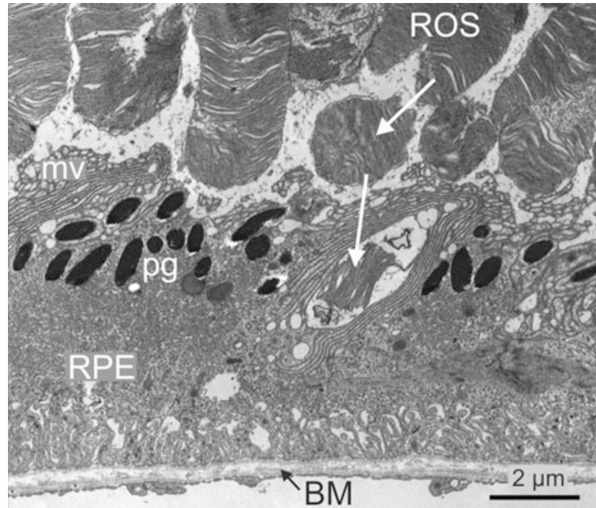

Fig. 20 The interface between rod outer segments (*ROS*) and retinal pigment epithelium (*RPE*) in the pig retina. *BM* Bruch's membrane, *mv* microvilli of RPE cells, *pg* pigment (melanin) granule. The *arrows* indicate a package of disks which was shed from the tip of a rod outer segment (*above*) and another package of disks which will be phagocytized by RPE cells (*below*)

constituent, blood vessels, is present only in vascularized retinas (i.e., in many but not all mammalian retinas and in some fish retinas such as that of the eel).

Photoreceptor, Bipolar, and Ganglion Cells Mediate the Forward Transmission of Visual Information

There are three types of neurons that lie in series and transfer the visual signal through the retina from the photoreceptor outer segments (where light is absorbed by the photopigments) at the outer surface of the neuroretina to the axons at the inner surface of the retina running toward the optic nerve: photoreceptor cells (rods and cones, the first-order neurons of the retina), bipolar cells (the major second-order neurons), and ganglion cells (the third-order neurons) (Figs. 2 and 19). Photoreceptor cells consist of three parts (Figs. 3, 10a, and 21), the somata of the cells with the cell nuclei located in the ONL, the sensory process that consists of an inner segment (containing the energy-producing mitochondria) and an outer segment (containing the molecular machinery that captures the photons and converts the light energy into neuronal activity), and an axon with a presynapse that lies in the outer plexiform layer. The presynapse is a part of a specialized type of synapses, the ribbon synapse (Fig. 22). The synaptic terminals of cones (*pedicles*) are greater than the terminals of rods (*spherules*). The sensory processes extend into the subretinal space where the outer segments are surrounded by microvilli of the RPE that are engaged in a lively exchange of molecules and even cell organelles with the outer segments (Figs. 19 and 20).

The somata of the bipolar cells are located in the INL (Fig. 19). The outer process, the dendrite, of the cells forms the postsynaptic elements of the ribbon synapses and receives the visual information from the photoreceptor cells (Fig. 22). The inner process or axon ends with presynapses in the IPL (Figs. 9, 19, and 23); these presynapses are ribbon synapses, similar to those of the photoreceptor cells.

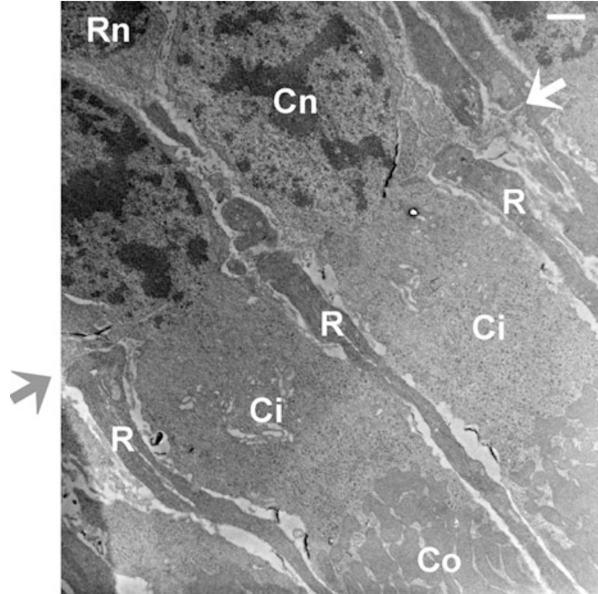

Fig. 21 Morphology of rods (*R*) and cones (*C*) at the level of the outer limiting membrane (*arrows*) in the pig retina. The perikarya of cones (containing the cell nuclei; *Cn*) are localized directly at the outer limiting membrane, while the perikarya of rods (*Rn*) are localized at more inner parts of the outer nuclear layer. The inner (*Ci*) and outer segments (*Co*) of cones are shown. Bar, 1 μm

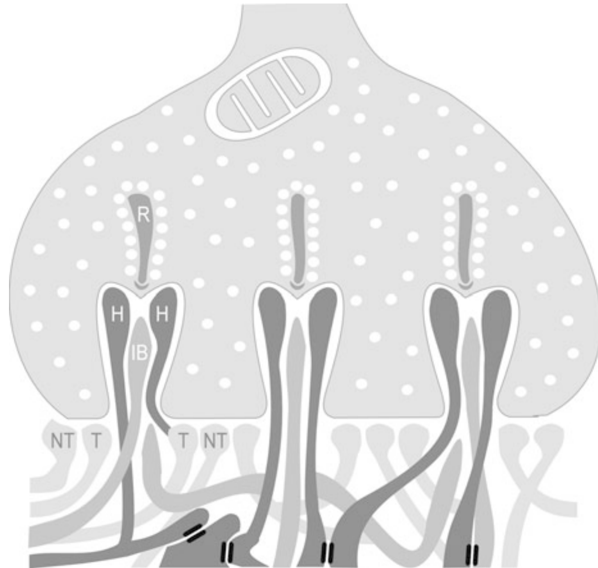

Fig. 22 Schematic structure of a cone pedicle in the primate retina which contains three triads. *H* horizontal cell, *IB* invaginating ON-bipolar cell, *NT* OFF-bipolar cell which is not associated with the triad, *R* ribbon which binds glutamate-containing synaptic vesicles, *T* triad-associated OFF-bipolar cell. The *black double lines* indicate the areas of gap junctional coupling (desmosome-like junctions)

The somata of the ganglion cells are located in the GCL (some displaced ganglion cells may be also located at the inner edge of the INL) (Fig. 19). The dendrites of these cells spread within the sublayers of the IPL where they end in postsynapses that receive the visual information from bipolar and amacrine cells (Fig. 9). The axons of

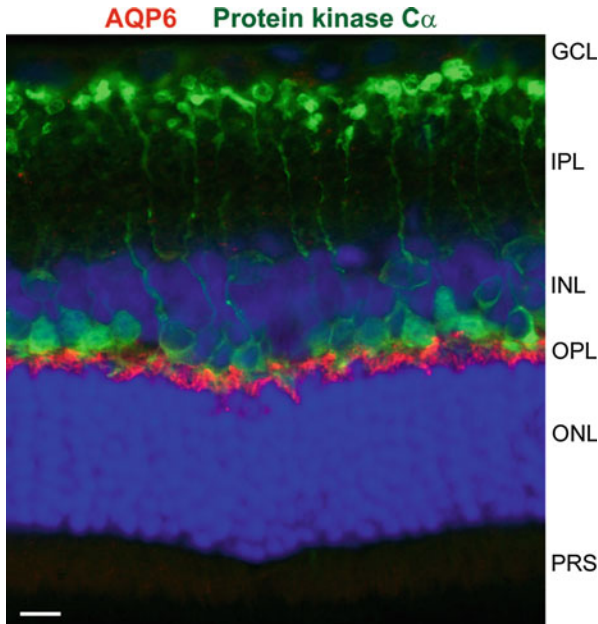

Fig. 23 In the rat retina, rod bipolar cells and a heterogeneous population of (predominantly tyrosine hydroxylase-negative) amacrine cells contain protein kinase Cα. The somata of bipolar cells are localized at the outer margin of the inner nuclear layer (*INL*), while the somata of amacrine cells are localized at the inner margin of the INL. The ribbon synapses of rod bipolar cells are localized at the inner margin of the inner plexiform layer (*IPL*), near the perikarya of ganglion cells. A slice of the rat retina was immunostained against protein kinase Cα (*green*) and aquaporin-6 (*red*) which marks the ribbon synapses in the outer plexiform layer (*OPL*). Cell nuclei were labeled with Hoechst 33258 (*blue*). *GCL* ganglion cell layer, *ONL* outer nuclear layer, *PRS* photoreceptor segments. Bars, 20 μm

all ganglion cells run in the NFL at the inner surface of the retina where they form nerve fiber bundles. These bundles meet at the optic nerve head (optic disk, the blind spot of the retina which is devoid of photoreceptors) and form the optic nerve which transfers the visual information from the retina to the brain.

Interneurons Mediate Processing of Visual Information

The interneurons of the retina, horizontal and amacrine cells (Fig. 19), are not primarily involved in the forward transmission of visual information (with the exception of the AII amacrine cell which connects the rod pathway to the cone output pathway). Horizontal and amacrine cells perform the processing of visual information. The axons and dendrites of these cells are arranged transverse to the "forward" direction, i.e., they extend within the OPL (horizontal cells; Fig. 24) and IPL (amacrine cells; Fig. 25), respectively. The cell bodies of horizontal cells are located at the outer border of the INL, and their dendrites and axons end at the ribbon synapses in the OPL where they regulate the effectiveness of the synaptic

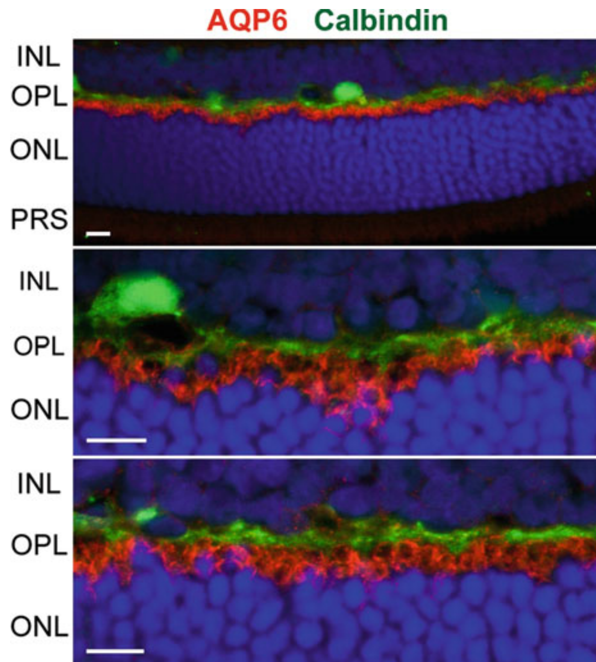

Fig. 24 Horizontal cells are localized at the outer margin of the inner nuclear layer (*INL*). Retinal slices of the rat were immunostained against calbindin (a protein selectively localized in horizontal cells) and aquaporin-6 which marks the ribbon synapses in the outer plexiform layer (*OPL*). Cell nuclei were labeled with Hoechst 33258 (*blue*). *ONL* outer nuclear layer, *PRS* photoreceptor segments. Bars, 20 μm

transmission from photoreceptor cells to bipolar cells (Figs. 19, 22, and 24). The somata of amacrine cells are located at the inner border of the INL (Figs. 19 and 25). Amacrine cells send dendrites and axons into the IPL where they regulate the activity of the bipolar-to-ganglion cell synapses. The chemical synapses of amacrine cells are of the conventional (i.e., nonribbon) type; they constitute the majority of synaptic elements in the IPL. In addition to the "normal" amacrine cells, there exists a considerable population of so-called displaced amacrine cells. Their perikarya are located in the GCL. A subtype of amacrine cells, the interplexiform cell, performs information processing between the two plexiform (synaptic) layers.

Rods and Cones as the Main Light Sensors in the Retina

Two types of specialized receptor cells absorb the environmental light and transduce the light stimuli into neuronal (electrochemical) signals, rods and cones. These cells are named according to the rod- and cone-like shape of the light-sensitive outer receptor segments (Figs. 2 and 10a). (A third type of light sensors, photosensitive retinal ganglion cells, are described below). The human retina has ~100 million rods and ~5 million cones. The phototransduction process is similar in rods and cones.

Photoreceptor cells have a characteristic shape. The perikarya of the cells are densely packed in the ONL (Figs. 23 and 26a, b). The perikarya of cones are located at the outer limiting membrane while rod cell perikarya are located at more inner

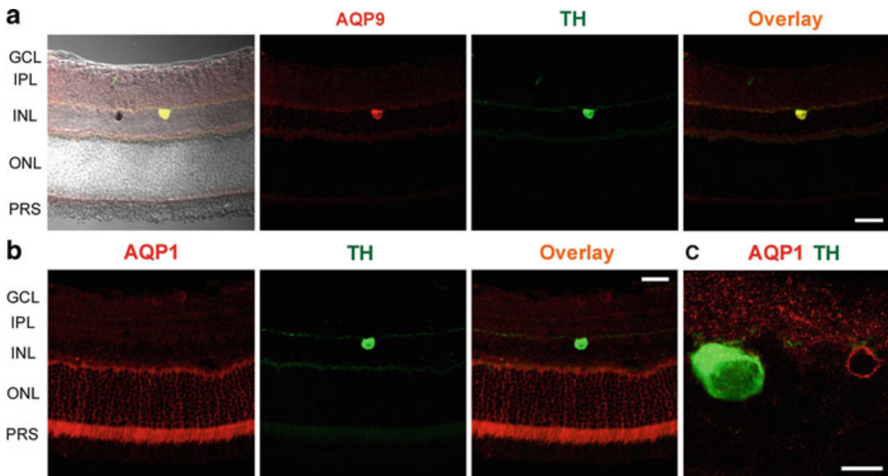

Fig. 25 Diversity of amacrine cells. Catecholaminergic amacrine cells in the rat retina express aquaporin-9 (*AQP9*) but not aquaporin-1 (*AQP1*). (**a**) Double immunostaining of a retinal slice against AQP9 and tyrosine hydroxylase (*TH*), the marker enzyme of catecholaminergic cells. Colabeling yielded a yellow overlay signal. TH-positive cell somata are localized at the inner margin of the inner nuclear layer (*INL*), while TH-positive nerve fibers draw within the stratum 1 of the inner plexiform layer (*IPL*). (**b**) Double labeling of a slice against AQP1 and TH. The TH-positive amacrine cells do not express AQP1, as indicated by the absence of a yellow overlay signal. (**c**) A soma of an AQP1-positive amacrine cell (*red*) and a soma of a TH-positive amacrine cell (*green*) in one slice. There is no overlap of the immunoreactivities for AQP1 and TH. *GCL* ganglion cell layer, *ONL* outer nuclear layer, *PRS* photoreceptor segments. Bars, 20 (**a**, **b**) and 10 μm (**c**)

sites within the ONL (Fig. 21). The axons of rod and cone cells draw to the OPL where they terminate as ribbon synapse (Figs. 19, 23, and 24). Photoreceptor inner and outer segments rise into the subretinal space. The segments of rods are connected by a nonmotile cilium (Fig. 3); the perikarya of cones are continuous with the inner segment (Fig. 21). The inner segments contain mitochondria and organelles of the metabolic machinery such as endoplasmic reticulum and Golgi complex. The outer segments contain membranous disks (Figs. 3 and 20) in which ~10^8 light-sensitive photopigments are embedded. The disks of rods are intracellular membrane stacks, whereas the disks of cones are invaginations of the plasma membrane (Fig. 10a). Each mammalian rod outer segment contains ~1,000 disks; the photopigment (rhodopsin) accounts for ~90 % of the protein in rod outer segments. Rod outer segments are continuously synthesized, with about 10 % of each segment assembled at its base and 10 % discarded from its tip each day (Fig. 20). The shed tips of photoreceptor outer segments are engulfed by RPE cells for degradation (Fig. 20).

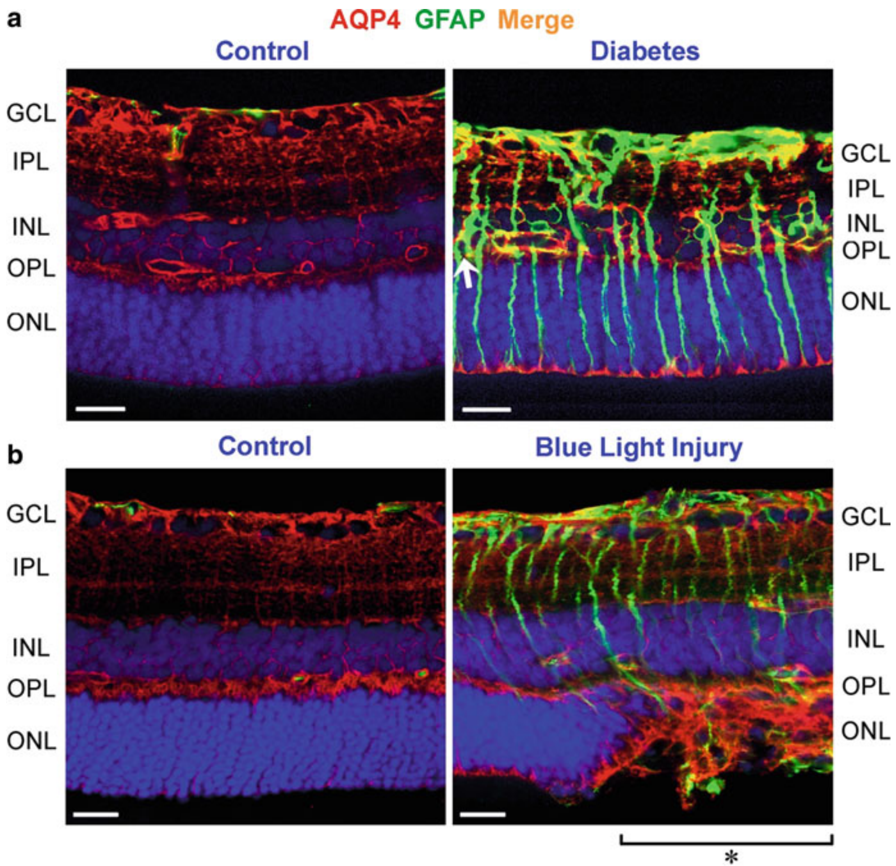

Fig. 26 Upregulation of intermediate filaments, e.g., of glial fibrillary acidic protein (*GFAP*), is a characteristic event of Müller cell gliosis under various pathological conditions. (**a**) Retinal slices from 6-month diabetic (*right*) and age-matched control rats (*left*) were immunostained against GFAP (*green*) and the glial water channel, aquaporin-4 (AQP4; *red*). Cell nuclei were labeled with Hoechst 33258 (*blue*). In the control retina, only astrocytes at the inner margin of the retina contained GFAP. In the retina of the diabetic animal, hypertrophied Müller cell fibers which traverse the whole retinal tissue displayed strong labeling for GFAP. (**b**) Retinal slices of a rat were stained 3 days after treatment of a circumscribed area of the retina with excessive blue light which resulted in apoptotic death of photoreceptor cells. The light-injured retinal area is shown *right* (*); the uninjured area is shown *left*. The strong upregulation of AQP4 in Müller cell processes within the outer nuclear layer (*ONL*) is likely a response to the outer retinal edema which develops due to the light-induced injury of the retinal pigment epithelium (resulting in leakage of the outer blood-retinal barrier) and the volume decrease of apoptotic cells which is mediated by an efflux of osmolytes (especially potassium and chloride ions) and water. *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *OPL* outer plexiform layer. Bars, 20 μm

Rods and Cones Operate Under Different Lighting Conditions

Rods are responsible for the low-contrast, achromatic vision with high sensitivity at low light intensities (*scotopic vision*). Rods are highly light sensitive; a rod can respond to a single photon. This high sensitivity underlies the remarkably low absolute threshold for human vision; five to seven absorbed photons are sufficient to register a response. Cones need hundreds of photons to become activated (Fig. 27). Because the central fovea of the human retina contains only cones and no rods, only the peripheral retina sees on a dark night, without color and with poor resolution, and the fovea is blind. (When looking at dim stars in the night, one can see stars in the periphery, but they disappear when one look at them with the fovea.) Cones are responsible for high contrast and color vision at bright daylight (*photopic vision*). In broad daylight, only the central fovea sees in detail and in color, and rods are saturated (inactivated) because the rod photopigment is "bleached." (Under *mesopic* conditions, i.e., at light intensities between scotopic to photopic levels, both rods and cones might be active.) Because under most lighting conditions rod and cone systems cannot operate simultaneously, there is an adaptation delay in moving from light to dark (slow) or vice versa (fast) (Fig. 27). Cones are able to perceive finer detail and more rapid changes in images because their response times to stimuli are five times faster than those of rods. In the mammalian retina, rods and cones have separate pathways to the ganglion cells (Figs. 2 and 10b, c). The rod pathway comprises rod bipolar cells and a special type of amacrine cell, the AII cell (Fig. 9).

Rod Vision Is Highly Sensitive and Cone Vision Has High Acuity

The higher sensitivity of rod vision as compared to cone vision is caused by (1) the high light sensitivity of rods and (2) the higher convergence from rods to ganglion

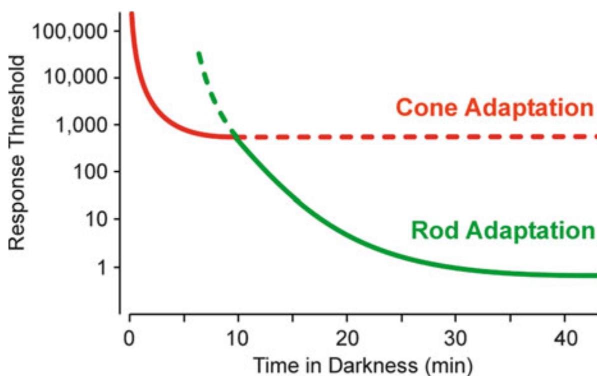

Fig. 27 In the course of the adaptation to darkness, the retinal sensitivity to light increases dramatically (i.e., the response threshold decreases; *solid lines*). The transition from photopic vision (*red solid line*) to scotopic vision (*green solid line*) occurs after 8–10 min (Kohlrausch bend). In the case of night blindness (caused, e.g., by vitamin A deficiency), the retina remains relatively insensitive to light (*red broken line*). Because cones and rods have different λ_{max} (555 and 498 nm, respectively), there is a sensitivity shift of the retina toward light with shorter wavelengths during dark adaptation (Purkinje shift)

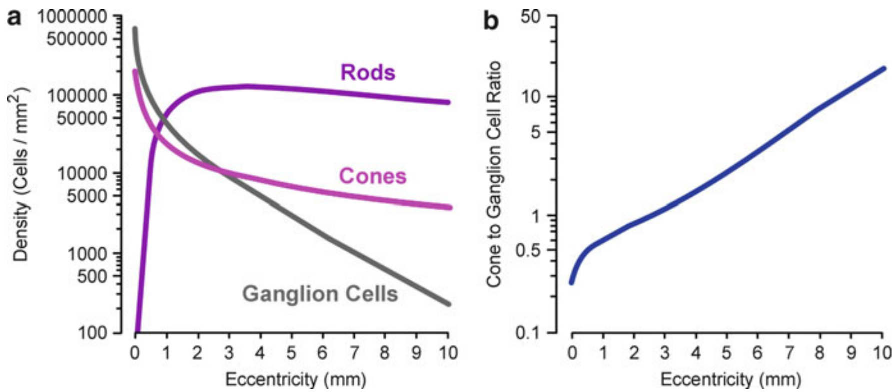

Fig. 28 Densities of rods, cones, and ganglion cells in the rhesus monkey retina (**a**) Zero eccentricity represents the fovea. (**b**) Cone-to-ganglion cell ratio

cells (120 to 1; eventually >1,000 to 1; Fig. 10b) than from cones to ganglion cells (6 to 1; in the fovea 1 to 3; Fig. 28b). In the central fovea, three midget ganglion cells (for the ON and OFF channels and color processing) are connected through three midget bipolar cells to a single cone (Fig. 10c). The 1-to-3 wiring of cones to ganglion cells and the small spacing of cones (the spatial density of cones is high and the receptor segments have a smaller diameter) are the basis for the high spatial resolution of the central fovea. Here, the cones are densely packed in a hexagonal pattern, with a minimum center-to-center spacing (a) of 2–3 μm. The resolution limit (Nyquist limit) is $\sqrt{3}a$ which corresponds to ~10,000 points for the whole fovea. Because the fovea is relatively small, the eyes must constantly shift their gaze when an object is large to bring different portions of the image into the fovea (as in reading).

The retinal periphery has poor acuity due to the large convergence from photoreceptor cells to ganglion cells and the lower density of photoreceptors and thicker receptor segments (large spacing). Outside the fovea, cone density decreases (Fig. 28a) and visual acuity is reduced accordingly (Fig. 29a, b). The density of ganglion cells decreases from the central to the peripheral retina by more than a factor of 1,000 in the primate retina (Figs. 28a and 29). The strong convergence of rods tends to make peripheral vision very sensitive to movement and is responsible for the phenomenon of an individual seeing something vague occur out of the corner of the eye.

The Optical Properties of the Tissue Are Important in the Inverted Retina

Although the retina is a transparent tissue, it consists of cells and cell processes that must interact with (e.g., scatter) light along its path toward the photoreceptor cells.

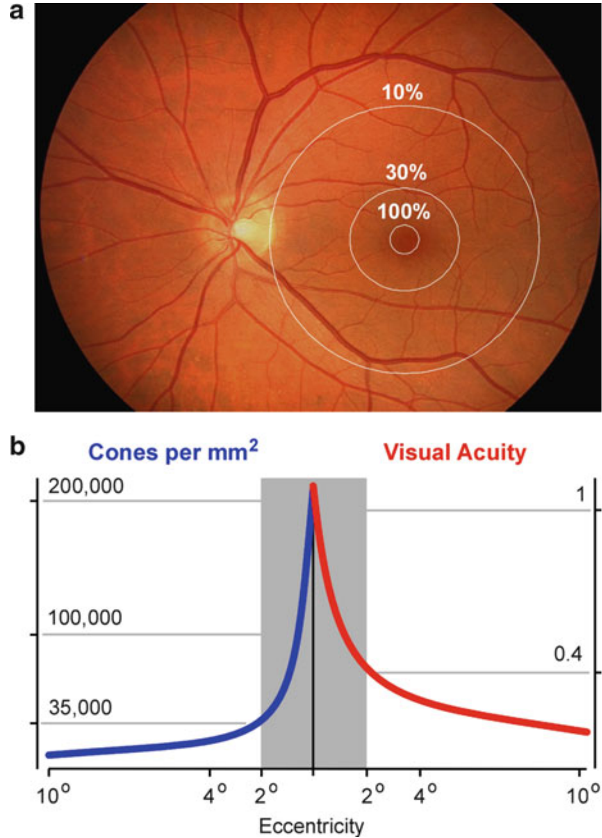

Fig. 29 The visual acuity depends upon the density of cones. (**a**) Relative visual acuity (in %) marked onto an angiographic image of the human retina. The fovea has the highest visual acuity. The retinal blood vessels draw to the optic disk (*bright yellow* area at *left*). (**b**) Cone density (*left*) and visual acuity (*right*) of the human retina. The *gray area* marks the acuity sufficient for reading

The Fovea Is an Adaptation to Increase the Sensitivity and Acuity of Cone Vision

The retinas of many predatory fish, reptilians, and birds contain one (or even two or three) retinal region(s) specialized for high visual resolution, a "fovea." Here, the densities of photoreceptors and neurons are particularly high, and the inner retina is deeply inclined in a funnel-shaped manner (Fig. 18a, b). In the retinas of nonprimate mammals, such a structure is missing; however, different regions of high cell density were developed, e.g., the "central area" in the cat retina and the "visual streak" in the rabbit retina. The different shapes of these regions are adaptations to the habitats of the animals. The visual streak of rabbits, e.g., is a high-density area that scans the horizon.

Because the vertebrate retina is inverted, light passes through the entire depth of the neuroretina before it is sensed by rods and cones. Often, the retina is considered as a transparent tissue through which light can pass without loss or scattering (Fig. 30). However, all cells and their processes and organelles are "phase objects," i.e., they must scatter the light. In particular, the synapses in the plexiform layers have diameters close to 0.5 μm, i.e., within the wavelength range of visible light

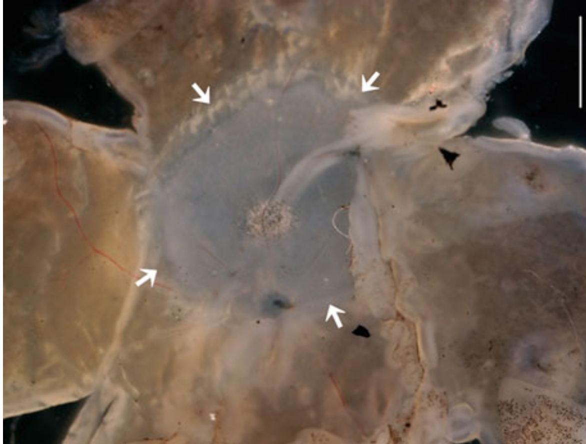

Fig. 30 The transparency of the neuroretinal tissue is decreased in retinal edema. The image displays a view onto a rat retina in an opened eye bulbous with removed lens and vitreous. Three days before, the retina was locally illuminated with excessive blue light. The *arrows* indicate the border between the light-injured (*middle*) and uninjured (*peripheral*) retinal tissue. In the center of the light-injured (*milky*) retina, the optical nerve head (blind spot) is visible. In the uninjured (*peripheral*) areas, the neuroretina is transparent, and one can see the brown retinal pigment epithelium (RPE) shining through the neuroretinal tissue. (One can also see large red blood vessels which draw at the inner surface of the retina.) The blue-light-injured retina (*middle*) lost the transparency and appears milky-cloudy. The retinal opacity is one main factor of the decrease in visual acuity in retinal diseases associated with edema. Blue light treatment results in subretinal edema due to the injury to the RPE (which leads to opening of the outer blood-retinal barrier) and the apoptotic death of photoreceptor cells (which is associated with a volume decrease of the cells mediated by an efflux of ions and water). However, the milky appearance makes it likely that the nontransparency of the retina is also caused by deteriorated light guidance through Müller glial cells with increased light reflection at the inner retinal surface and at inner retinal structures. Bar, 1 mm

(0.38–0.77 μm), which makes them strong light-scattering structures (Fig. 31). Indeed, light scattering by the retinal tissue is evidenced by the mere fact that optical coherence tomography delivers images of retinal layers in the living eye (Fig. 18c). Light scattering at inner retinal structures will decrease light sensitivity and visual acuity. The primate fovea is an adaptation to increase the sensitivity in the region of high-acuity vision. Within the central fovea (foveola), all inner retinal layers are absent, and the light reaches directly the photoreceptor cells (Fig. 18a). Because the inner retinal neurons are displaced away from the central foveal region (up to 0.3 mm laterally), the axons of the cones must run centrifugally until they make synapses with bipolar cells (Fig. 18a). These laterally running cone axons form the Henle fiber layer in the primate fovea; they are surrounded and bound together by the outer processes of Müller glial cells.

The funnel shape of the fovea walls (Fig. 18b, c) constitutes a means to expand the image, resulting in a magnified image at the level of the photoreceptors. This magnification allows more (cone) photoreceptors to share the same (region of the) image and thus to detect image details with enhanced spatial resolution. The

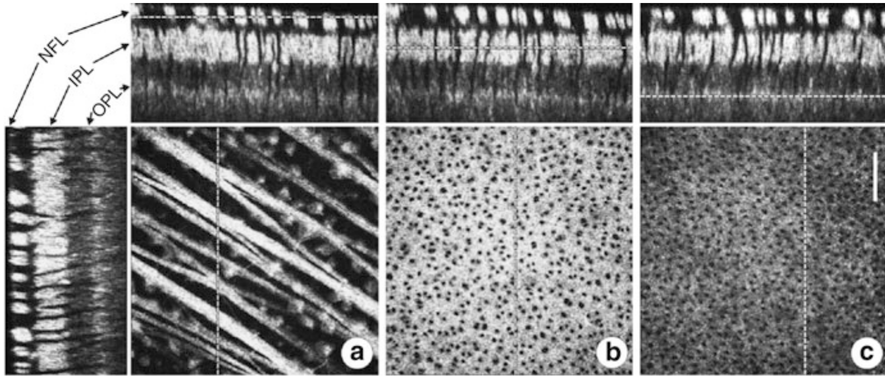

Fig. 31 Light backscattering occurs in the retinal neuropil but not in Müller (radial glial) cells. Confocal images were taken in reflection mode from a living unstained guinea-pig retina oriented with the vitreal side up. Backscattering (reflection) of light from various retinal layers is indicated by the *white* structures. At the *black* structures, the light is not reflected. Three different retinal levels are shown, (**a**) nerve fiber layer (*NFL*), (**b**) inner plexiform layer (*IPL*), and (**c**) outer plexiform layer (*OPL*). At the *top* of (**a**–**c**), orthogonal z-axis reconstructions (side views) of the confocal image stacks are shown; the *dotted* horizontal lines indicate the levels at which the images (**a**–**c**) were taken. A regular pattern of nonreflecting (*black*) tubes is apparent that traverses the entire neuroretina. Large parts of the innermost retinal layers (*NFL*) are nonreflecting (*black*) Müller cell end feet. Individual retinal ganglion cells and axon bundles in the NFL can be seen by their strong (*white*) reflection (**a**). Both plexiform layers (**b**, **c**) display a strong uniform (*white*) background reflection while the many circular cross sections through Müller cell processes are nonreflecting (*black*). Bar, 25 μm

steep, convexiclivate foveas of some predatory birds, lizards, and fish do not contain a fovea centralis; here, the fovea walls act as magnifiers of the images. The primate fovea consisted of the flat fovea centralis (which lacks inner retinal layers) and the lateral fovea walls which provide a magnification of the image (Fig. 18b, c). The latter mechanism compensates the optical problems of the fovea walls where the retina is very thick (i.e., many light-scattering layers are in front of the cones).

Müller Glial Cells Are Living Optical Fibers Which Increase the Sensitivity and Acuity of Peripheral Vision

Another mode which increases the light sensitivity of scotopic vision and the acuity of photopic vision in the retina outside the fovea is light guidance by the cellular fibers of Müller (radial glial) cells. Müller cells pass vertically through the entire neuroretinal tissue from the outer to the inner limiting membrane (Fig. 19). The funnel-shaped end feet of Müller cells, which contact the inner limiting membrane, act as light collectors at the vitreal surface of the retina. The end feet are "softly coupled" to the vitreous body due to a rather low refractory index which reduces the light reflection (i.e., light loss) at the inner retinal surface. The radial fibers of Müller cells directly transfer the light (and the image of the environment) toward the photoreceptor cells (Fig. 31). (In addition, the inner and outer photoreceptor

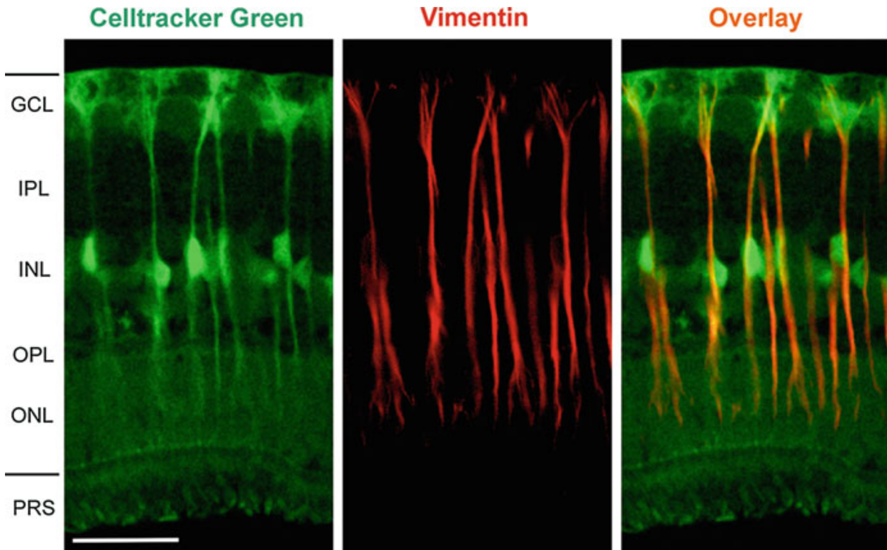

Fig. 32 The somata of Müller glial cells are localized out of the cell axis; this may support the light-guiding function of the cells. Müller cells in a freshly isolated slice of the guinea-pig retina were loaded with the vital dye Celltracker Green; after fixation, the slice was immunostained against the glial intermediate filament vimentin which marks the axis of Müller cells. Müller cell somata lie in the middle of the inner nuclear layer (*INL*). *GCL* ganglion cell layer, *IPL* inner plexiform layer, *ONL* outer nuclear layer, *OPL* outer plexiform layer, *PRS* photoreceptor segments. Bar, 25 μm

segments are light-guiding fibers.) By light guidance, Müller cells transport an image directly from the inner retinal surface to the photoreceptors which is resolved in “pixels” (corresponding to individual Müller cells; Fig. 31b, c). This improves the optical properties of the inverted vertebrate retina. Because the local densities of cones and Müller cells are roughly equal, every cone may have its “personal” Müller cell which delivers the light. In contrast to cones, several (up to >20) rods are illuminated by the same Müller cell (this contributes to the low spatial resolution of the scotopic vision.)

The molecular basis of the light-guiding capacity of Müller cells is unknown; however, bundles of intermediate filaments are arranged along the light path (Fig. 32). In tissue edema (a characteristic of many ischemic and inflammatory diseases of the retina), the neuroretina loses the transparency (Fig. 30). The milky opacity of the edematous tissue makes it likely that the light guidance through Müller cells is deteriorated, resulting in increased light scattering at the inner retinal surface and at inner retinal structures. Perhaps, the upregulation of intermediate filaments (Fig. 26a, b) is one reason.

The retinas of reptiles and birds contain Müller cells with many thin branches which appear not to be suitable for light guidance. It may be speculated that this is (one of) the reason(s) for the presence of up to three foveas in the same retina in some birds.

Rows of Rod Nuclei Act as Chains of Lenses

The outer stem process of Müller cells in the ONL is very thin and irregularly shaped, and intermediate filaments are absent in the (outer part of the) ONL (Fig. 32). Thus, the outer stem process of Müller cells is not suitable as optic fiber. Instead, the light is guided through the ONL by the rod nuclei which are arranged in linear vertical rows (Figs. 23 and 26a), providing a chain of lenses which collect and transport the light like a light fiber. In nocturnal animals, which have a thick ONL (Fig. 10d), this function of rod nuclei might be supported by the spherical shape of the nuclei and the inverted arrangement of chromatin (i.e., heterochromatin localizes to the nuclear center and euchromatin lines the nuclear border rather than vice versa as in "normal" nuclei). To increase light perception, some nocturnals like cats have also a "reflective tapetum" behind the photoreceptors.

At the border between OPL and ONL, the light is transferred from Müller cells to photoreceptor nuclei. Here, the outer stem process of Müller cells taper and split into thin branches. A similar tapering can be observed on the outer segments of cones in many species including the human retinal periphery. Such tapered cylinders are light radiators which, in the case of cone outer segments, transfer the light (which has been guided but not absorbed in the outer segment) to the adjacent rod outer segments. In analogy, the tapered outer processes of Müller cells may act as light radiators illuminating the chains of rod nuclei.

Vertebrate Photoreceptors Are Inactivated by Light

Rods and cones contain photopigment molecules that react to light; this results in a chain reaction that modifies the release of the neurotransmitter glutamate from the presynapse of the photoreceptor cell. The glutamate release from rods and cones (as well as from bipolar and horizontal cells, and many amacrines) is graded (i.e., these cells are not capable of generating action potentials). Photoreceptor cells are exceptional sensory neurons as they are activated in the absence of their adequate stimulus. In the dark, their cell membrane is depolarized, and glutamate is released by their presynapse. Exposure to light of the adequate wavelength hyperpolarizes the membrane and thus inhibits glutamate release.

Light Induces Bleaching of Vertebrate Photopigments

Light-absorbing photopigments in the human retina (rhodopsins in rods, photopsins in cones) are composed of two molecular parts, an opsin protein and the chromophore retinal (the aldehyde of vitamin A). Retinal absorbs the photons. Before light hits the molecule, both parts are connected through a Schiff-base linkage, and retinal exists as the nonactivated 11-*cis* retinal. Absorption of a photon induces a conformational change of retinal from *cis* (bent) to *trans* (straight). All-*trans* retinal separates from the opsin protein. The remaining photopigment loses its color (it is "bleached"). The *cis*-to-*trans* photoisomerization of retinal is the initial event in phototransduction.

In bright light, much of the rhodopsin is broken up into opsin and retinal, and the rod sensitivity is much reduced so that vision is primarily provided by cones (cone-based vision; Fig. 27). At low light levels, only rods are activated by the ambient radiation because the light sensitivity of cones is 100-fold lower than that of rods (rod-based vision). Under conditions of "snow blindness," both rhodopsin and cone pigments are bleached.

Phototransduction Results in a Decrease of the Dark Current

When no light is absorbed by photoreceptors, a constant current circulates between the outer and inner segments of photoreceptor cells. This "dark current" is caused by an influx of Na^+ from the subretinal space into the outer segments and an efflux of K^+ from the inner segments into the subretinal space. The Na^+ influx occurs through cGMP-activated nonspecific cation channels (cyclic nucleotide-gated [CNG] channels) (Fig. 33). These channels are permeable to Na^+, K^+, and Ca^{2+}, and the channels stay open when three cGMP molecules bind to the inner side of the channel. The K^+ efflux from the inner segments occurs through nongated K^+ channels. Because the reversal potential of nonspecific cation currents is near 0 mV, and the reversal potential of K^+ currents is −70 to −80 mV, photoreceptor cells have a membrane potential of −30 to −40 mV in the dark. Under these depolarized conditions (the resting potential in other nerve cells is usually −65 mV), voltage-gated Ca^{2+} channels are open (the L-type Ca^{2+} current that controls exocytosis begins to activate around −45 mV). The steady-state Ca^{2+} influx causes a continual vesicular release of glutamate from the ribbon synapses of photoreceptor cells.

When the photopigment absorbs a photon, the photoisomerization of retinal induces a rapid (<1 ms) conformational change in the opsin protein (rhodopsin converts into the active metarhodopsins I and II) which is followed by activation of the phototransduction cascade (Fig. 33). The conformational change enables the opsin to bind and to activate transducin, a heterotrimeric G-protein. GDP is exchanged for GTP on the G_a subunit of transducin, and the G_aGTP subunit binds to and activates phosphodiesterase (PDE) enzymes which are located in the disk membranes of the outer segment. Active PDE hydrolyzes cGMP to 5′GMP; the level of free cGMP in the outer segment decreases which results in a dissociation of cGMP from the cGMP-gated cation channels and closure of the channels. The closure of the cation channels reduces the circulating dark current (reduction in Na^+ and Ca^{2+} influx) and hyperpolarizes the photoreceptor membranes because the K^+ currents through the open K^+ channels in the inner segment membranes shift the actual membrane potential toward the reversal potential of K^+ currents (−65 mV). The hyperpolarization of the photoreceptor cell membrane inactivates voltage-gated Ca^{2+} channels. Because Ca^{2+} is required for the glutamate-containing presynaptic vesicles to fuse with the membrane and to release their contents, the decrease in cytosolic Ca^{2+} inhibits the release of glutamate from the ribbon synapses within the OPL. The magnitude and duration of hyperpolarization increase with increase in light intensity. ATP provided by the mitochondria in the inner segments powers the Na^+-K^+ pump. This pump is necessary to reset the initial state of the outer segment by pumping Na^+ ions out of the cells and K^+ into the cells.

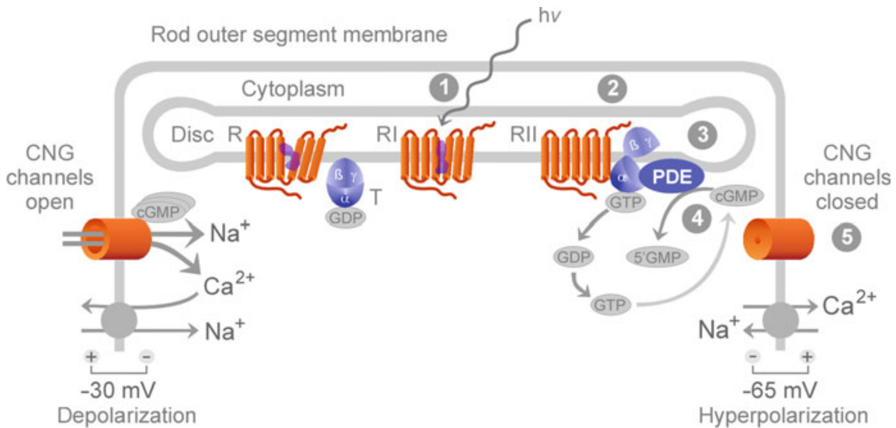

Fig. 33 Molecular steps in phototransduction. Depicted is an outer membrane disk in a rod. Step *1*: A photon (hν) is absorbed and activates a rhodopsin in the disk membrane by conformational change to metarhodopsin II (*RII*). Step *2*: RII makes repeated contacts with transducin (*T*) molecules, catalyzing its activation by dissociation into G_a and $G_{\beta,g}$ subunits (via exchange of the bound GDP by cytoplasmic GTP). Step *3*: G_aGTP binds inhibitory γ subunits of the phosphodiesterase (*PDE*) activating its α and β subunits. Step *4*: Activated PDE hydrolyzes cGMP. Step *5*: Reduced levels of cytosolic cGMP cause cyclic nucleotide-gated (*CNG*) channels to close, preventing further influx of Na^+ and Ca^{2+}. cGMP, the second messenger in the phototransduction cascade, is synthesized from GTP by the guanylate cyclase

Invertebrate Photoreceptors Are Activated by Light

While photoreceptors in vertebrates are inactivated by light, photoreceptors in invertebrates (as well as photosensitive retinal ganglion cells of the mammalian retina; see below) are activated by light (Fig. 3). Here, light stimulates ionic currents in the photoreceptor membrane, i.e., photopigments are coupled via a G_q protein to a phospholipase C signaling cascade, leading to the opening of nonselective cation channels (such as TRP channels) resulting in cellular depolarization and induction of neurotransmitter release. The photopigments of many invertebrates are bistable, i.e., the isomerized chromophore does not dissociate from the opsin. Instead, the thermally stable inactive and active ("meta") states of the pigment interconvert, primarily through light absorption. Thus, these pigments are not bleached by light, but light alters the color of the pigment.

Phototransduction Is Turned Off by Inactivation of Transducin and Metarhodopsin

The phototransduction cascade is deactivated by inactivation of transducin and metarhodopsin. Transducin is inactivated by the GTPase-activating protein (GAP). GAP activates the intrinsic GTPase activity of the G_a subunit of transducin; hydrolyzation of the bound GTP to GDP results in inactivation of transducin. Metarhodopsin is inactivated by the rhodopsin kinase and arrestin. When Ca^{2+} is present, recoverin is bound to the rhodopsin kinase. When the Ca^{2+} level falls during light stimulation, Ca^{2+} dissociates from recoverin, and rhodopsin kinase

phosphorylates metarhodopsin II. The phosphorylation decreases the affinity of metarhodopsin for transducin and increases the affinity for arrestin. Arrestin binds the phosphorylated metarhodopsin which results in complete inactivation of metarhodopsin. By this way, phototransduction is deactivated, and the dark current and glutamate release is restored. The inactivation of metarhodopsin is relatively rapid (0.5–1 s) and controls the amplitude of the photoreceptor response while the inactivation of transducin is slow and controls the recovery of light sensitivity (i.e., is responsible for the saturation of rods by bright light).

Phototransduction Is Amplified at Several Steps

Phototransduction uses an amplification cascade to convert a small stimulus (one absorbed photon) into a large membrane voltage signal. One molecule of metarhodopsin activates ~100 transducin molecules. One PDE molecule hydrolyzes ~1,000 cGMP molecules. Thus, one photon induces the hydrolyzation of ~10^5 cGMP molecules, resulting in the closure of ~30,000 cation channels. Normally, a single photon produces a reduction of the dark current by ~1 pA (the reduction peaks 1 s after photon absorption), resulting in cellular hyperpolarization by ~1 mV. Thirty photons suppress half of the dark current. The amplification of phototransduction is five times faster (but 100-fold smaller) in cones than rods. Rods respond relatively slowly to light, and the stimuli received are added over ~100 ms. This makes rods more sensitive to smaller amounts of light but decreases their capability to sense temporal changes, such as quickly changing images ("you can walk through the forest with nothing but starlight, but you cannot run").

The Light Sensitivity of Photoreceptors Is Controlled by Ca^{2+}

To maintain light sensitivity of photoreceptors over a wide dynamic range (i.e., to prevent full inactivation or overstimulation of photoreceptors), there are light adaptation mechanisms mediated by Ca^{2+}. In the dark, when the cytosolic Ca^{2+} level increases due to the Ca^{2+} influx through the cGMP-gated cation channels, the activity of the guanylate cyclase (which synthesizes cGMP from GTP) is inhibited, and the level of free cGMP in the outer segments decreases. Inhibition of the guanylate cyclase is mediated by Ca^{2+} binding to the guanylate cyclase-activating protein (GCAP). Ca^{2+} binding induces a dissociation of GCAP from the guanylate cyclase resulting in inactivation of the guanylate cyclase. Light closes the cation channels which results in a decrease of the cytosolic Ca^{2+} level (because the Ca^{2+} efflux through the Na^+-Ca^{2+}, K^+ pump continues). At low Ca^{2+} level, GCAP activates the guanylate cyclase which replenishes the cGMP levels, resulting in reopening of cation channels. Ca^{2+} also inhibits the binding of cGMP on the cation channels (which increases the affinity of the channels for cGMP). In addition, Ca^{2+} controls the amplitude of the light response by prolongation of the time period required for inactivation of metarhodopsin.

The Recovery of Light Sensitivity Is Slower in Rods than in Cones

The kinetics of the regeneration of 11-*cis* retinal, which contributes to the recovery of the photoreceptor sensitivity to light, is different between rods and cones. Rods

recover more slowly than cones (Fig. 27). Therefore, dark adaptation is slow; 100 % adaptation is achieved after 30–100 min (one can be temporarily blind when moving from light into dark). Cones reach full sensitivity after 5–12 min.

Rod-derived all-*trans* retinal is slowly regenerated to 11-*cis* retinal by the RPE (*retinoid cycle*). After dissociation of all-*trans* retinal from the opsin, it is reduced to all-*trans* retinol within the photoreceptor outer segment. All-*trans* retinol diffuses into the subretinal space where it is transported to the RPE by a chaperone protein (interphotoreceptor retinoid binding protein, IRBP). In the RPE, all-*trans* retinol is reisomerized via all-*trans* retinyl ester to 11-*cis* retinol and further oxidized to 11-*cis* retinal. 11-*cis* retinal is then transported to photoreceptors by IRBP. Cone-derived all-*trans* retinol is processed in Müller glial cells. Müller cells convert all-*trans* retinol to 11-*cis* retinol which is subsequently oxidized to 11-*cis* retinal by a retinol dehydrogenase and released into the extracellular space for uptake by cone photoreceptors. For the transport of retinoids, Müller cells contain cellular retinol binding protein (CRBP) that binds all-*trans* retinol and cellular retinal binding protein (CRALBP) that binds 11-*cis* retinol and 11-*cis* retinal. The transfer of retinoids between cones and Müller cells is likely mediated by IRBP. All-*trans* retinol cannot be synthesized by humans and must be supplied by vitamin A in the diet. Deficiency of all-*trans* retinol can lead to night blindness.

Mutations in the enzymes of the retinoid cycle (e.g., of the isomerohydrolase RPE65 which catalyzes the formation of 11-*cis* retinol from all-*trans* retinyl ester) are one reason for the blinding disease retinitis pigmentosa which is characterized by an early degeneration of rods and a delayed death of cones. Other reasons include mutations in the rhodopsin gene which also lead to congenital night blindness caused by overactivations of rhodopsin and transducin.

The Type of Opsin Defines the Spectral Sensitivity of Photopigments

Visual pigments are G-protein (transducin)-coupled seven transmembrane domain receptors (similar to odor receptors; retinal is like an odor molecule that is bound to the receptor). The light-wavelength (color) selectivity of a distinct photoreceptor is dependent on the kind of opsin expressed in the cells. Seventy to eighty-five percent of the environmental white light reaches the sensory retina. The human retina receives photons with wavelengths between ~380 nm (violet) and ~770 nm (red). UV light (300–400 nm) is absorbed by the lens (and the cornea) of the eye. In contrast to man, various vertebrates including fish, birds, and eventually mice see also UV light. Some human subjects were capable of seeing UV light when the lenses were removed during cataract surgery.

Human rods contain rhodopsin composed of scotopsin (OPN2) and 11-*cis* retinal. Rhodopsin has an absorption maximum at light of a wavelength (λ_{max}) of 498 nm (blue–green light; Fig. 34). The human retina (and retinas of Old World monkeys) usually contains three classes of cones for color vision (*trichromatic vision*). The cones contain photopsins composed of 11-*cis* retinal and one of the following iodopsins:

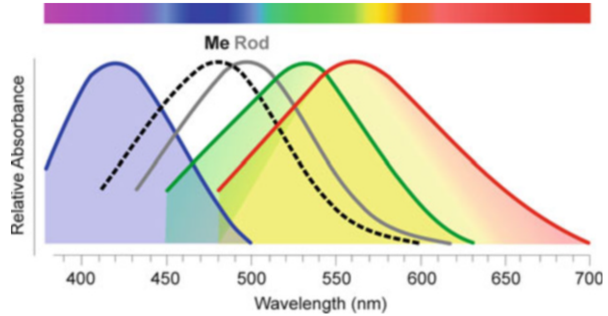

Fig. 34 Spectral sensitivities of primate photoreceptors. *Rod* rhodopsin, *Me* melanopsin

1. L-(red) cones: long-wavelength-sensitive opsin (OPN1LW; λ_{max} 560 nm)
2. M-(green) cones: middle-wavelength-sensitive opsin (OPN1MW; λ_{max} 530 nm)
3. S-(blue) cones: short-wavelength-sensitive opsin (OPN1SW; λ_{max} 420 nm) (Fig. 34)

The spectral sensitivity of cone opsins is relatively wide, and the sensitivities of different cone opsins overlap (Fig. 34). "Blue" cones are sensitive to wavelengths between 380 and 500 nm, "green" cones to wavelengths between 450 and 630 nm, and "red" cones to wavelengths between 480 and 700 nm. In particular, the light sensitivities of red and green opsins overlap considerably because the amino acid sequences of both opsins are very similar (equal to 96 %). (Because the λ_{max} of the three cone types does not exactly match the three colors (Fig. 34), the terms "L-, M-, and S-" cones are used in the literature rather than "red, green, and blue" cones).

In the human retina, blue cones constitute ~10 % of the entire cone population. The red-to-green cone ratio is 2:1 (however, substantial interindividual differences in the cone ratio were found). In the fovea, the numbers of each cone type are not equal. Usually, red cones are most numerous and blue cones least numerous because eyes transmit more red light (longer wavelength) than blue light (shorter wavelength). The very center of the fovea has no blue cones. Cones of the same type form clusters.

When the light intensity decreases in the dusk (mesopic conditions), the peak sensitivity of the human retina shifts toward the blue end of the spectrum because the rods (which are most sensitive to blue–green light; Fig. 34) increasingly contribute to vision. This fact is responsible for the *Purkinje effect* in which blue colors appear more intense relative to reds at twilight ("blue hour" at the end of the day).

Color Is Processed in the Retina, Thalamus, and Cortex

Because the spectral sensitivities of cone opsins overlap and because the amplitude of cone responses varies with both the wavelength and the intensity of light, interactions between at least two types of cone are necessary to perceive color. Trichromatic color vision is accomplished by using combinations of cell responses. The visual cortex compares the signals from each type of cone and determines the intensity and color of light. It is estimated that each of the three cone types in the human retina can pick up about 100 different gradations and that the brain can

combine those variations such that the human can distinguish about one million different colors.

The question of how color is processed in the retina and brain is still far from complete. Color processing is carried out by using color-opponent mechanisms (the opposing effects of red–green, blue–yellow, and light–dark). The color opponency is created in the retina at the level of the color-coded ganglion cells. The dorsal lateral geniculate nucleus (dLGN) in the thalamus consists of the M-zone (containing M-cells) and P-zone (containing P-cells). M- and P-cells constitute the principal sublayers, while each layer has a koniocellular (K) sublayer. M- and P-cells receive relatively balanced input from both red and green cones. The K sublayer receives axons from the *bistratified ganglion cells* and the giant monostratified ganglion cells. These cells carry blue-ON/yellow-OFF signals, i.e., they compare the output of blue cones and red/green cones, giving rise to the blue-yellow opponent mechanisms (Fig. 35). In the primary visual cortex (V1), double-opponent cells (red–green cells and blue–yellow cells) form clusters likely associated with the "blobs" of the hypercolumns. Red–green cells compare the relative amounts of red–green in one part of a scene with the amount of red–green in an adjacent part of the scene, responding best to local color contrast (red next to green). The double-opponent cells in the cortex may also contribute to color constancy.

Red-Green Blindness Is Evolutionary Conserved

Red and green opsins are encoded on the X chromosome (the blue opsin gene is on chromosome 7, the rhodopsin gene on chromosome 3). Because red and green informations are opponents in color processing, mutations in one of these genes result in

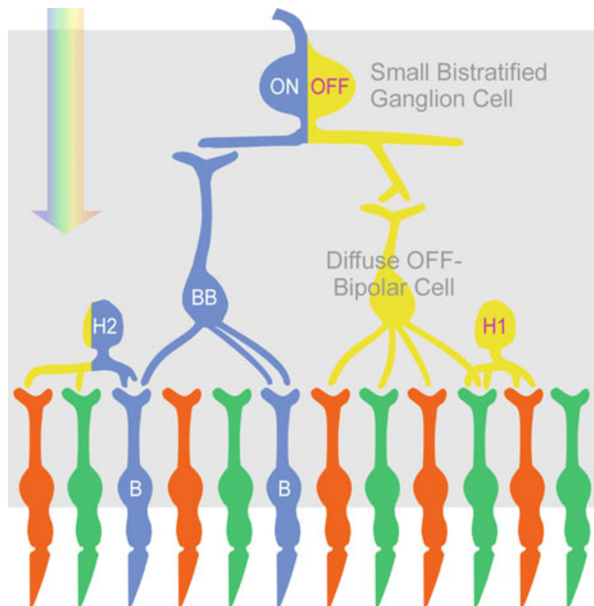

Fig. 35 *Blue*-ON/*yellow*-OFF signaling in the primate retina. *B* blue cone, *BB* blue bipolar cell, *H* horizontal cell

red-green blindness, and the subjects are dichromats (Fig. 12). Mutations in the blue opsin gene are very rare. The gene of red opsin is highly polymorphic; 2–3 % of women have an extra type of color receptor (containing an opsin with λ_{max} that lies between that of the standard red and green opsins). Because the different alleles are expressed in different cone populations (due to random X-inactivation), these women have a distinct degree of tetrachromatic vision that increases color differentiation. In certain situations, color-blind individuals have an advantage over those with normal color vision. For example, red-green color-blind subjects have a better contrast perception under mesopic conditions; this might be the reason for the higher prevalence of red-green blindness in regions with longer (Caucasians, 8 %) than shorter twilight (Africans, 4 %).

The Yellow Macula Acts as a Sunglass

The macula lutea is a yellow pigmented area (diameter, ~5 mm) around the center of the human retina which includes the fovea, the parafovea, and perifovea. The yellow pigments (the carotenoids lutein and zeaxanthin) are arranged in front of the photoreceptors within the cone axons of the Henle fiber layer. The pigment area absorbs excess blue and UV lights that enter the eye and is probably an evolutionary adaptation to the problem of chromatic aberration. By light absorption, the macula acts as a natural sunglass for the central retina. Because blue and UV lights are energy rich, excess illumination with light of these short wavelengths causes retinal (macular) degeneration characterized by damage to the RPE and apoptotic death of photoreceptor cells (Figs. 26b, 36, and 37), ultimately leading to blinding. Blue light is responsible for solar retinitis (blinding after long-term looking directly into the sun) and plays a role in the pathogenesis of age-related macular degeneration (thus, sunglasses should especially eliminate blue light from the visible spectrum). The pigments are so neatly arranged that they slightly polarize the light. Humans can only detect polarized light when it is continuously changing. By contrast, invertebrates can see polarized light and use polarization in the sky for navigation. Some fish are also sensitive to the polarization of light.

Retinal Neurons Mediate Processing of Visual Information Before It Is Transmitted to the Optic Nerve

Horizontal Cells Provide Contrast Enhancement Through Lateral Inhibition

Horizontal cell bodies are located at the outer margin of the INL, and their dendrites and axons form a dense network in the OPL (Figs. 19 and 24). Most mammalian retinas contain two types of horizontal cells.

Horizontal cell dendrites contact the terminals of cones (*cone pedicles*) in a characteristic synaptic complex, the *triad* (Fig. 22). Each cone pedicle in the primate retina has 20–100 triads. These triads are presynaptic invaginations that contain a *ribbon* (presynaptic dense body which binds glutamate-containing synaptic vesicles)

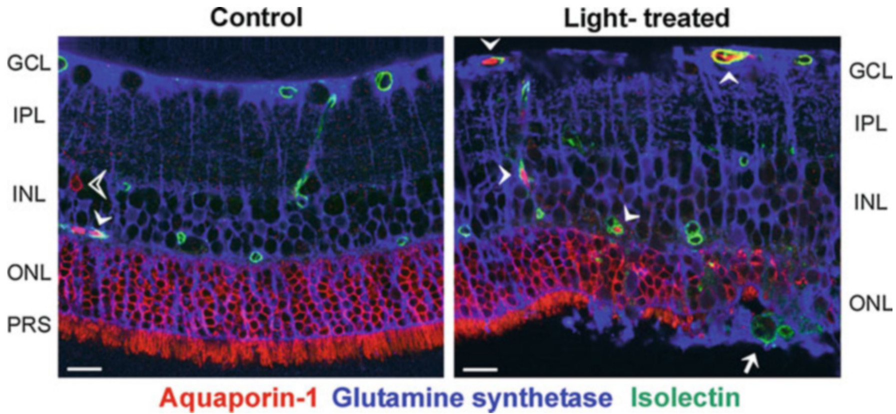

Fig. 36 Glutamine synthetase is distributed in the whole cytosol of Müller glial cells. The images show slices of a control retina of the rat (*left*) and of a rat retina obtained 3 days after local blue-light treatment (*right*), which were immunostained against aquaporin-1 (*red*) and glutamine synthetase (*blue*). Blood vessels and activated immune cells were stained with isolectin (*green*). The *right* image shows the transition zone between the blue-light-injured and uninjured tissue. The degeneration of photoreceptor cells induced by irradiation with excessive blue light is associated with a disappearance of aquaporin-1 immunoreactivity in the outer retina. *Arrow*, isolectin-stained immune cell (invading macrophage or activated microglia). *Filled arrowheads*, aquaporin-1 expressing erythrocytes. *Unfilled arrowhead*, aquaporin-1-positive amacrine cell. *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *ONL* outer nuclear layer, *PRS* photoreceptor segments. Bars, 20 μm

that is opposed to three postsynaptic processes. The central element within the triad is an ON-bipolar cell dendrite, and the two lateral processes are horizontal cell dendrites. (OFF-bipolar cell dendrites contact the basis of the cone pedicles outside the triads.) There are between 40 and 200 horizontal cell dendritic terminals in each cone pedicle, and these come from 6 to 8 horizontal cells, which allow any individual horizontal cell to make up to 10 contacts within one cone pedicle. In a distance of ~1.5 μm from the cone pedicles, horizontal cells of the same type are coupled by gap junctions (Fig. 22). The degree of junctional coupling is diurnally regulated by dopamine, nitric oxide, and retinoic acid. Cone pedicles have also gap junctions for electrical contacts with other cone pedicles and rod spherules.

In the mammalian retina, the *rod* synaptic terminal (*spherule*) is substantially smaller than the cone pedicle. There is only one invagination, and up to three dendritic tips of rod bipolar cells are inserted as central elements into a spherule. The lateral elements come from the fine branches of the horizontal cell axon terminal systems.

Horizontal cells are activated by photoreceptor-derived glutamate in the dark via opening of ionotropic glutamate receptors of the AMPA/kainate subtype (composed of GluR2/3 and GluR4); these receptors are glutamate-gated nonspecific cation channels. In response to light, they give hyperpolarizing responses (like photoreceptor cells). When horizontal cells are activated by glutamate, they release GABA which provides a negative feedback onto cone pedicles and rod spherules. The

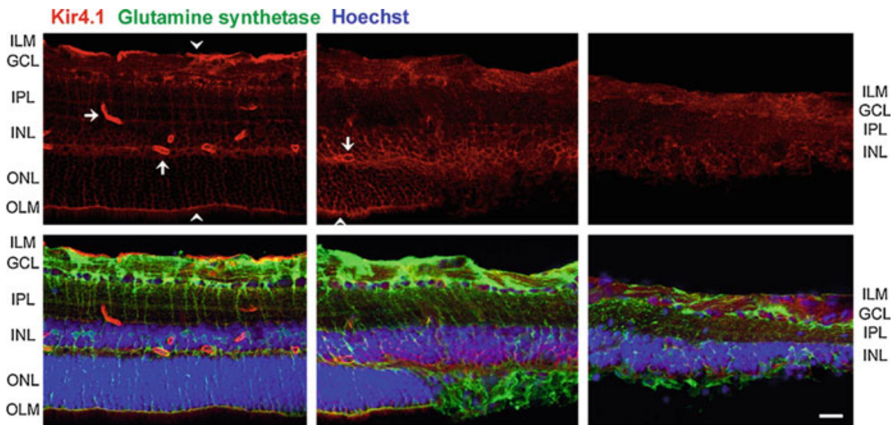

Fig. 37 Local irradiation of the rat retina with excessive blue light causes degeneration of the photoreceptor cells and gliotic alterations of Müller cells, as indicated by the alteration in the immunolocalization of the potassium channel Kir4.1. Retinal slices were stained against Kir4.1 (*red*) and glutamine synthetase (*green*) 3 days after light exposure. Cell nuclei were labeled with Hoechst 33258 (*blue*). Injured retinal areas are shown *right*, uninjured areas are shown *left*, and transition zones are shown in the *middle*. The *arrows* point to perivascular labeling of Kir4.1, and the *arrowheads* indicate the inner (*ILM*) and outer limiting membranes (*OLM*). *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *ONL* outer nuclear layer. Bar, 20 µm

primary function of horizontal cells is to produce lateral inhibition of photoreceptor and bipolar cells. This lateral inhibition is involved in contrast enhancement, the adaptation of the retinal activity to the intensity of the environmental light, and the generation of the center-surround antagonism of the receptive fields of bipolar cells (with the center signal representing the direct contact from cones to bipolar cells and the opposite surround signal stemming from the negative feedback of horizontal cells).

In the retina of dichromatic mammals, there is no chromatic selectivity in horizontal cells, i.e., they does not contribute to color perception. A-type horizontal cells contact red and green cones, while B-type horizontal cells contact rods as well as red and green cones. In the cat retina, B-type horizontal cells have a thin, long (up to 400 µm) axon, which unfolds into a huge axon terminal system innervating up to 3,000 rods. In the trichromatic primate retina, H1 cells contact rod spherules and the pedicles of red and green cones, whereas H2 cells contact predominantly blue cones and only few red and green cones (Fig. 35). Retinas of lower vertebrates have also horizontal cells with chromatic responses which are depolarized by distinct wavelengths and hyperpolarized by other wavelengths.

Bipolar Cells Transfer Visual Information to Ganglion and Amacrine Cells

Bipolar cells are so named because they have a central perikaryum from which two main processes arise in opposite directions (Fig. 2). Bipolar cells have a dendritic

tree in the OPL where they receive input signals from rods or cones and an axon that terminates in the IPL and that produces graded release of glutamate to ganglion and amacrine cells. There are several distinct forms of cone bipolar cells and only one rod bipolar cell (Figs. 2, 9, 19, and 23). The primate retina contains nine types of *midget and diffuse cone bipolars* and one type of *rod bipolars*. The small midget bipolars contact only one cone pedicle, diffuse bipolar cells (having greater dendritic fields) contact 5–10 neighboring cone pedicles, and rod bipolar cells contact at least 15 rod spherules.

Different Glutamate Receptors in Bipolars Underlie the ON- and OFF-Dichotomy of the Cone Pathway

In the mammalian retina, rod bipolar cells are ON-center cells, while cone bipolars are either ON- or OFF-center cells. ON-cells depolarize and OFF-cells hyperpolarize in response to a light stimulus projected onto their receptive field center. (The "receptive field" of a cell is determined by the dendritic field and represents the area of the retina where light stimuli changes the activity of the cell.) Light responses consist of graded and sustained changes of the membrane potential by 15–20 mV from a dark potential of −40 to −45 mV. The ON/OFF-dichotomy is caused by two different glutamate receptors in the membranes of bipolar cell dendrites. Photoreceptor-derived glutamate opens ionotropic glutamate receptors of the AMPA/kainate subtype (composed of GluR1, GluR5–7, and/or KA2) in the postsynaptic membranes of *OFF-bipolar cells* and thus causes depolarization of the cells. *ON-bipolar cells* express metabotropic glutamate receptors (mGluR6) in their postsynaptic elements in the OPL; activation of these G-protein-coupled receptors results in closure of nonspecific cation channels, leading to hyperpolarization of the cell. ON-bipolar cells are depolarized by light (when the photoreceptor cells stop glutamate release; "sign inverting") and OFF-bipolar cells are excited in the dark (when the photoreceptor cells are depolarized; "sign conserving"). Thus, the expression of different glutamate receptors in the postsynaptic membranes of bipolar cells is the physiological basis of the functional ON- and OFF-dichotomy in the retina.

The axons of cone bipolar cells terminate in the IPL where they make specialized ribbon synapses called "dyads." Two postsynaptic elements are opposed to a ribbon in the bipolar terminal. One of the postsynaptic elements is an amacrine cell process which often makes a reciprocal synapse back onto the bipolar terminal; the other element is a ganglion cell dendrite. There are also dyads where both postsynaptic processes stem from amacrine cells or, more rarely, from ganglion cells. In the IPL, the terminals of OFF-bipolar cells (which contact the dendrites of OFF-center ganglion cells) are located close to the INL, whereas the terminals of ON-bipolar cells (which contact the dendrites of ON-center ganglion cells) are located in a stratum closer to the GCL (Figs. 9 and 35).

The transmitter of bipolars is glutamate. ON- and OFF-bipolars have excitatory effects on ganglion and amacrine cells. ON-center ganglion cells receive excitatory input from ON-bipolar cells and inhibitory input mainly from amacrine cells. OFF-center ganglion cells receive excitatory input from OFF-bipolar cells. In retinas

of lower vertebrates, most classes of ON-bipolar cells collect input from rods and cones, and the light responses from both rods and cones inhibit the cells.

Midget Bipolar Cells Underlie the Color-Opponent Receptive Fields of Ganglion Cells

Cone bipolar cells comprise different morphological types which provide a more complex operation than just simply exciting ganglion cells. The *color-coded ganglion cells* in the primate retina have in the center of their receptive fields an input from only one type of cone, while their surround is color antagonistic. Cone-opponent retinal ganglion cells come in four varieties, red-ON/green-OFF, green-ON/red-OFF, blue-ON/yellow-OFF (Fig. 35), and yellow-ON/blue-OFF. (Horizontal cells of dichromatic mammals receive input from both cone types and thus cannot produce a color-coded surround, while in the primate retina, H1 and H2 horizontal cells might contribute to the blue-yellow opponency.) Midget bipolar cells can make exclusive contact to only one (red or green) cone; thus, one type of midget bipolar cells provides the excitatory signal for the center, whereas the other type of midget bipolar cell, connected to a spectrally different cone type, provides the signal for the inhibitory surround. However, even foveal midget cells might have centers that are impure because gap junctions could mix signals between red and green cones. In the peripheral retina, both the center and surround of midget ganglion cells sample from multiple cones.

Each red and green cone pedicle in the primate central fovea makes synapses with at least three different bipolar cells, including an ON- and an OFF-midget bipolar cell. (Outside the central fovea, one red or green cone pedicle contacts two midget bipolar cells and 10–15 diffuse bipolar cells.) The axons of ON- and OFF-bipolar cells end in different strata in the IPL (Fig. 9). Because midget bipolar cells are connected to only one cone, they carry a chromatic signal. Diffuse bipolar cells, even within the fovea, are connected to several neighboring cone pedicles, and they therefore have mixed cone inputs. Thus, in the primate retina, the color and luminosity signals are likely transferred from cones to ganglion cells by different types of bipolar cells (midget and diffuse). The blue cone pathway is different from the green and red cone pathways. Distinct subtypes of bipolars innervate several blue cone pedicles (Fig. 35). These cells are always ON to the blue cone, and the axon terminals contact the dendrites of the bistratified and the giant monostratified blue ON-ganglion cells. (However, OFF-midget ganglion cells also appear to carry blue cone signals to the brain.)

Rod Bipolars Are Not Directly Connected to Ganglion Cells

Rod bipolar cells (Fig. 23) are morphologically different from cone bipolars, e.g., the dendrites of rod bipolars are finer and the dendritic trees are bushier because they innervate many rod spherules (in cats and primates, ~15–40 according to the eccentricity; in the rabbit, up to 100). In rod-dominated retinas of nocturnal mammals such as cats, the great majority of all bipolar cells are rod bipolars. In the central primate retina, which is cone-dominated, rod bipolar cells are <20 % of the total bipolar cell population. Rod bipolar cell axons terminate close to the ganglion cell

perikarya (Fig. 9); however, they make no direct output synapses onto ganglion cells but contact two types of amacrine cell processes, (1) processes of the AII amacrine cell, which is a small-field, glycinergic, local-circuit neuron, and (2) a wide-field GABAergic amacrine cell (e.g., the A17 cell) which makes a reciprocal synapse. AII amacrine cells "superimpose" the rod pathway onto the cone output pathway (Fig. 9). Light responses of rod bipolars are depolarizing. Both AII and A17 amacrine cells are also depolarized by light.

In contrast to the mammalian retina, nonmammalian retinas also contain bipolar cells that receive direct input from both rods and cones. Here, rod bipolar cells might synapse directly with ganglion cells.

Amacrine Cells Process Visual Information According to Alterations in the Environmental Visual Signals

The human retina contains ~2 millions amacrine cells which mediate lateral interactions between retinal ganglion cells. Amacrine cells are responsible for 70 % of input to retinal ganglion cells. The effectiveness of the ribbon synapses of bipolar cells (which are responsible for the other 30 % of input to ganglion cells) is regulated by amacrine cells. Amacrine cells form a dense network in the IPL where they make synapses with the processes of bipolar, ganglion, and other amacrine cells. There are two populations, one with perikarya in the INL and another with perikarya in the GCL ("displaced" cells). Displaced amacrines comprise up to 20 % of all amacrine cells. Cajal introduced the name "amacrine" which means cells lacking an axon. Amacrine cell processes both receive synaptic inputs and make synaptic outputs. Amacrine cells are a very heterogeneous cell population; their cell shape and transmitter vary in dependence on the function of the cell. There are at least 30 distinct types of amacrine cells in the mammalian retina, and there is probably no transmitter in the central nervous system that is not found in some retinal amacrines. About a half of amacrines are GABAergic whereas the other half is glycinergic. In addition to these inhibitory transmitters, many amacrines release further neuromodulatory transmitters including acetylcholine, dopamine, serotonin, and different peptides. The *GABAergic amacrines* have often wide dendritic fields which can span >2 mm on the retina. They give depolarizing light responses and form reciprocal synapses at rod bipolar cell dyads.

One major function of amacrines is the adaptation of the retinal activity to specific visual environments. Though many adaptations of the vision to alterations in color and light intensity are carried out in the visual cortex, the presence of retinal adaptation mechanisms may account for the great number and diversity of amacrine cells. During a day, there is a spectral shift of sunlight (in the morning, the spectral composition of sunlight is shifted to the red part of the visible spectrum, at noon shorter wavelengths are predominant, and by the evening it has returned to a red shift). The visual system has to compensate for this spectral shift and must weigh the three color channels accordingly (chromatic adaptation or *color constancy*). Another example is the regulation of retinal sensitivity according to differences in the light intensity within the image. When there is bright light in any part of the visual field, the overall sensitivity of the retina is decreased. Thus, it is not a good idea to put a

computer screen against bright background like a window. The cell bodies and dendritic processes of *somatostatin-containing amacrine cells* are largely confined to the lower part of the retina. However, the cells have a long axon-like process which projects to the upper part of the retina. Somatostatin increases the responsiveness of ganglion cells to light. Under natural viewing conditions, the upper part of the retina receives light from the ground and the lower part receives light from the sky; thus, there are great differences in luminance between the upper and lower retina. The somatostatin projection from the lower to the upper retina might be used to equilibrate these differences in illumination.

Another type of specialized amacrine cells is the *interplexiform cell*. These cells receive input into their dendritic field within the IPL and have processes which leave the IPL, pass through the INL, and reach the OPL. Here, the processes branch and make conventional synapses onto dendrites of bipolar cells. Some amacrine cells make synapses within the NFL (which may therefore represent a third plexiform layer). These cells receive input in the IPL and make output synapses onto the somata and axons of ganglion cells.

AII Amacrines Connect the Rod Pathway to the Cone Output Pathway

Cajal grouped amacrine cells into two main types according to the distribution of their processes in the IPL, (1) *diffuse cells* (the processes branch vertically throughout the IPL; e.g., AQP1-positive cells as shown in Fig. 25c) and (2) *stratified cells* (the processes are predominantly on one plane; e.g., tyrosine hydroxylase [TH]-positive cells as shown in Fig. 25a, b). *Glycinergic amacrine cells* have diffuse dendritic fields. These cells produce vertical interactions between the three major functional subdivisions of the IPL, the ON-sublamina, the OFF-sublamina, and the "rod" sublamina (Fig. 9).

One example of diffuse amacrine cells is the glycinergic *AII cell* which connects the rod pathway to the cone output pathway under scotopic conditions. AII cells are the most numerous amacrine cell type in the mammalian retina comprising ~10 % of total amacrine cells. AII cells receive their major synaptic input from rod bipolar cells (Fig. 9) and are depolarized by light. (A second synaptic input comes via reciprocal synapses from cone bipolar cells.) The outputs of AII cells are located in two sublaminas of the IPL, (1) at about the middle of the IPL, they make electrical synapses (gap junctions) with ON-cone bipolar cells which provide excitatory contacts onto ON-ganglion cells, and (2) in the outer part of the IPL, they make conventional inhibitory chemical synapses with OFF-cone bipolar and OFF-ganglion cells which inhibit the information output from the OFF channel (Fig. 9). In this way, AII amacrine cells produce signals of opposite polarity in ON- and OFF-ganglion cells, i.e., sign-conserving signals in the ON-pathway and sign-reversing signals in the OFF-pathway. There is a fundamental difference between the rod and cone pathways in respect to the creation of the ON/OFF-dichotomy. In the cone pathway, the ON/OFF-dichotomy is created in the OPL by different glutamate receptors in the bipolar cell membranes, whereas in the rod pathway, two different types of synapses of AII cells (a chemical glycinergic and

an electrical synapse) provide the polarity change in the IPL. The density of AII amacrine cells defines the limits of scotopic visual acuity.

A further mode of the connection of the rod pathway to the cone pathway might be active under mesopic light conditions. Under these conditions, gap junctions between rod spherules and cone pedicles provide a direct route for the rod signal into the cone pathway. During photopic or scotopic illumination, the separate pathways for the rod and cone signals are active.

AII amacrine cells have narrow dendritic fields (i.e., the processes lie within a small column of the retina) with a minimum overlap. The narrow, nonoverlapping dendritic tree provides a local-circuit function that does not degrade spatial resolution (a light spot projected onto the retina is represented in not more than three to four AII cells). Other glycinergic amacrine cells, which all have small dendritic fields and diffuse stratification (e.g., A3, A4, and A8 cells), might be involved in the mutual inhibition between the ON- and the OFF-channel. Though glycinergic amacrine cells are inhibitory interneurons, they facilitate the forward transmission of visual information, i.e., they amplify and quicken the response of bipolar cells (via gap junctions) and, as a result, enhance the light responses of ganglion cells.

Cholinergic Amacrines Play a Role in Movement Detection

Cholinergic amacrine cells are stratified amacrines. Their cell bodies are found in about equal numbers at both sides of the IPL, i.e., they exist as two mirror-symmetric populations. Their dendrites stratify in two narrow bands within the IPL. The cells have a radially symmetric morphology ("starburst" cells; Fig. 16). The processes of cholinergic amacrine cells with cell bodies in the INL form a narrow stratum in the outer part of the IPL, whereas those of the displaced population (with cell bodies in the GCL) stratify in the inner part of the IPL. The dendritic trees of starburst amacrines are larger than those of AII cells, so that the processes of 30–60 cholinergic cells overlap every point of the retina. Cholinergic amacrines are excited by light "off" and light "on," respectively.

Cholinergic amacrines also release GABA, i.e., they release both an excitatory and inhibitory transmitter. Most cholinergic amacrines receive inputs all over their dendritic fields, but the output synapses are confined to a region near the perikaryum. Cholinergic amacrines produce direction selectivity in *direction-selective ganglion cells*, i.e., they act (in cooperation with the ganglion cells) as movement detectors. Direction-selective magnocellular ganglion cells (having large receptive fields) respond optimally to movement in a "preferred" direction and show little or no response to movement in the opposite direction. The responses of directionally selective ganglion cells depend on both GABAergic and cholinergic innervation. Directionality is achieved because the dendrites of cholinergic amacrines display different responses when a light stimulus moves toward the origin or the end of the dendrite. Though cholinergic cells are morphologically a homogenous population, physiologically they comprise different subclasses depending on the direction of movement to which they are tuned. Thus, several different physiological classes could be hidden within the 30–60 cholinergic cells overlapping any retinal point.

Dopamine and Melatonin Are the Chemical Surrogates of Night and Day in the Retina

In mammals, the synchronization of daily activities and sleep depends predominantly upon the retinal influence on the suprachiasmatic nucleus of the hypothalamus (the master pacemaker of circadian rhythms). The vertebrate retina contains circadian oscillators (mediated by neuromodulators such as adenosine, dopamine, glutamate, serotonin, and melatonin) and intrinsically photosensitive ganglion cells (see below). They work together to temporally organize retinal and organism physiology. There are many retinal parameters such as levels of photopigments, phagocytosis of photoreceptor outer segments, and visual sensitivity that display circadian fluctuations. The cyclic variations occur even under constant light and after lesioning of the suprachiasmatic nuclei, indicating that the mammalian retina contains its own autonomous circadian clocks.

Dopamine and melatonin are the main light and dark signals in the mammalian retina. *Melatonin* activates the disk shedding from rods. It is synthesized by photoreceptors and ganglion cells at night and is inhibited by light and dopamine. *Dopamine* is synthesized in subsets of amacrine cells; the production of dopamine is stimulated by light and inhibited by dark and melatonin. Because of these rhythms, rod outer segment disks are predominantly shed at the onset of light (in the morning) and cone outer segments are mainly shed at the onset of night. Dopamine also inhibits the coupling between rods and cones so that rod-cone coupling is weak during the day and increased during the night. The dopaminergic amacrines are driven by light via their inputs from rods and cones and from photosensitive retinal ganglion cells (see below). Dopaminergic amacrine cells can be detected by staining against the key enzyme in dopamine synthesis, TH (Fig. 25a, b). Their processes form a dense plexus at the INL-IPL border (Fig. 25a, b) which contains characteristic "rings" which encircle the cell bodies of AII amacrines. The AII cell perikarya receive synapses within these rings, and dopamine modulates the activity of these interneurons of the rod pathway.

In addition to dopamine- and melatonin-producing cells, other cell types of the retina have autonomously generated circadian rhythms which influence, e.g., the biosynthesis of phospholipids and prostaglandins. In the mammalian retina, circadian clocks are preferentially localized in inner retinal neurons, while in amphibian and avian retinas, this function is localized to photoreceptor cells.

Retinal Ganglion Cells Act as Parallel Operating Filters That Extract Distinct Aspects of the Image

Ganglion cells are the only output cells of the retina. The ~1.3 million axons of ganglion cells in each human eye project through the optic nerve to the brain. Different ganglion cell classes project into different visual centers of the brain, including the dLGN of the thalamus (image vision), the midbrain pretectal area (pupillary reflex), the superior colliculus (eye movements, saccades), and the suprachiasmatic nucleus (circadian rhythm).

The density of ganglion cells decreases from the retinal center to the periphery (Fig. 28a), but the size of the dendritic field (Fig. 16) increases at the same rate.

Consequently, the product of dendritic field size and density, the coverage factor, remains constant with changing eccentricity. The same inverse relationship has been found for many other neuronal cell types of the retina.

Ganglion cells receive their input from bipolar and amacrine cells. They convert visual information, coded by graded potential changes in bipolar and amacrine cells, into alterations of their action potential frequency. Ganglion cells have concentric center-surround receptive fields. Corresponding to their contacts with bipolar cells, there are ON-center cells, ON/OFF-cells, and OFF-cells. ON-ganglion cells are postsynaptic to ON-bipolar cells and increase their action potential rate when the light intensity increases in their receptive field center. The responses of ganglion cells show a variety of kinetics. One type (X) summed visual inputs approximately linearly over space and exhibited maintained changes in firing rate in response to a change in light intensity; the second type (Y) performed a more complex nonlinear computation over space and exhibited more transient responses (e.g., responding most strongly just after the light onset).

The visual information is preprocessed in the retina before it reaches the brain by coding in several distinct information channels. Such a channel is defined as a population of ganglion cell axons carrying together a special type of information extracted from visual image of the environment; the information from distinct channels usually is analyzed by distinct neuronal domains in the brain. One of these channels, the ON–OFF channel, is realized within the forward transmission. Other channels are devoted to transport information about contrast, color, and spatial orientation including direction selectivity of moving visual objects. The extraction of information for these channels requires lateral information processing by horizontal and amacrine cells. The parallel processing of different aspects of the light signal starts at the first synapse of the retina, the cone pedicle. Midget bipolar cells contact only one cone and, thus, provide high spatial resolution. Diffuse bipolar cells get convergent input from many cones and thus exhibit high contrast sensitivity.

Retinal ganglion cells can be subdivided into at least 17 functional classes according to their receptive fields, projections, and functions. In the primate retina, there are five main classes, small (ON- and OFF-) *midget cells* (*parvocellular, P, or Pß cells*; 80 % of all ganglion cells), large (ON- and OFF-) *parasol cells* (*magnocellular, M, or Pa cells*; 10 % of all ganglion cells), and very small *bistratified cells* (*koniocellular or K cells*; “koniocellular” means “cells as small as dust”). *Midget* (*P*) *ganglion cells* project via the ventral pathway to the parvocellular layers 3–6 of the dLGN. They receive inputs from relatively few rods and cones; in many cases, they are connected to midget bipolars which are linked to one (red or green) cone. They are tonic cells, have slow conduction velocity, and respond to changes in color but weakly to changes in contrast. They have simple ON- or OFF-center receptive fields. *Parasol* (*M*) *cells* project via the dorsal pathway to the magnocellular layers 1 and 2 of the dLGN. Parasol cells have large center-surround receptive fields and are responsible for orientation and movement detection. They receive inputs from relatively many rods and cones (in the primate fovea, there is convergence of 30–50 cones onto each M cell). They have phasic light responses, fast conduction velocity, and a high contrast sensitivity, but a low color

sensitivity. Small (*K*) and large *bistratified cells* (as well as giant monostratified ganglion cells) project to the koniocellular layers of the dLGN. K cells receive inputs from intermediate numbers of rods and blue cones. K cells have very large receptive fields that have only centers (no surrounds) and are always ON to the blue cone and OFF to both the red and green cone. Because the resolution limit depends both on the spacing and the dendritic field size, only cells with high density and small dendritic fields, i.e., midget (P) ganglion cells, are capable of providing high visual acuity and can transmit fine details. The large receptive fields of M and K cells permit higher contrast sensitivity.

Many other subtypes of ganglion cells were discovered in the last years. In addition to the five major types (ON- and OFF-parasol, ON- and OFF-midget, and small bistratified), 12 additional cell types with distinctive morphology have been identified.

Retinal Ganglion Cells Are Coincidence Detectors for Weak Visual Signals

Under dim light conditions, single-photon responses can be recorded from rods, and in the absence of light, there are rare single-photon-like responses due to thermal isomerization of 11-*cis* retinal. The retinal ganglion cell solves the problem of reliably detecting a weak signal in the presence of thermal noise by acting as a coincidence detector. Under conditions of complete dark adaptation, five to seven single-photon responses must arrive within a few milliseconds to be considered a real light flash and not just background thermal noise.

Photosensitive Ganglion Cells Control Non-image-forming Vision

A third class of photoreceptors in the mammalian retina, intrinsically photosensitive ganglion cells, was discovered in the early 1990s. The human retina contains ~1.3 million ganglion cells; 1 % of them are intrinsically light sensitive. These cells are sparsely distributed within the retina but usually have large dendritic fields. They are less sensitive to light than rods and cones (because the quantity of photopigments in the plasma membrane is low), respond to light very slowly (20 times more slowly than rods), are long-lasting, and signal the overall intensity of long-term light. A long integration time allows summation of photons arriving many seconds apart while making the cell insensitive to rapid fluctuations in light intensity. Thus, photosensitive ganglion cells are visual brightness detectors and control mainly the nonimage vision implicated in reflexive responses of the brain and body to light, such as circadian rhythm and pupil constriction. In addition, because these cells receive excitatory and inhibitory inputs from rods and cones (via bipolar and amacrine cells), they may play a role in color constancy (in primate, these cells exhibit a yellow-ON/blue-OFF spectral signature obtained by opposing inputs from blue cones and red/green cones). Photosensitive ganglion cells are morphologically diverse; one subclass is displaced to the INL. In primates, a subset of photosensitive ganglion cells are the largest ganglion cells of the retina.

Photosensitive ganglion cells contain a photopigment constituted of retinal and melanopsin (OPN4). Melanopsin belongs to the rhabdomeric group of photopigments which are usually found in invertebrates. Because melanopsin is

most sensitive to blue light (λ_{max} 480 nm; Fig. 34), melanopsin-containing ganglion cells may also play a role in mesopic vision. Melanopsin resembles invertebrate opsins also functionally. Photosensitive ganglion cells respond to light by depolarization, opening of nonselective cation (likely TRP) channels, and increase in the action potential frequency. The terminals of the cells release glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP).

The axons of photosensitive retinal ganglion cells terminate in various brain regions including the dLGN of the thalamus (image-forming vision), the olivary pretectal nucleus of the midbrain (pupillary reflex), the ventrolateral preoptic nucleus (a control center for sleep), and the suprachiasmatic nucleus (circadian rhythms). The majority of these targets are also innervated by conventional retinal ganglion cells. Photosensitive ganglion cells contribute to the photic suppression of melatonin release from the pineal gland; blue light in the range of 440–480 nm is highly effective in phase-shifting the human circadian clock, can increase alertness, and may be used to treat seasonal affective disorders. The projection to the dLGN suggests that photosensitive ganglion cells also influence the image vision. Blind rodless/coneless patients who rely exclusively on melanopsin signals for photodetection have lost image vision, but some are still able to photoentrain their circadian rhythm, constrict their pupils, and even have some reported visual awareness. Photosensitive retinal ganglion cells also influence the retinal activity; they modulate the signal processing of rods and cones, and make electrical synapses with conventional retinal ganglion cells. During the day, dopaminergic cells sensitize photosensitive ganglion cells by upregulating melanopsin expression.

Visual Cortex Preserves a Topological Map of the Retina

The dLGN and the primary visual cortex are organized in a retinotopic fashion. The size of the representation of a distinct retinal area in the dLGN and the primary visual cortex depends upon the density of ganglion cells in this area. Despite occupying only ~0.01 % of the visual field, ~10 % of the axons in the optic nerve are derived from ganglion cells within the fovea.

The axons from ganglion cells of the nasal half of the retina cross at the optic chiasma to join with axons from ganglion cells of the temporal half of the retina in the contralateral eye before passing into the dLGN. Ganglion cell axons from the ipsilateral eye terminate in the layers 2, 3, and 5 of the dLGN, and ganglion cell axons from the contralateral eye terminate in the layers 1, 4, and 6.

Visual Information Is Compressed in the Retina

The visual information processing within the retina has several aims including adaptation to the environmental light conditions (to achieve a stable image despite different lighting) and compression of the amount of visual information by extraction of key features of visual objects including color, contour, contrast, and movement. Information compression is required because the number of axons in the optic nerve is limited. Compression occurs in several ways including convergence (in the human retina, the information collected from ~105 millions photoreceptor cells is transferred to ~2 millions bipolars and further to ~1.3 million ganglion cells), the

transfer of a detailed image only from a small part of the retina (the fovea), and the transfer of only the changes in color, brightness, and contrast. This also contributes to image constancy because the contrast (the change in intensity between adjacent parts of an image) remains constant independent of lighting conditions. Compression of the visual information is mainly mediated by lateral inhibition which enhances image contours and edges and which occurs at two levels, i.e., in the OPL (where horizontal cells create the center-surround receptive fields of bipolar cells) and in the IPL (where bipolar and amacrine cells create the center-surround receptive fields of ganglion cells).

Most Retinal Neurons Produce Graded Potentials

Few amacrine cells and all ganglion cells generate action potentials, whereas rods and cones and horizontal, bipolar, and many amacrine cells produce only graded changes in membrane potential. Graded changes in potential allow continuous and more rapid transmission of information than information transmission by action potentials. Ganglion cells generate action potentials because they transmit information over a long distance to the brain. Graded changes in potential could not travel over such long distances.

At low light intensities, graded potentials are small. In the dark adapted retina, the absorption of a single photon generates a signal of ~0.25 mV in a depolarizing bipolar cell receiving synaptic inputs from rods. These small signals are reliably transmitted to ganglion cells. In conventional synapses, such small potentials would produce only a very small release of neurotransmitter. This problem is overcome by the presence of *ribbon synapses* in the terminals of rods, cones, and bipolar cells. Ribbon synapses have a high sensitivity to small alterations in the membrane potential and a high speed of neurotransmitter release. One ribbon synapse contain 20–100 ribbons (Fig. 22). Each ribbon tethers approximately thousands synaptic vesicles and holds the vesicles very close (several nanometers) to the active zone of the presynaptic membrane. In addition, there are about one million synaptic vesicles in the bulk of the synapse.

RPE Cells Are the Major Photoreceptor-Supporting Cells

Per definition, RPE cells are ependymoglial cells which makes them close relatives of Müller cells. In fact, at the extreme (blind) periphery of the neuroretina, the poorly differentiated (Müller) radial glial cells can hardly be discriminated from the underlying (also poorly differentiated) RPE cells. The RPE consists of highly specialized glial cells which are responsible for a wealth of crucial interactions with the neuroretina, particularly with the photoreceptor cells. These involve (1) the maintenance of the outer blood-retinal barrier and, thus, the controlled exchange of nutrients, waste products, ions, and other molecules between the choroid and the neuroretina; (2) a contribution to the metabolism/recycling of (rod) photopigments; (3) the phagocytosis of the "worn" tips of photoreceptor outer segments which are shed in a daily schedule (Fig. 20); (4) the absorption by melanin granules (Fig. 20) of

"excess light", i.e., of photons that were not absorbed by the photopigments of the outer segments along their path (blockade of light reflection), as well as vice versa; (5) the back-reflection of such photons by guanine crystals in the retinas of deep-sea fish specialized for maximum light sensitivity; (6) the maintenance of hypotension in the subretinal space and, thus, prevention of a detachment of the neuroretina from the RPE; (7) immunological and inflammatory reactions; and (8) many others.

RPE Cells Support the Function of Photoreceptors

In addition to the isomerization of photoreceptor-derived all-*trans* retinal to 11-*cis* retinal, RPE cells stabilize the ion composition of the subretinal space fluid. Photoreceptors release in the dark high amounts of K^+ while light induces (due to the decrease in the dark current of photoreceptors) a decrease in K^+ in the subretinal space fluid. Because the alterations in the ionic conditions impair the excitability of photoreceptors, RPE cells compensate these alterations by ion transports across their apical plasma membranes. In the dark, RPE cells take up excess subretinal K^+, and in the light, K^+ is released from the cells into the subretinal space.

RPE Cells Maintain the Structural Integrity of Photoreceptors

RPE cells deliver components for the resynthesis of photoreceptor outer segments including polyunsaturated ω3-fatty acids (in particular docosahexaenoic acid). RPE cells secrete further factors which maintain the structural integrity (and inhibit apoptosis) of photoreceptors, including ATP, pigment epithelium-derived factor (PEDF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). To maintain the integrity of choroidal blood vessels, and to keep their endothelium fenestrated, RPE cells secrete from their basolateral membranes factors like vascular endothelial growth factor (VEGF), Fas ligand, and PEDF.

RPE Cells Protect Photoreceptors from Photooxidative Damage

The macular retina is the tissue with the highest cell density in the body. Photoreceptor cells use three to four times more oxygen than other retinal and CNS neurons and are probably the cells of the body with the highest rate of oxidative metabolism. The high level of metabolic activity is associated with high oxygen and glucose demands. Therefore, the blood flow rate through the choriocapillaris is very high (higher than in the kidney). (A second reason for the high choroidal blood flow rate is the removing of heat because absorption of light heats up the RPE to temperatures above 40 °C.) In the night (when the photoreceptors are active), the photoreceptor inner segments consume all oxygen supplied from the choriocapillaris. During the day, the retina withdraws only little oxygen from the choroidal blood (~10 %). Thus, the outer retina containing the photoreceptor segments is hypoxic in the night and hyperoxic in the day. The oversupply of oxygen during the day together with the high light energy density (and the presence of high polyunsaturated fatty acid levels in the disk membranes) causes photooxidative damage to the outer photoreceptor segments. (In particular, excess illumination with high-energy blue light damages the photoreceptor segments, which is followed by apoptotic death of photoreceptor and

RPE cells; Figs. 26b, 36, and 37.) The tips of the photoreceptor outer segments, which contain disks injured by oxygen radicals, are shed and phagocytized by RPE cells for degradation (Fig. 20).

There are also other mechanisms by which RPE cells protect the photoreceptors from photooxidative damage. Melanin granules within the RPE (Fig. 20) absorb excess light. In addition, RPE cells contain the carotenoids lutein and zeaxanthin (which absorb blue and UV light), antioxidant enzymes, and agents and repair mechanisms for injured proteins and DNA. The impairment of the protective and repair mechanisms provided by the RPE (which results, e.g., in the accumulation of lipofuscin in RPE cells) is a causative factor of age-dependent macular degeneration (which impairs the cone vision in the fovea). Incomplete degradation of disk-derived lipids by RPE cells, as well as changes in immunological factors, results in impaired clearance of the degraded lipids by monocytes/macrophages into the choroidal blood. Because the oxygenation of the photoreceptors is barely adequate under night conditions, even slight hypoxic conditions have serious metabolic consequences. The high local oxygen demands, together with the presence of an avascular zone in the central fovea, and the relatively long distance for oxygen diffusion from the choroid across the photoreceptor outer segments which are longer in the macula than in the peripheral retina, explain the special vulnerability of the macula under conditions of perturbed oxygen supply due to vascular disease or smoking.

RPE Cells Absorb Photoreceptor-Derived Water and Lactate

The high rate of oxidative metabolism in photoreceptors is accompanied by the formation of huge amounts of water and lactate. The lactate levels in the subretinal space may achieve 20 mM. Water accumulation in the subretinal space (subretinal edema) impairs the tight connection between photoreceptor segments and RPE and is a major cause of reduced vision in ischemic-hypoxic and inflammatory retinal diseases. Subretinal water and lactate are transported through RPE cells into the choroidal blood. The transcellular water transport is facilitated by ion channels and transporter molecules, as well as by water-selective channels (aquaporins, AQPs). The transmembrane water transport is coupled to a transport of osmolytes, in particular to the active transport of Cl^- ions (Fig. 38). The Cl^- transport is driven by the Na^+ gradient across the plasma membrane generated by the Na^+-K^+ ATPase. The Na^+ gradient drives the Na^+/K^+/2Cl^- cotransporter, resulting in intracellular accumulation of Cl^-; this is followed by a Cl^- efflux from the cells through the Cl^- channels in the basolateral membrane. The transcellular Cl^- transport drives osmotically water through the cells and causes a potential difference between both sides of the cells, i.e., the basolateral side is 5–15 mV more negative than the apical side. Functional impairment of the Cl^- transport (e.g., due to mutation of the Cl^- channel gene) is one cause of retinitis pigmentosa. The water transport through the RPE results in hypotension within the subretinal space; this is the main cause of the adhesion of the neuroretina to the RPE. Impairment of the Cl^- and water transport causes a detachment of the neuroretina from the RPE, followed by decreased vision and retinal degeneration.

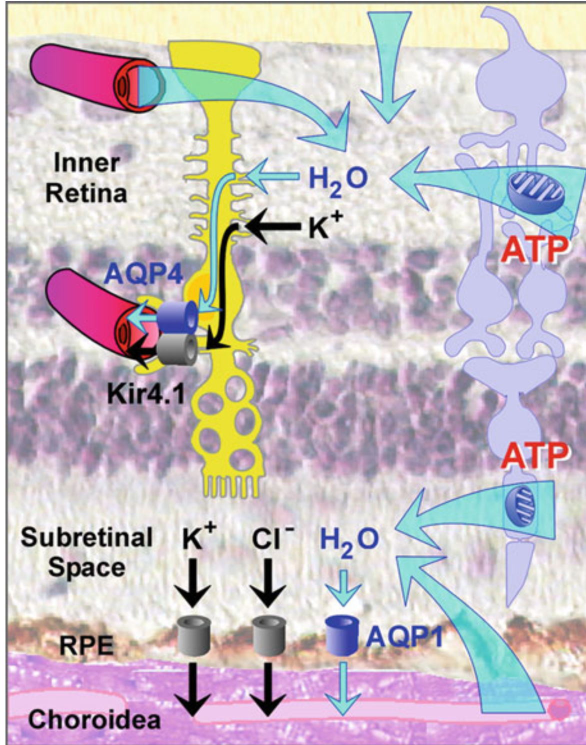

Fig. 38 Water fluxes through the retina. Under normal conditions, water accumulates in the neural retina and subretinal space due to an influx from the blood (coupled to the uptake of nutrients such as glucose) and vitreous chamber (due to the intraocular pressure) and the oxidative synthesis of ATP in the mitochondria that generates carbon dioxide and water. The excess water is redistributed into the blood by a transcellular water transport through Müller cells and the retinal pigment epithelium (*RPE*). The water transport across cell membranes is facilitated by aquaporin (*AQP*) water channels. RPE cells express AQP1, while Müller cells express AQP4. The transcellular water transport is osmotically coupled to the transport of osmolytes, especially of potassium and chloride ions. The ion fluxes across the cell membranes are facilitated by transporter molecules and ion channels. In Müller cells, the Kir4.1 potassium channel is colocalized with AQP4 in membranes that contact the vessels and both limiting membranes of the retina

Retinal Glial Cells Mediate Neuronal Nutrition, Waste Management, and Inflammation

The neuroretina contains microglial cells and one or two types of macroglial cells (independent of whether or not the retina is vascularized). A further type of glial cells, oligodendrocytes, are present in myelinated nerve fiber bundles of birds, rabbits, hares, and some fish. In humans, myelination of the optic nerve fibers commences at the optic disk.

Microglial Cells Are the Blood-Borne Resident Immune Cells of the Retina

Microglial cells are blood-borne macrophages which invade the retina during the development of the retinal vasculature. In adult mice, there is also a complete turnover of resident retinal microglia within 6 months by recruitment of blood-derived precursor cells. Microglial cells play (in close relationship to other types of glial cells; Fig. 39) important roles in host defense against microorganisms, initiation of inflammation, and tissue repair. Resting microglia act as highly motile patrolling cells constantly surveying their microenvironment. Once a pathogenic stimulus is detected, microglia become activated, proliferate (Fig. 40), and migrate toward the region of damage, where they kill bacteria, release cytotoxic agents, and phagocytize cellular debris. However, excessive or prolonged activation of microglial cells contributes to chronic inflammation and retinal degeneration. Microglial cells release proinflammatory cyto- and chemokines. Inflammation is associated with an opening of the blood-retinal barrier which allows monocytes/macrophages (Fig. 39) and neutrophil granulocytes to invade the retina where they release cytotoxic cytokines and reactive oxygen species (which normally kill microorganisms but are also harmful to photoreceptors and neurons).

With age, microglia migrate into the subretinal space to support the RPE in the clearance of age-dependent debris (in particular, of photooxidized receptor disks). Subretinal microglia contain autofluorescent lipofuscin granules (which is a "waste" material in the aging retina and implicated in the development of age-related macular degeneration). Subretinal microglia form "crystallization" points for cellular deposits and complement-containing immune complexes and activate RPE cells. Proinflammatory factors secreted from activated RPE cells stimulate further microglial migration and activation. The highly activated microglia containing phagocytosed photoreceptor debris may actively exit the subretinal space via retinal and choroidal vessels and may reach the spleen, where they act as antigen-presenting cells and elicit systemic immune responses against the retina. Elevated levels of antiretinal antibodies have been found in the serum of patients with age-dependent macular degeneration. At present, it is unclear why microglia are sometimes damaging and other times protective.

Macroglial Cells Provide Metabolic and Functional Support for Retinal Neurons

Macroglial cells support the function and metabolism of retinal neurons. For this reason, they constitute an anatomical and functional link between retinal neurons and blood vessels (and the vitreous cavity). Most nutrients, waste products, ions, and other molecules are transported through macroglial cells between the inner retinal blood vessels and neurons. Vascularized mammalian retinas like the human retina contain astrocytes and Müller glial cells. Astrocytes play a crucial role in the retinal vascularization; the localization of the cells is restricted to the innermost retinal layers (NFL and GCL) (Fig. 19). Avascular mammalian retinas contain only Müller

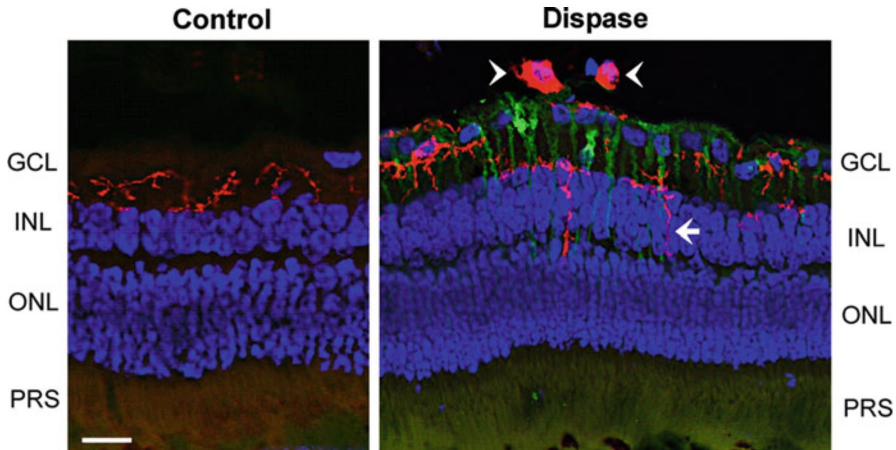

Fig. 39 Interaction of blood-derived monocytes/macrophages and retinal glial cells in a rabbit model of early proliferative vitreoretinopathy induced by intravitreal injection of the protease dispase. Retinal slices were stained against immune cells (*red*), GFAP (*green*; a marker of activated Müller glial cells), and cell nuclei (*blue*). Under control conditions, microglial cells (*red*) are restricted to the innermost retinal layers, and Müller cells do not express GFAP. The retinas from dispase-treated eyes display "hot spots" of glial cell reactivity characterized by an upregulation of GFAP in Müller cells (*green*) and activated microglia that begin to migrate toward the outer retina (*arrow*). Blood-borne monocytes/macrophages adhere to the vitreal surface of such hot spots (*arrowheads*), suggesting a relationship between the attachment of macrophages and glial cell activation. *GCL* ganglion cell layer, *INL* inner nuclear layer, *ONL* outer nuclear layer, *PRS* photoreceptor segments. Bar, 20 μm

cells as neuron-supporting glial cells, and the retinal tissue is fed by the choroidal blood and (to a lesser extent) the vitreal fluid.

The human retina contains about five million Müller cells. Müller cells are specialized radial glial cells which span the entire thickness of the retina from the inner to the outer limiting membrane (Fig. 19). Müller cell membranes surround all retinal vessels, neuronal cell bodies, and synapses. Müller cells play a wealth of crucial roles in supporting neuronal information processing. They provide trophic substances to neurons and remove metabolic waste. Müller cells play a critical role in the regulation of the extracellular space volume, ion and water homeostasis of the retina, and the maintenance of the inner blood-retinal barrier (which is formed by tight junctions between vascular endothelial cells). They release gliotransmitters and other neuroactive substances, regulate the synaptic activity by neurotransmitter recycling, and provide precursors of neurotransmitters to neurons (Fig. 41). All of these functions may directly or indirectly modulate neuronal excitability and transmission. Müller cells support the survival of retinal neurons and photoreceptor cells, are responsible for the structural stabilization of the retina, and are modulators of immune and inflammatory responses. They guide the light to the photoreceptors and buffer mechanical deformations in the tissue. Müller cells become activated upon virtually all pathogenic stimuli. Reactive Müller cells are neuroprotective but, on the

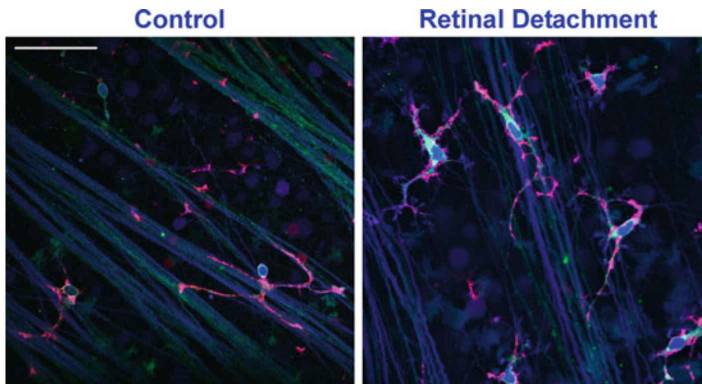

Fig. 40 In the avascular regions of the rabbit retina (retinal areas outside the medullary rays), microglial cells are located in the innermost retinal layers. Acutely isolated wholemounts of a control retina (*left*) and a retina obtained 2 days after experimental retinal detachment (*right*) were stained with Cy3-tagged *Griffonia simplicifolia* agglutinin isolectin I-B_4 (to label microglial cells) and viewed at the vitreal surface (i.e., onto the nerve fiber/ganglion cell layers). The isolectin labels D-galactose residues on microglial/immune cells. Elongated structures are light reflections on nerve fibers. Under pathological conditions like detachment of the neuroretina from the RPE, microglial cells become rapidly activated, as indicated by increased cell number and morphological alterations such as hypertrophy of cell somata, a decrease of the process number per cell, and shortening and thickening of cell processes. Strongly activated, amoeboid microglial cells do not bear cell processes. Bar, 50 μm

contrary, may also stop their neuron-supportive functions and contribute to neuronal degeneration.

As the whole CNS, the retina is structurally and functionally compartmentalized into columnar units or cellular “domains.” Every Müller cell constitutes the core of a cellular column as the smallest functional unit for the forward processing of visual information. In the rabbit retina, such a column contains (in addition to the Müller cell) ~15 neurons, including 1 cone, 10 rods, and 2 bipolar and 1 amacrine cells. In the human retina, one unit contains 1 (fovea centralis) to 11 photoreceptors (retinal periphery).

Müller Cells Are Involved in Synaptic Signaling by Uptake of Neurotransmitters

Precise shaping of the synaptic activity depends upon the kinetics of both the presynaptic release of neurotransmitter and the reuptake of the transmitter into the cells. In the retina, photoreceptor cells, neurons, and macroglial cells express high-affinity transporters for neurotransmitters. Müller cells express uptake and exchange systems for various neurotransmitters including glutamate and GABA (Fig. 41).

The major glutamate uptake carrier of Müller cells is the glutamate-aspartate transporter (GLAST or EAAT1). This carrier system mediates a cotransport of 3 Na^+ and 1 H^+, and the countertransport of 1 K^+, with each glutamate anion. The transport of an excess of Na^+ into the cell generates an inward current (i.e., the transporter is

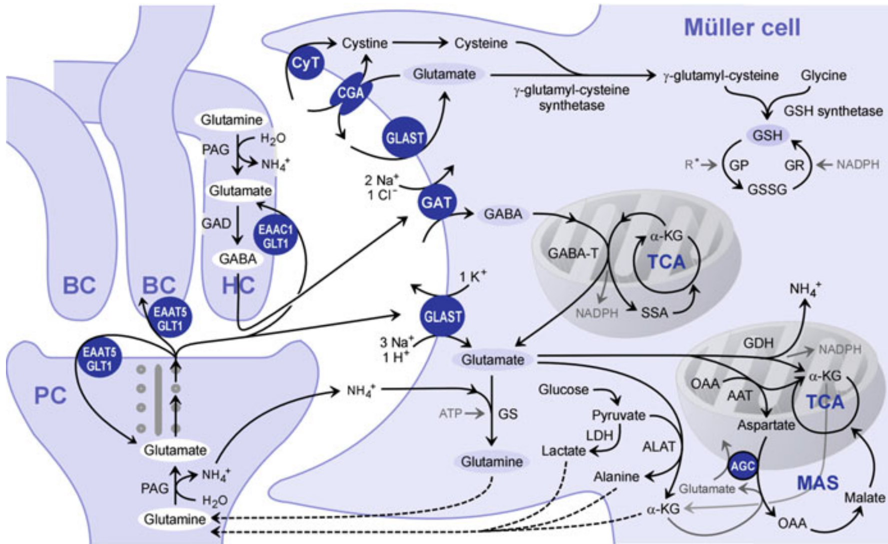

Fig. 41 Recycling of amino acid neurotransmitters in the outer plexiform (synaptic) layer of the mammalian retina. The ribbon synapse of a photoreceptor cell (*PC*) synthesizes glutamate which is continuously released during darkness. The postsynaptic elements are dendrites of bipolar (*BC*) and horizontal cells (*HC*). Horizontal cells release GABA which is formed from glutamate. The synaptic complexes are surrounded by Müller cell sheets; the *right* side shows neurotransmitter uptake systems and some metabolization ways of Müller cells. Glutamate, GABA, and ammonia (NH_4^+) are transported into the Müller cell and transformed to glutamine, alanine, and α-ketoglutarate (*α-KG*). These products are released from Müller cells and taken up by neurons. Glutamine serves as precursor for the transmitter synthesis in neurons (glutamate–glutamine cycle). Lactate, alanine, α-ketoglutarate, and glutamine are utilized by neurons as substrates for their energy metabolism. Another metabolic way is the production of glutathione (*GSH*) which is an intracellular antioxidant, released from Müller cells and taken up by neurons under oxidative stress conditions. *ALAT* alanine aminotransferase, *AAT* aspartate aminotransferase, *CGA* cystine-glutamate antiporter, *CyT* cystine transporter, *EAAC1* excitatory amino acid carrier 1, *EAAT5* excitatory amino acid transporter 5, *GABA-T* GABA transaminase, *GAD* glutamic acid decarboxylase, *AGC* aspartate–glutamate carrier, *GAT* GABA transporter, *GDH* glutamate dehydrogenase, *GLAST* glutamate–aspartate transporter, *GLT-1* glutamate transporter-1, *GlyT* glycine transporter, *GP* glutathione peroxidase, *GR* glutathione reductase, *GS* glutamine synthetase, *GSH* glutathione, *GSSG* glutathione disulfide, *LDH* lactate dehydrogenase, *MAS* malate–aspartate shuttle, *OAA* oxaloacetate, *PAG* phosphate-activated glutaminase; $R^{\bullet}$ free radicals, *SSA* succinate semialdehyde, *TCA* tricarboxylic acid cycle

“electrogenic”; Fig. 42a). Na^+-dependent carriers allow uphill transport of substrates into the cells against a concentration gradient. The driving force for these transporters is the electrochemical gradient of Na^+ across the plasma membrane that is generated by the energy-consumptive activity of the Na^+-K^+ ATPase. Other transporters, e.g., for GABA (Fig. 42b) are also electrogenic.

In the CNS, glial glutamate transporters are responsible for the bulk of glutamate uptake, while neuronal glutamate transporters have more specialized roles, ensuring a high signal-to-noise ratio for synaptic transmission. Müller cells remove the bulk of glutamate from extracellular sites within the inner retina (IPL, GCL). Glutamate

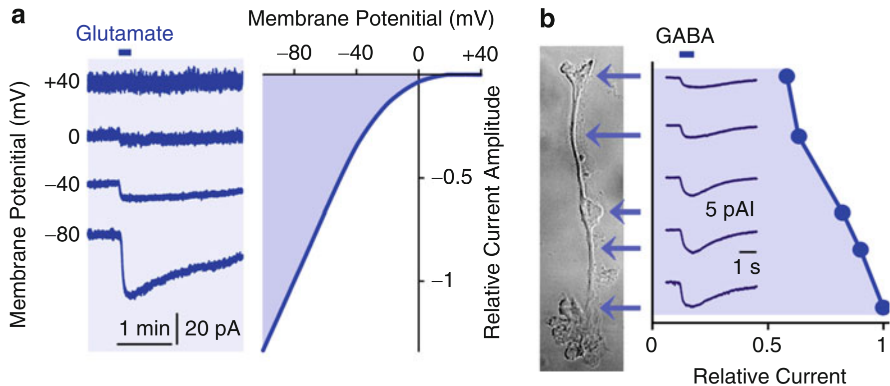

Fig. 42 The main glutamate and GABA uptake carriers of Müller cells are electrogenic transporters. The membrane currents were recorded in whole Müller cells. (**a**) Administration of glutamate (1 mM) to a Müller cell of the rabbit retina evokes inward currents at negative membrane potentials (*left*). The current–voltage relation of the glutamate transporter currents in guinea-pig Müller cells (*right*) shows that the efficiency of glutamate transport increases with increasing membrane potential. (**b**) Subcellular distribution of the GABA transporter currents in a Müller cell of the guinea pig. The distribution of the currents was determined by focal ejections of GABA (1 mM) onto the following membrane domains of the cell: end foot, inner stem process, soma, and inner and outer parts of the outer stem process

released from rod spherules and cone pedicles is mainly removed by photoreceptor, horizontal, and bipolar cells. However, Müller cells take up glutamate which is diffused out of the synaptic clefts, thereby preventing a lateral spread of the transmitter and ensuring visual resolution. The glutamate uptake by Müller cells in the IPL contributes to the shaping and rapid termination of the postsynaptic action of glutamate in nonspiking retinal neurons and ganglion cells. Here, Müller cells are an active part in synaptic transmission. A malfunction and even reversal of glial transporters under pathological conditions contribute to glutamate-induced neurotoxicity. GABA is taken up by amacrine and Müller cells in the inner retina and exclusively by Müller cells in the outer retina of mammalian species. This corresponds with the greater GABA transport currents in the outer stem process of Müller cells (Fig. 42b).

In addition to the uptake of neurotransmitters, Müller cells regulate the synaptic transmission more directly by the release of "gliotransmitters," in particular glutamate and ATP. Glia-derived glutamate and ATP might activate retinal ganglion cells, while adenosine (which is extracellularly formed by degradation of ATP) suppresses neuronal activity.

Müller Cells Provide Glutamine as Precursor of Neuronal Transmitter Synthesis

The uptake of glutamate and GABA by Müller cells links neuronal excitation with the release of lactate and other molecules that nourish retinal neurons, as well as with the defense against oxidative stress. After being taken up by Müller cells, glutamate is rapidly converted to glutamine by the enzyme glutamine synthetase (Fig. 41)

which is exclusively localized to astrocytes and Müller cells (Fig. 36). Glutamine is released from Müller cells and taken up by neurons as a precursor for the synthesis of glutamate and GABA (*glutamate-glutamine cycle*; Fig. 41). The production of glutamate in bipolar and ganglion cells depends almost entirely on Müller cell-derived glutamine. The glutamate synthesis in photoreceptor cells depends in part on Müller cell-derived glutamine, and the other part is synthesized within the photoreceptor cells by transamination of α-ketoglutarate. In addition, a significant amount of GABA in amacrine cells is synthesized from Müller cell-derived glutamine. Pharmacological blockade of the glutamine synthetase in Müller cells results in a rapid loss of the glutamate content of bipolar and ganglion cells, and the animals become (within 2 min) functionally blind.

In addition to the production of glutamine, glutamate in Müller cells is utilized for the production of the antioxidant glutathione (Fig. 41). Oxidative stress induces a rapid release of reduced glutathione from Müller cells and its uptake by neurons. The metabolization of glutamate in Müller cells is also tightly coupled to the nutritive function of glia. Müller cells produce various substrates for the oxidative metabolism of photoreceptors and neurons such as glutamine, lactate, alanine, and α-ketoglutarate (Fig. 41).

Müller Cells Maintain the Ion and Osmohomeostasis in the Neuroretina

Neuronal activity causes rapid alterations in the composition of the extracellular space fluid which should be regulated to avoid disturbances of the information processing. In addition to the release of neurotransmitters, activated neurons release K^+, water, and CO_2 (the two latter are derived from the oxidative metabolism of glucose; Fig. 43). Via transcellular transport, Müller cells remove these "waste products" into the blood and vitreal fluid (Fig. 38). In addition, neuronal activity is associated with rapid alterations in the volume of neuronal cell structures (in particular of synapses). Activation of ionotropic receptors such as AMPA/kainate and NMDA receptors causes an influx of cations (Na^+, Ca^{2+}) through the postsynaptic membranes which is (for osmotical reasons) coupled to an influx of water. The ion influx into neuronal cells (and of excess K^+ into Müller cells) causes a decrease in the osmolarity of the extracellular fluid, and the influx of water causes a swelling of neuronal cell structures and a decrease in the extracellular space volume. To regulate the extracellular space volume, Müller cells inhibit their swelling (despite the presence of a hypoosmotic environment) or even decrease the volume of their perisynaptic processes. Rapid alterations in cell shape are supported by the biomechanical features of Müller cells which are softer than neurons.

Müller Cells Clear Excess K^+ from the Extracellular Fluid

Light-evoked increases in extracellular K^+ occur in the plexiform (synaptic) layers and (after cessation of illumination) in the subretinal space. To avoid overexcitation of retinal neurons, Müller cells buffer the local increases in extracellular K^+ by a transcellular K^+ transport mediated by inwardly rectifying K^+ (Kir) channels (Fig. 38). In domains which abut neuronal cell structures, Müller cell membranes contain Kir channels which mediate inward K^+ currents (Kir2.1, Kir4.1/Kir5.1)

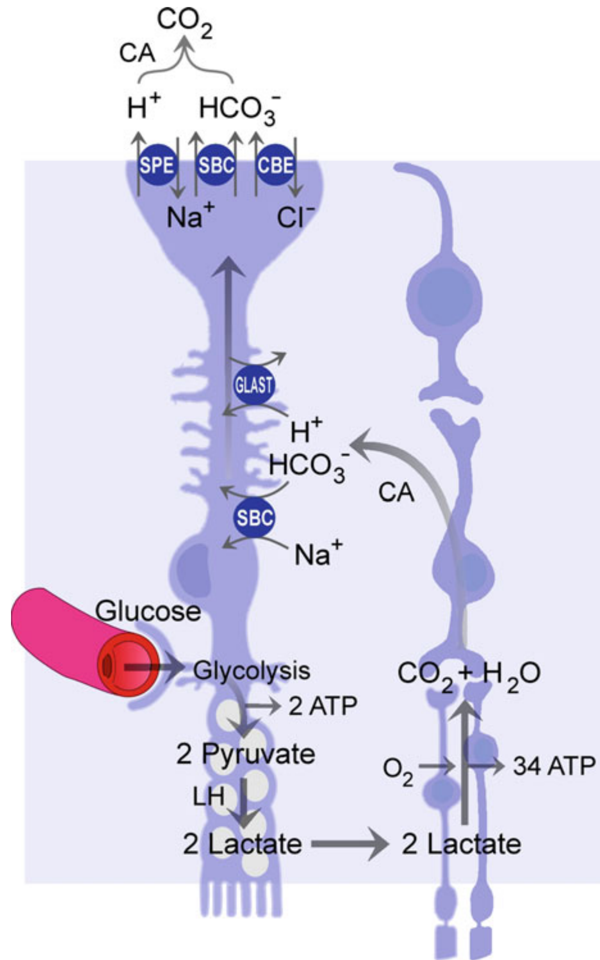

Fig. 43 Carbon dioxide siphoning by Müller cells. The oxidative metabolism of retinal neurons and photoreceptors results in the formation of carbon dioxide and water from lactate/pyruvate which are in part produced in Müller cells. The carbonic anhydrase (*CA*) at the surface of Müller cells converts carbon dioxide and water into bicarbonate and protons which are taken up by the sodium bicarbonate cotransporter (*SBC*) and the glutamate transporter GLAST, for example. Bicarbonate and protons are then preferentially released into the blood vessels and the vitreous by the concerted action of SBC, the chloride-bicarbonate exchanger (*CBE*), and the sodium-proton exchanger (*SPE*). *LH* lactate dehydrogenase

while the membranes which contact blood vessels, the vitreous cavity, and the subretinal space (i.e., fluid-filled compartments outside the neuroretina) contain Kir4.1 channels that allow bidirectional K^+ currents (Fig. 44a, b). The passive K^+ transport through Kir channels clears excess K^+ from the extracellular fluid around excited neurons into the fluids outside the neuroretina (Fig. 44c).

Under ischemic-hypoxic and inflammatory conditions, the perivascular Kir4.1 is downregulated and dislocated (Figs. 37 and 44b). The inactivation of Kir4.1 channels impairs the transcellular K^+ transport (and thus, the retinal K^+ homeostasis) and results in a depolarization of the cells (from about −80 to about −50 mV). The depolarization impairs the glutamate uptake by Müller cells (which is dependent on the membrane potential: Fig. 42a), and high extracellular K^+ causes overexcitation of neurons. Both events facilitate glutamate-induced neurotoxicity under pathological conditions.

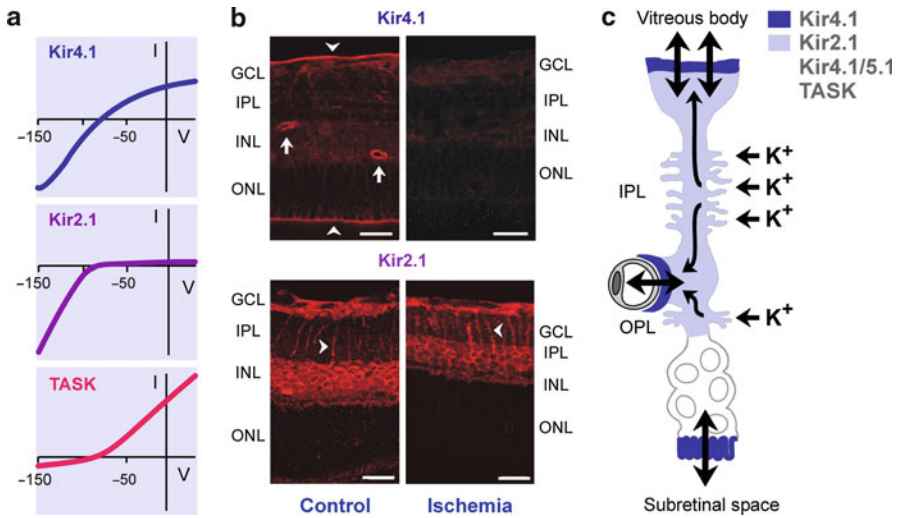

Fig. 44 The subcellular localization of different Kir channel subtypes determines the direction of spatial buffering potassium currents through Müller cells. (**a**) Current–voltage (I–V) relations of various glial potassium channels. The Kir4.1 channels mediate inward and outward currents with similar amplitudes, whereas the Kir2.1 channels mediate inward currents, and two-pore domain (TASK) channels mediate outward potassium currents. (**b**) Immunolocalization of glial Kir channels in the normal and postischemic rat retina. The Kir4.1 protein is predominantly localized at the limiting membranes of the neuroretina (*arrowheads*) and around the blood vessels (*arrows*). The Kir2.1 protein is localized in the inner retina in membrane domains of Müller cells that abut on neuron compartments, e.g., in the processes that traverse the inner plexiform layer (IPL) (*arrowheads*). Seven days after a 1-h transient retinal ischemia, the expression of Kir4.1 protein is largely downregulated, whereas the localization of the Kir2.1 protein is unaltered. Note the decrease in the thickness of the inner retina which is a characteristic feature of retinal ischemia-reperfusion injury. (**c**) Scheme of the potassium buffering currents that flow through Müller cells during neuronal activation. Activated neurons release potassium ions which are absorbed by Müller cells through Kir2.1 and Kir5.1/4.1 channels and distributed into the blood vessels, the vitreous, and the subretinal space through Kir4.1 channels. Kir4.1 channels mediate in- and outward currents and, thus, contribute to the osmohomeostasis between the neuroretina and extraretinal fluid-filled spaces. *GCL* ganglion cell layer, *INL* inner nuclear layer, *ONL* outer nuclear layer, *OPL* outer plexiform layer. Bars, 20 μm

Müller Cells Dehydrate the Inner Retina

The retinal clearance of water (mainly derived from oxidative degradation of glucose) is mediated by an osmotically driven transcellular water transport that is coupled to a transport of osmolytes, in particular Cl^- (RPE cells) and K^+ (Müller cells) (Fig. 38). The transcellular water transport is facilitated by AQPs. Generally, the inner retina and the OPL is dehydrated by water transport through AQP4 expressed in astrocytes and Müller cells, while the osmotic balance in the ONL and subretinal space is mediated by water flux through AQP1 expressed by photoreceptor and RPE cells (Figs. 36 and 45). The colocalization of AQP4 (Fig. 45) and

Kir4.1 (Figs. 37 and 44b) in Müller cell membranes that surround the vessels suggests that the osmotic pressure of the Müller cell interior is adapted to the blood osmolarity. Under conditions associated with edema in the outer retina (e.g., after blue-light illumination resulting in injury to photoreceptor and RPE cells; Figs. 36 and 37), Müller cell processes within the ONL increase strongly their expression of AQP4 (Fig. 26b). Under these conditions, Müller cells are also responsible for the osmohomeostasis in the ONL. The downregulation of Kir4.1 observed under ischemic-hypoxic and inflammatory conditions (Figs. 37 and 44b) also disrupts the water transport through Müller cells. The impairment in the water clearance through Müller cells contributes to the development of retinal edema which is a major factor of decreased vision in cases of ischemic and inflammatory retinal diseases.

Outlook

As it has been stated at the end of the "History" section, this article definitely is aimed at stimulating its readers to search for new discoveries in the retina although it has been intensely studied over the last centuries. Still, there are many questions to be answered. These involve, among others, the developmental mechanisms, the interindividual shape variability, and the distinctive functional and metabolic features of the fovea centralis, including its high susceptibility against age-related degenerations. Furthermore, many details of intraretinal information processing (in color vision, movement detection, etc.) need to be elucidated in order to understand both the normal functioning of the visual system and disease-related functional defects. Another unresolved problem is the retinal control of eye growth, including emmetropization. Although in certain Asian populations up to 90 % of the children become myopic, and despite of intense research over the decades, still we need to clarify the complex signaling chains if we want to develop a causal therapy of high-grade myopia. On the other side of the medal, many promising new techniques have been – and still are being – developed which will enable retina scientists to achieve breakthroughs in these and other areas. Optical coherence tomography, in combination with adaptive optics and other current techniques such as two-photon microscopy, allows high-resolution imaging of the retina in the intact patient, close to the resolution of microscopy on retinal preparations. Transgenic animal models, in combination with micromethods of electrophysiology, functional imaging, biochemistry, and molecular biology, provide novel insights into the (patho-)mechanisms of retinal functioning and dysfunctioning which have been impossible a few years ago. Innovative therapeutic approaches such as gene therapy, stem cell delivery, or hybrid technologies (e.g., implantation of electronic retina chips) are on the way. Thus, retina research will remain one of the most exciting fields in neurobiology and is waiting for young talented researchers willing to enter the boat.

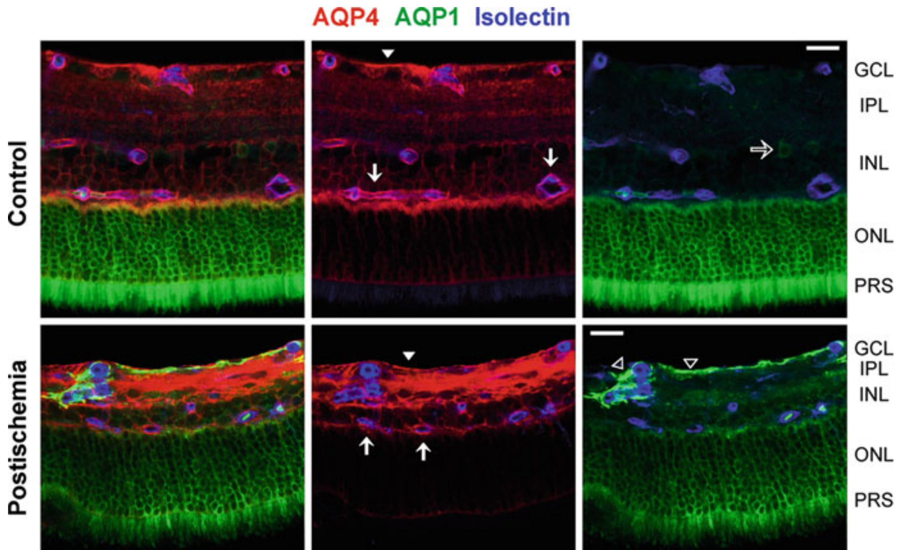

Fig. 45 The water homeostasis of the outer neuroretina is mediated by aquaporin-1 (*AQP1*) expressed by photoreceptor cells, while the water homeostasis of the inner retinal tissue is mainly mediated by glial aquaporin-4 (*AQP4*) in the normal retina (*above*), and by AQP4 and AQP1 in the postischemic retina (*below*). Retinal slices of the rat were immunostained against AQP1 (*green*) and AQP4 (*red*) and were labeled with isolectin (*blue*) to show the blood vessels. The tissues were derived from a control animal (*above*) and from an animal at 7 days after transient retinal ischemia induced by elevation of the intraocular pressure for 1 h (*below*). AQP4 is strongly expressed by Müller cells in the inner retina and the outer plexiform layer (*OPL*), whereas the expression of the protein in the outer nuclear layer (*ONL*) is faint. AQP4 is enriched in Müller cell membranes that surround the vessels (*filled arrows*) and in the vitreous-abutting end feet membranes (*filled arrowheads*). Photoreceptor cells express AQP1. In the inner retina, AQP1 is expressed by subpopulations of glycinergic and GABAergic amacrine cells under normal conditions (*unfilled arrow*) and additionally by glial processes around the vessels and at the inner retinal surface after ischemia (*unfilled arrowheads*). In addition, erythrocytes within the retinal vessels express AQP1. *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *PRS* photoreceptor segments. Bar, 20 μm

Glossary

AII Cell Subtype of glycinergic amacrine cells which connects the rod pathway to the cone output pathway.

Amacrine Cell Interneuron in the IPL which lacks an axon.

Bipolar Cell Cell with bipolar morphology (two main processes evolve from the perikaryum in opposite direction) transfers visual information from the synaptic terminals of photoreceptor cells to amacrine and ganglion cells.

Bistratified Cell Cell with processes that branch in two distinct sublayers of the IPL.

Choriocapillaris Inner sublayer of the choroidea which contains capillaries.

Ciliar Photoreceptor Vertebrate-type photoreceptor which contains photopigments in invaginations of the cilium membrane.

Cone Cone-like photoreceptor responsible for color vision under bright light conditions.

Dark Current Current between photoreceptor outer and inner segments which is active in the dark.

Diffuse Amacrine Amacrine cell with processes that branch vertically in different sublayers of the IPL.

Diffuse Bipolar Bipolar cell which has a relatively large dendritic field and contacts several cone pedicles.

Displaced Cell Cell with a perikaryum that is localized in an unusual retinal layer.

Dyad A ribbon-containing structure of the synaptic terminal of a bipolar cell.

Fovea Centralis The center of the fovea with the highest visual acuity, contains only cones.

Ganglion Cell Output cell of the retina; its axon draws in the optic nerve to the brain.

Horizontal Cell Interneuron that makes lateral visual information processing in the OPL.

Interplexiform Cell Subtype of amacrine cells that regulates the activity of bipolar dendrites in the OPL in dependence on the activity of the IPL.

K Cell Koniocellular cell.

Kir Channel K^+ channels of Müller cells implicated in spatial buffering of the extracellular K^+ concentration.

Koniocellular Cell Cell as small as dust.

M Cell Large magnocellular (parasol) cell.

Macula Lutea Yellowish area of the primate retina which includes the fovea, parafovea, and perifovea.

Melanopsin Photopigment of intrinsically light-sensitive retinal ganglion cells.

Mesopic Vision Rod and cone vision at middle to low light intensities.

Metarhodopsin Light-activated rhodopsin that activates the phototransduction cascade.

Midget Cell Small cell with small receptive field.

Müller Cell The main macroglial cell of the retina.

Nuclear Layer Layer that contains perikarya.

OFF-Cell Cell which is inactivated when the center of the receptive field is illuminated.

ON-Cell Cell which is activated when the center of the receptive field is illuminated.

Optic Disk Optic nerve head; blind spot of the retina which does not contain photoreceptors.

P Cell Small parvocellular (midget) cell.

Parasol Cell Large cell with large receptive field.

Pedicle Synaptic terminal of a cone.

Photoisomerization Light-induced conformation change of 11-*cis* retinal to all-*trans* retinal.
Photopic Vision Cone vision under bright light conditions (daylight).
Photopsin Photopigment of a cone composed of iodopsin and retinal.
Phototransduction Transduction of light energy into neuronal activity.
Plexiform Layer Synaptic layer.
Purkinje Shift Shift of the retinal sensitivity from yellow toward blue during adaptation to darkness (mesopic vision).
Receptive Field Area of the retina where light stimuli alter the activity of a cell.
Retinoid Cycle Metabolic cycle which regenerates 11-*cis* retinal from all-*trans* retinal.
Rhabdomeric Photoreceptor Invertebrate-type photoreceptor which contains photopigments in the membranes of microvilli.
Rhodopsin Photopigment of rods composed of scotopsin and retinal.
Ribbon Presynaptic dense body binds glutamate-containing synaptic vesicles.
Rod Rodlike photoreceptor responsible for achromatic vision under low light conditions.
Scotopic Vision Rod vision at low light intensities (night vision).
Spherule Synaptic terminal of a rod.
Starburst Amacrine Amacrine cell with large dendritic field which is implicated in movement detection.
Stratified Cell Cell with processes that branch in a distinct sublayer of the IPL.
Subretinal Space Fluid-filled space between the neuroretina and RPE.
Transducin G-protein involved in the phototransduction cascade.
Triad A ribbon-containing structure of the cone pedicle.

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