The evolution of vision

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In this review, the evolution of vision is retraced from its putative origins in cyanobacteria to humans. Circadian oscillatory clocks, phototropism, and phototaxis require the capability to detect light. Photosensory proteins allow us to reconstruct molecular phylogenetic trees. The evolution of animal eyes leading from an ancestral prototype to highly complex image forming eyes can be deciphered on the basis of evolutionary developmental genetic experiments and comparative genomics. As all bilaterian animals share the same master control gene, Pax6, and the same retinal and pigment cell determination genes, we conclude that the different eye-types originated monophyletically and subsequently diversified by divergent, parallel, or convergent evolution.

INTRODUCTION

The eye is a most fascinating organ, and the elucidation of eye evolution is one of the most challenging tasks in evolution biology. In ‘The Origin of Species’ Charles Darwin devoted an entire chapter to ‘Difficulties of the Theory’ and the eye as an organ of extreme perfection is discussed extensively. At first sight, it seems absurd that an eagle’s eye with all its perfections could have evolved simply by random variation and selection. However, Darwin found a possible solution to this enigma and proposed that the complex, highly perfected eyes like the eagle’s eyes originated from a relatively simply prototypic organ consisting of an optic ‘nerve (photoreceptor cell) surrounded by pigment cells and covered by translucent skin, but without any lense or other refractive body’. Such a prototypic eye would enable its carrier to determine the direction of the incoming light and provide a substantial selective advantage over those organisms which can only distinguish between light and dark. Darwin postulated this prototype merely on theoretical grounds, but subsequently it was found in e.g. the planarian Polycelis auricularia and trochophora larvae. Darwin also clearly stated that the evolution of the prototype cannot be explained by selection, because selection can only act on organs having at least a minimal function. Therefore, the evolution of the prototypic eye must have been a purely stochastic and hence a very rare event. Once the prototype was formed, random variation and selection could lead to the evolution of the various more complex eye-types.

By contrast the neodarwinists like Ernst Mayr assumed that the various eye-types found in different animal phyla arose 40–60 times independently. Their arguments were mainly based on morphology and physiology, but also on the different modes of development. However, more recent genetic experiments indicate that the various metazoan eye-types are controlled by the same set of transcription factors, in particular, Pax6 which serves as a master control gene for eye morphogenesis in taxa as different as insects and mammals and is found in all bilaterian phyla. This demonstrates that the various eye-types can be ‘built’ with the same tool kit. In 2001, Mayr admitted that his former notion was not quite correct.

The evolution of vision will be traced all the way back to its putative origins in cyanobacteria, the oldest known fossils on earth. Cyanobacteria have evolved a circadian clock and phototaxis both of which depend on vision. They are the first organism with ‘eye spots’, i.e. organelles for photoreception. The evolution of circadian clocks and of phototaxis and phototropism are discussed, followed by the phylogenetic analysis of the three major classes of photosensory proteins, phycobiliproteins, cryptochromes, and rhodopsins. The evolution of the various animal eye-types will be followed from their prototypes to their adaptive radiation. Finally, some general genetic mechanisms governing eye evolution are proposed.
THE ORIGIN OF VISION: CYANOBACTERIA

Cyanobacteria are among the oldest macroscopic fossils on earth. They are known as stromatolites, which are sedimentary rocks formed by layers of cyanobacteria and represent the earliest evidence for life on earth. Stromatolites from Pilbara in Western Australia were dated to be approximately 3500 million years old. Living stromatolites can still be seen today in Western Australia for example at Shark Bay or in Yellowstone National Park in America. Cyanobacteria are prokaryotic eubacteria capable of photosynthesis. They use the photosystems I and II, chlorophyll a and b, the Calvin-cycle for CO2 fixation, H2O as an electron donor, and they produce O2. They contribute substantially to the oxygen content of the atmosphere. Their cells contain stacks of thylakoid membranes on which photosynthesis takes place. They can be single-celled, like Gloeobacter or Synechococcus or filamentous, like Anabaena and Nostoc. They represent a large fraction of the phytoplankton of the oceans and also occur in fresh water and terrestrial habitats and can grow under extreme conditions. In the course of evolution, some were taken up as symbionts by eukaryotes and became chloroplasts.

Light detection confers a considerable selective advantage to photosynthetic organisms which tend to be attracted by sunlight and repelled by light of excessively high intensity surpassing the capacity of the photosynthetic machinery, and they also avoid UV light which causes DNA damage (see cryptochromes).

There are two lines of evidence that cyanobacteria can sense light: circadian clocks calibrated by the light–dark cycle of the sun, and Phototaxis have both been found in cyanobacteria. The observation that stromatolites are deposited in a regular centimeter-scale spacing similar between modern and ancient stromatolites suggests that they both were deposited rhythmically with a period of approximately 20 h corresponding to their circadian clock.

The terrestrial cyanobacterium, Leptolyngbya sp., forms long, slender trichomes (filaments) and moves very slowly by parallel movements of its trichomes toward a light source (positive phototaxis). The apical cell is characterized by an orange spot at its distal tip which resembles the ‘eye spot’ or ‘stigma’ of carotenoid-rich lipid globules which is almost invariably present in phototactic flagellated algae. Microspectrophotometric analysis showed an absorption spectrum with a major peak at 456 nm and a second peak at 504 nm, which was tentatively identified as rhodopsin. Hydroxylamine treatment which reacts with all aldehydes including retinal in aqueous solution to form stable oximes, abolishes this peak at 504 nm and prevents the phototactic movements of the trichomes. This suggests that these cyanobacteria have evolved photoreceptor organelles endowed with rhodopsin as we find them in flagellated algae, e.g. Chlamydomonas (Figure 2).

THE EVOLUTION OF CIRCADIAN CLOCKS

Virtually, all organisms from cyanobacteria to humans have developed cellular oscillations and mechanisms to synchronize these cellular oscillations to environmental cycles, in particular, to the light–dark cycle of sunlight. Internal timing mechanisms may facilitate physiological and behavioral adaptations to these environmental conditions. These timing mechanisms involve an internal molecular oscillator, a clock, which is synchronized, entrained, in response to environmental signals, the Zeitgeber (German for time-giver). The pioneering genetic work of Konopka and Benzer in Drosophila led to the discovery of numerous genes involved in the control of circadian rhythms. Genetics in combination with recombinant DNA technology and biochemistry, led to the isolation of the first clock genes and to the elucidation of the mechanisms of the biological clocks (for review, see Ref 12).

Circadian Oscillators in Cyanobacteria

Cyanobacteria such as Synechococcus have an endogenous timing mechanism that can generate and maintain a 24 h (= circadian) rhythm controlling expression of the entire genome (see Ref 13 for review). This rhythmicity extends to many other physiological functions. The periodicity of these processes reflects the periodicity of sunlight, the primary source of energy for these photoautotrophic organisms.

The basis of the circadian rhythm is a timing mechanism called circadian clock. The timing mechanism must keep time and continue to drive oscillatory behaviors even under constant environmental conditions.

The best studied model organism is the photoautotrophic cyanobacterium Synechococcus elongatus. To synchronize and reset the circadian clock, the cultures are kept in the dark for 6–12 h before exposing them to constant illumination. Timing under these constant conditions is designated as a ‘free-run’. The period of any oscillation during the free-run is called the free-running period. The first half of the free-running period is the subjective day, the latter half the subjective night. In addition to free-running self-sustained oscillations, true circadian clocks can be
entraîné. L’entrainement est le processus par lequel le cycle lumineux–nocturne détermine la période et la phase d’une oscillation autrement auto-sustainnée. L’entrainement nécessite un mécanisme de perception de la période environnementale (lumineux–nocturne) i.e. une forme de perception lumineuse. Un oscillateur circadien authentique doit également compenser les changements de température tels que la période libre dépendante constant du domaine de température physiologique.

La méthode la plus avancée dans l’étude de l’oscillateur circadien a été effectuée dans S. elongatus, une cyanobactérium qui est amenable à l’analyse moléculaire. Premièrement, un grand ensemble de gènes circadiens a été isolé en utilisant une lignée reporter qui transporte une luciférase attachée à un promoteur de photosystème II. Les gènes circadiens ont été identifiés et caractérisés biochimiquement. L’oscillateur circadien peut être divisé en trois composants: l’oscillateur qui génère la périodicité de base, l’entrée par voie qui transmet les signaux de l’environnement au oscillateur (synchroniser) l’oscillateur, et l’entrée par voie qui transmet l’information temporelle de l’oscillateur à diverses fonctions cellulaires, comme la transcription de gènes. L’oscillateur cyanobactérium a été identifié16 et reconstruit in vitro.17

Les gènes circadiens (oscillateur circadien) encodent trois gènes contigus, KaiA, KaiB, et KaiC, et ces gènes sont exprimés à partir de deux promoteurs: KaiA a son propre promoteur, whereas B et C sont transcrits comme un message dicistronique à partir d’un promoteur immédiatement en amont de KaiB. Les informations de transcription ne sont pas nécessaires pour le fonctionnement circadien. Une séquence de processus de phosphorylation de KaiC forme la base de l’oscillateur circadien. Le cycle de phosphorylation consiste en quatre étapes (1) phosphorylation de threonine (T432), (2) phosphorylation de serine 431 (S 431). Ce dual phosphorylation convertit KaiC en un autokinase à dephosphorylation de T432 et (4) dephosphorylation de S431 et reconversion de l’oscillateur circadien in vitro. Nakajima et al.17 réussirent à reconstituer un oscillateur circadien de KaiC par phosphorylation in vitro de KaiA, KaiB, KaiC, et ATP. So far, a complete reconstruction of the oscillator clock in vitro has only been accomplished in cyanobacteria.

Bien que le mécanisme d’oscillation ait été élucidé, notre compréhension des chemins d’entrée et de sortie est toujours limité. En particulier, le photorécepteur moléculaire doit être identifié. Sur le côté d’entrée, CikA a été identifié comme un composant de base pour fournir des informations environnementales.

### The Circadian Clock in Plants

Le cycle circadien dans les plantes régule un grand nombre de processus biologiques, tels que les mouvements rythmiques de la tige, les végétations des tiges, les mouvements de la tige de l’hypocotyle, et les mouvements de la tige de l’hypocotyle. Le cycle circadien est utilisé pour mesurer les informations environnementales contre les pathogènes (voir Ref 21 pour revue). Les phénomènes coor- dent la floraison lors de la saison appropriée (photopériode) pour induire des méristèmes, de sorte que la floraison se déroule lors de la saison correcte (photopériode florale).22 Ces phénomènes contribuent à l’augmentation de la fiabilité et donc, conférer un avantage sélectionnel.

Les gènes circadiens sont responsables des phases matinal, de la veille et du coucher, qui contribuent à une plus grande performance et donc, conférer un avantage sélectionnel.20

Les gènes circadiens de *Arabidopsis* ont été isolés par une approche génétique et il a été clairement démontré expérimentalement que des expériences de compétition qui ressonnent les oscillateurs circadiens confèrent un avantage sé- lectionnel.18,19

Sur le côté d’entrée, SasA, un kinases histidinique, interagit directement avec l’oscillateur KaiC et active le facteur de transcription RapA, qui peut activer tous les promoteurs dans le génome de *Synechocystis*. RapA est en retour négativement contrôlé par LabA pour contrôler KaiBC dans une façon circadienne.18,19

Le question de savoir si le cycle circadien offre un avantage sé- lectionnel a été approché expérimentalement et il a été clairement démontré par compétition des expériences qui ressonnent les oscillateurs circadiens confèrent un avantage sélectionnel.20

Les phénomènes co-or- dentnt contribuent à l’augmentation de la fiabilité et donc, conférer un avantage sélectionnel.

Le cycle circadien de *Arabidopsis* a été isolé d’un nombre substantiel de gènes circadiens (voir Ref 21 pour revue). Cependant, ces gènes sont d’un type différent de ceux des cyanobactéries. Ils contiennent en fait des séquences cryptiques et les plantes, le cycle est utilisé pour mesurer les informations environnementales photothorpe d’induction des méristèmes, de sorte que la floraison se déroule lors de la saison correcte (photopériode florale).22 Ces phénomènes contribuent à l’augmentation de la fiabilité et donc, conférer un avantage sélectionnel.

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Une approche génétique a conduit à l’isolement d’une substance de nombre de gènes circadiens (voir Ref 21 pour revue). Cependant, ces sont un peu différents de ceux des cyanobactéries. Ils génèrent un feedback cycle composé de transcriptions répressrices: protéines de phase matinale, protéines de phase nocturne, et protéines de phase intermédiaire. La oscillation circadienne est dépendante de similar decay rates for mRNAs and proteins, and large amounts of proteins at their peak level. Whether a posttranslation oscillator as found in cyanobacteria functions also in plants is an open question.

A genetic approach also led to the isolation of a cryptochrome blue light receptor.23 *Arabidopsis* seedlings grown under light have a shorter hypocotyl than seedlings grown in the dark; this response is mediated by blue (420–500 nm), red, or far-red (700–750 nm) light. Certain *by* mutants have
selectively lost the capacity to respond to certain portions of the spectrum. The cry4 (= cry1) mutant has selectively lost its response to blue light. The isolation and sequencing of the cry1 gene showed that it encodes a protein with sequence similarity to DNA photolyases. The CRY1 protein was shown to be a flavoprotein, but it lacks photolyase activity and is the long-sought blue light receptor and was named cryptochrome 1 (CRY1). Subsequent experiments by Somers et al. showed that it functions in the entrainment of the circadian clock of Arabidopsis. Since then cryptochromes have been found in many flowering plants and also in ferns and Chlamydomonas algae. They also play a major role in animals.

Circadian Oscillators in Fungi

Our knowledge about the circadian clock in fungi is again limited to model organisms which are amenable to molecular genetic analysis, in this case, mostly Neurospora (see Ref 25 for review). Like in cyanobacteria the biological clock consists of interconnected positive and negative feedback loops: The GATA-type transcription factors WC-1 and WC-2 assemble to form the White-Collar complex (WCC), which activates the clock gene frequency (frq). FRQ protein then inhibits the activity of WCC and thereby feeds back on its own synthesis. Owing to this feedback, regulation frq RNA and FRQ protein display high-amplitude circadian abundance rhythms constituting the biological clock. In an interconnected positive feedback loop, FRQ supports accumulation of high levels of WCC at the posttranslational level. The negative feedback loop takes place in the nucleus, whereas the positive loop is confined to the cytosol. The maturation of FRQ from a nuclear repressor toward an inactive cytoplasmic repressor is due to phosphorylation by casein kinase 1a. In the cytoplasm, WCC becomes dephosphorylated and reactivated by the protein phosphatase PP2A.

On the input side, the WCC is directly activated by light, which resets the clock. WC-1 protein binds the flavin (FAD) as a chromophore and serves as a circadian blue light receptor. In addition, a hierarchical cascade of transcription factors activates light-induced transcription of hundreds of target genes. To prevent disturbances of the clock by changes in light intensity during the day and to ensure proper synchronization, the cells are desensitized to ambient light by photoadaptation. In Neurospora, photoadaptation depends on the blue light receptor vivid (VVD) which accumulates immediately after light activation and rapidly silences the expression of WCC-dependent genes.

Although some of these mechanisms are reminiscent of the cyanobacterial clock, the clock genes are completely different suggesting that the clock may have evolved several times separately.

The Biological Clocks in Animals

We shall limit the discussion of animal clocks to Drosophila and the mouse, the two model organisms which are best studied (see Ref 29 for review). The isolation of mutants in the period (per) gene of Drosophila provided the first evidence that daily rhythms in the sleep-wake cycle of animals are genetically controlled. One mutation (per^short) reduced the period of activity rhythm, whereas per^long prolonged the period of activity, and a third mutation per^01 abolished the locomotor rhythm altogether. It took another 15 years until the per gene was cloned and available for molecular analysis. showed that the per mRNA levels are cycling in circadian rhythm, and that the cycling was advanced in the per^short mutant, whereas it was delayed in per^long flies indicating that the PER protein must feed-back to regulate its own expression.

During the past 20 years research on the clock in Drosophila and in parallel in the mouse has revealed that the clock mechanism is remarkably conserved between insects and mammals, including humans.

The regulatory scheme of the molecular clock is outlined in Figure 1. It is basically a bimodal switch of two negative feedback loops. The cycle is driven by a heterodimer protein CLOCK-CYCLE (CLK-CYC). This core heterodimer coordinates the antiphasic expression of two sets of genes, the morning and the evening genes that generate a bimodal switch of two negative feedback loops. The cycle is driven by a heterodimer protein CLOCK-CYCLE (CLK-CYC). This core heterodimer coordinates the antiphasic expression of two sets of genes, the morning and the evening genes that generate a bimodal switch which is self-sustaining and takes approximately 24 h (circadian) to complete. During daytime the heterodimer CLK-CYC is active and the E-box genes (including per, tim, vri, pdp-1e, and cwo) are switched on, whereas the V/P-box genes (including Clk and Cry) are turned off due to rapid accumulation of the VRI-repressor (see Figure 1). At night, when CLK-CYC is inactive, E-box genes are turned off, because the PER-TIM heterodimer blocks CLK-CYC and direct repression of E-boxes by CWO, whereas V/P-box genes are switched on by PDP1e which replaces VRI repression. The activity of clock proteins is modulated by phosphorylation by protein kinases leading to protein degradation, whereas protein phosphatases stabilize the respective proteins. The phosphorylation status also guides the subcellular localization of the clock proteins which accumulate either in the nucleus or the cytoplasm.

Light input to the clock is mediated by the blue-light receptor protein called Cryptochrome

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The central clock cells are neuronal cells, i.e. photoreceptor cells and nine groups of specific interneurons in the brain. However, there are also non-neuronal clock cells (glial cells, cells of the Malpighian tubules, and the rectum). There are also clock cells in the abdomen, thorax, legs, wings, antennae, proboscis, ocelli, retina, and brain. These peripheral clock cells also synchronize with the external light–dark cycle, which suggests that they all express cryptochrome. However, those neurons which do not express cryptochrome (CRY?) and cannot synchronize with the external light–dark cycle, receive light input from photoreceptor cells in the eyes and ocelli. 

The biological clock mechanism in mammals is remarkably similar to that of Drosophila. Many of the core factors of the clock are conserved between Drosophila and mouse. The mammalian clock also consists of a bimodal switch (interlocked-feedback-loop). However, during vertebrate evolution two genome duplications have occurred, so that several clock genes have been duplicated. For example, there are two mammalian Clock (Clk) genes, and two cyc genes, whose protein products form heterodimers and activate E-box genes. There are also two cryptochrome genes (Cry1 and Cry2) whose protein products no longer respond to light. The only clock cells that receive light input are located in the suprachiasmatic
nuclei (SCN) of the brain. Light input to the SCN comes from a specialized group of Melanopsin-expressing retinal-ganglion cells in the eye.\textsuperscript{35,36} In response to light, these retinal ganglion cells switch the \textit{Per} genes on in SCN neurons.\textsuperscript{37} Increased levels of Per-Cry reset the circadian clock. The most striking difference between \textit{Drosophila} and mouse involves feedback that generates the interlocked-feedback-loop, which involves a different class of genes than those of \textit{Drosophila}. However, the conservation of the clock mechanisms between insects and mammals is quite remarkable.

Comparing the clock mechanisms of cyanobacteria and plants, fungi, and animals, we have to assume that there have been multiple circadian clock origins during evolution.\textsuperscript{12} However, there are also highly conserved elements, particularly between insects and mammals. Also, the use of cryptochrome for resetting the clock is very ancient and has been substituted by melanopsin only in mammals. We shall trace cryptochrome evolution further in the discussion of visual pigments.

THE EVOLUTION OF PHOTOTAXIS AND PHOTOTROPISM

The second line of evidence that cyanobacteria can detect light comes from studies of phototaxis and comparative genomics.

Phototaxis\textsuperscript{38} is a locomotory movement of a whole organism in response to a light stimulus. Movement toward the light is called positive phototaxis, whereas avoidance of illumination is designated as negative phototaxis. In sessile organisms like plants and fungi, the term phototropism is used for the corresponding light response, meaning that growth is determined by the direction of the light source. In both cases, a mechanism for sensing light is required.

Some prokaryotes are phototactic. They often use a random walk strategy, using a type I sensory rhodopsin for light sensing and a two-component signaling system to induce a reversal of the flagellar beat. This strategy only allows phototaxis along a steep light gradient, as found in microbial mats or sediments. Some filamentous bacteria have evolved the ability to steer toward a light vector. This allows them to navigate in two dimensions, gliding on a surface.

In contrast, eukaryotes have evolved the capacity to follow a light vector in three dimensions in open water. This capability requires a polarized organism with a defined shape, helical swimming driven by cilia, and a shading device for the light sensor which allows the organism to sense the direction of light. Such an eye spot or prototypic eye confers a considerable selective advantage, and precedes the evolution of more sophisticated eye-types.

Phototaxis in Cyanobacteria

Cyanobacteria, like \textit{Synechococcus} and \textit{Anabaena} can slowly orient along a light vector.\textsuperscript{39} This phototactic orientation occurs in filaments or colonies. It is a slow gliding motility, a uniform motion on a solid surface in a direction parallel to the cell’s long axis and occasionally interrupted by reversals.\textsuperscript{40} The unicellular cyanobacterium \textit{Synechocystis} sp. PCC 6803 shows gliding motility on agar plates or glass slides, a small and intermittent translocation on a solid surface, in which the direction of movement often changes. This species can be transformed genetically and a number of genes involved in phototactic motility have been identified.\textsuperscript{41,42} Wildtype \textit{Synechocystis} cells form flat sheet-like irregularly shaped colonies which can move on solid surfaces. Mutant cells with disrupted \textit{pil} genes involved in the formation of type IV pili form round colonies and have lost motility. Type IV pili are 5–7 nm in diameter and several micrometers in length and are involved in several functions, including gliding movement, phototaxis, and DNA transfer to other cells.

The action spectra for phototaxis are quite complex, depending on the species of cyanobacteria used. However, in most species they overlap with the absorption spectra of phycobilins, which serve as chromophores in phytochromes. In \textit{Thermosynechococcus}, the action spectrum shows several peaks at 530, 570, 640, and 680 nm, but at higher fluence rates the red action peak (at 640–680 nm) disappeared and far-red peaks at 720 and 740 nm emerged. When cells are illuminated simultaneously with red and far-red light, phototaxis is drastically reduced, which also suggests that phytochromes are involved.\textsuperscript{43} Two classes of cyanobacterial phytochromes have been found and were designated as \textit{cyanobacteriochromes} classes 1 and 2. In \textit{Synechococcus}, mutations in the PixJ1 mutations in the PixJ1 (= Tax1) gene which abolish positive phototaxis, but show instead negative phototaxis, have identified a photoreceptor for phototaxis.\textsuperscript{44} It contains GAF and PAS domains as they are typically found in phytochromes, which are the major red/far-red light receptors in plants (see below). It has been shown that the Tax1 homolog of \textit{Thermosynechococcus} is localized at the poles of this rod-shaped cyanobacterium, which suggests that it may be involved in sensing the light direction.\textsuperscript{43} Negative phototaxis is controlled by another cyanobacteriochrome locus\textsuperscript{42} and two adjacent response regulator loci which together encode
a UV-A-activated signaling system. The cyanobacteriochrome receptor gene encodes a protein with three transmembrane domains two PAS, a GAF, and a histidine kinase domain, which is characteristic for phytochromes. Illumination by UV-A light (360 nm) induces strong negative phototaxis which represents a UV-avoidance reaction (see also cryptochromes). In cyanobacteria, cyanobacteriochromes have undergone adaptive radiation and at least 41 different genes have been identified encoding these phytochromes involved in light perception. Whole genome sequencing of other cyanobacteria has identified several other photoreceptors: In *Anabaena*, one sensory rhodopsin (a green light receptor) has been found and two Cryptochromes/Photolyases (putative blue light receptors). *Gloeobacter* has a photorhodopsin gene closely similar to that found in dinoflagellates (see Russian Doll Hypothesis). *Gloeobacter* also has a cryptochrome-like gene, similar to animal cryptochromes which are related to (6-4) photolyases (see cryptochromes).

**Phototaxis in Halophytic Archaea**

Archaea like *Halobacterium salinarum* have sensory rhodopsins for phototaxis. Rhodopsins are seven transmembrane proteins which bind retinal as a chromophore. Light triggers the all-trans/cis isomerization of retinal, which leads to phototransductive signaling through a two-component phosphotransfer relay system. In *H. salinarum* two types of rhodopsins, sensory rhodopsin I (SRI) and SRII have been found, which signal via the halobacterial transducer proteins Htrl and Htrl. Signaling in phototactic archaia involves CheA, a histidine kinase, which phosphorylates the response regulator CheY. Phosphorylated CheY induces swimming reversals. SRI functions as an attractant receptor for orange light and a repellent receptor for near-UV light, whereas SRII is repellent receptor for blue light. This mechanism of phototaxis only works with a steep light gradient.

**Phototaxis in Algae and Protists**

*Red algae* lack cilia in all stages of their life cycle and therefore, the ability of helical swimming. Also, they lack eye spots (stigmata) and as a consequence are incapable to perform three-dimensional phototaxis. However, they find their optimal light conditions by surface gliding and two-dimensional phototaxis as described for *Porphyridium*. *Green algae* (*Chlamydomonas*) have a photoreceptor organelle called eye spot or stigma located in the outermost portion of the chloroplast, directly underneath the two chloroplast membranes indicating that it is derived from cyanobacteria (see unicellular photoreceptor organelles). Indeed, stigma-like structures have been found in certain cyanobacteria (see: The Origin of vision: cyanobacteria). The stigma consists of tens to hundreds of lipid globules containing carotenoid pigments which provide a screening function, shielding the light from one side and serving as a light reflector (Figure 2). The stigma is not to be mistaken for the photoreceptor. The stigma only provides directional shading for the adjacent membrane-bound photoreceptors. Stigma can also reflect and focus light onto the photoreceptors like a concave mirror, thereby increasing sensitivity (Figure 2). Carotenoids can also serve as light antennae: Xanthorhodospin of the eubacterium *Salinibacter ruber* contains a single energy-donor carotenoid (sali-xanthin) noncovalently bound to a small 25 kDa membrane protein with a single retinal acceptor.

In the best studied green alga *Chlamydomonas reinhardtii* phototaxis is steered by a rhodopsin pigment. This was first shown by restoring phototaxis in blind mutants by retinal analogs. Genomic sequencing revealed two Channelrhodopsins-1 and 2. These proteins have an N-terminal 7-transmembrane domain similar to archaeabacterial proteorhodopsin and a C-terminal membrane-associated domain. They act as light-gated cation channels and trigger depolarizing photocurrents. Channelrhodopsin-1 was shown to localize in the stigma region by immunofluorescence studies.

*Dinoflagellates* have evolved the most sophisticated photoreceptor organelles starting out from relatively simple stigmata with caroten-containing vesicles to some species like *Cryptoperidinium foliaceum* with lamellar bodies consisting of regularly stacked membranes strikingly resembling mammalian rod and cone cells. Some dinoflagellates like *Warnowia* and *Erythropsis* even have lenses for focusing the incoming light to a retina-like structure, the retinoid (see Unicellular Photoreceptor Organelles). *Dinoflagellates* possess two cilia, a transverse cilium in the groove around the equator of the cell and a longitudinal one inserting directly behind the eye organelle, serving as a steering cilium. Genomic studies have identified a proteorhodopsin (type I rhodopsin) in *Pyrocystis lunula*, in *Oxyrrhis* (Ernst Bamberg, personal communication) and in *Erythropsis* (Suga et al., unpublished data) which are closely related to cyanobacterial proteorhodopsins from *Gloeobacter* indicating that the eye organelle is indeed derived from their chloroplasts.

*Euglena* belongs to a diverse group of biciliated protists which have taken up a secondary plastid by endosymbiosis from an algal prey. They are autotrophic, also phototactic and have
The eyespot of *Chlamydomonas*. (a) A *Chlamydomonas* cell with two flagella, a large green chloroplast and a yellow-orange eyespot (eye organelle). (b) Function of the eyespot, Channelrhodopsin 1 (ChR1) is a light-gated proton channel located in the plasma membrane above the pigment spot. The entering protons diffuse laterally and activate a voltage or proton-gated Ca$^{2+}$ channel (VGCC). Channelrhodopsin 2 (ChR2) is conductive for H$^+$, Na$^+$ and Ca$^{2+}$. The voltage change $\Delta \Psi$ is transmitted along the membrane and sensed by the VGCC channels in the flagellar membrane. A sudden Ca influx induces a switch of flagellar motion. The carotene reflects the light to activate the ChRs.54 (Reprinted with permission from Ref 54. Copyright 2004 American Physiological Society)
The discovery of additional mad mutants and their detailed characterization led to the identification of 10 unlinked genes, mad A through mad I.72,73 Mutants of mad A and mad B are defective in phototropism and other light responses suggesting that their gene products play key roles in Phycomyces photobiology.70

Most of our understanding of fungal photobiology comes from Neurospora (see above). Mutations in wc-1 and wc-2 disrupt all the responses of Neurospora to blue light. The WC-1 protein contains a zinc-finger motif, a chromophore-binding domain (called LOV) and PAS domains for protein–protein interactions.27 The LOV domain binds the flavin chromophore (FAD) allowing WC-1 to function as a circadian blue light receptor.

In Phycomyces madA encodes a protein similar to the Neurospora blue light receptor WC-1, and madB corresponds to WC-2. The proteins MAD A and MAD B also contain a zinc-finger motif and can interact to form a complex. These findings indicate that phototropism and other responses to light in Phycomyces are mediated by a photoresponsive transcription complex as in Neurospora.74

Phototaxis in Metazoa

Phototaxis in Sponges
In the demosponge Reneira75 the embryos develop in brooding chambers inside the sponge. The chambers contain 20–150 embryos which develop into larvae. The larvae have an outer layer of monolayered cells possessing 20 μm long cilia, but there are two protruding bare patches of cells at both poles of the larva. At the posterior pole there is a ring of pigmented cells bearing much longer (120–150 μm) cilia, which are thought to be the photoreceptor cells with steering cilia. The larvae are released from the brooding chamber and in the presence of light, swim continuously forward rotating clockwise (as observed from the posterior end of the larva). Upon release, the larvae swim first upward from the adult sponge, but they are negatively phototactic until at least for 12 h after release. A sudden increase in light intensity causes the long cilia to freeze which presumably allows the larval to steer away from areas of bright light toward darker areas, to settle down and form a new sponge underneath the corals in the reef. Although all other metazoan phyla have rhodopsin as photoreceptor pigment, the genome of the sponge Amphimedon queenslandica76 does not contain a rhodopsin gene, and searches for rhodopsin genes in the marine sponge Microciona as well as the freshwater sponge Ephydatia were negative.77 The action spectrum of photosensitivity of Reneira75 has a maximum in blue light (440 nm) and a smaller, secondary response peak (at 600 nm) in orange-red light. This suggests that the photoreceptor pigment in sponges may be a flavin or carotenoid.

This is in line with findings in adult Demospongiae (Suberites domuncula)78–80 which have identified cryptochrome as photoreceptor pigment based on the light-reactive behavior of these siliceous sponges. They have identified an intriguing quartz glass-based system of spicules, which can generate light enzymatically with a luciferase; the spicules serving as optical waveguide and cryptochrome as photosensor. This suggests that also the larvae of demosponges use cryptochrome as a photoreceptor. Cryptochrome does have a flavin chromophor as suggested by the spectral analysis. Whether sponges never had a rhodopsin gene or whether they have lost it, remains an open question.

Phototaxis in Cnidarians
In the planula larva of the box jellyfish Tripedalia the most primitive kind of ‘eye’ has been found in the form of single pigmented photoreceptor cells which are interspersed between the ciliated epithelial cells covering the larva81 (Figure 2). These photoreceptor cells are also carrying a flagellum (cilium) and microvilli on their surface and they contain pigment granules. The microvilli presumably contain rhodopsin and the flagellum is involved in steering the larva which is phototactic. The structure of these cells suggests that they are the most primitive rhabdomeric photoreceptors, whereas the adult box jellyfish has highly evolved eyes with multiple photoreceptor and pigment cells, as well as lenses. The photoreceptors of the adult are of the ciliary-type (see below). In adult Tripedalia, the eyes are integrated together with a statocyst (gravity sensory organ) into so-called rhopalia, batteries of sensory organs. Several lens and slit eyes are combined with a statocyst in the four rhopalia, one on each side of the box jellyfish. The sensory input is integrated by nerve connections,82 which represent the first stages of brain evolution. Therefore, we can assume that the eye as a sensory organ has evolved before the brain, providing sensory information which subsequently is processed by the brain.4,83

Phototaxis in Bilateria
As in cnidarians the larvae of bilateria show the most primitive optical apparatus. Trochophora larvae of the marine annelid Platynereis possess a pair of prototypic eyes consisting of two cells only, a photoreceptor cell and a shielding pigment cell.84 These simple eyes mediate phototaxis. Selective illumination of one eye changes the beating of adjacent cilia resulting in a locally reduced water flow. Similar to Chlamydomonas, the Platynereis larvae swim in
a right handed helix, i.e. in a clockwise forward movement, rotating around their antero-posterior axis. The shielding by the pigment cells results in an on–off illumination of the photoreceptor cell, if the larvae swims at 90° to the incident light, and a constant illumination, if it swims toward the light source. This allows the larva to determine the direction of the light source, which confers a considerable selective advantage. For negative phototaxis, shielding by the pigment cell seems not to be required. The eye spots of unicellular organisms have to be considered as cellular organelles, whereas these larval photoreceptors have to be designated as eye organs because they consist of at least two cell-types a photoreceptor cell and a pigment cell. These prototypic eyes mediate phototaxis as shown by laser ablation experiments. Ablation of both eyes leads to a complete loss of phototaxis, whereas larvae in which only one eye was ablated are still capable of swimming toward the light in most cases.

A first step of cellular differentiation leads to the formation of a pigment cell and a photoreceptor cell. The photoreceptor is a sensory-motor neuron which regulates phototactic steering by acetylcholine and is connected via its axon to two ciliated cells which contain acetylcholine receptors. Therefore, phototaxis in Platynereis larvae is due to direct sensory-motor coupling between the photoreceptor cell and the locomotor ciliary cell. In Tripedalia jellyfish larvae, light detection and ciliary locomotor output are still combined in a single cell (see above). In adult Platynereis, the eyes are much more elaborate and the sensory-motor neurons differentiate into sensory and motor neurons and are connected by interneurons which eventually become integrated in the brain. Subsequently, the brain plays an increasingly important role in processing the visual information.

PHOTOSENSORY PROTEINS

Phytochromes

The red/far-red regulators of photomorphogenesis in higher plants are called phytochromes (see Ref 85 for review). Phytochromes share a conserved photosensory protein core which comprises a PAS domain, a GAF domain, and a Phy domain to which a linear tetrapyrrole chromophore bilin is covalently linked. There are three major bilins: phycocyanobilin (PCB), phytochromobilin (ΦΦB), and biliverdin (BV). Phytochrome-related photosensory proteins have not only been found in higher plants, but also in cyanobacteria, nonoxygenic photosynthetic bacteria, nonphotosynthetic bacteria, unicellular algae, diatoms, and fungi. The bilin chromophore is covalently attached to a conserved cysteine residue in the phytochrome apoprotein. Organisms using PCB or ΦΦB have a conserved cysteine in the GAF domain, whereas those using BV have a conserved Cys N-terminal to the PAS domain. Plant and some algal phytochromes lack a specific histidine residue which serves as a phosphorylation site for bona fide histidine protein kinases which are involved in signaling. The photoswitching arises via photoisomerization of the bilin chromophore about its 15/16 double bond, followed by a protein–chromophore relaxation process that shifts the absorbance spectrum further. In most phytochromes, the photoproduct is metastable and slowly reverts to the stable dark state. This process is known as dark reversion. The conformational changes occurring upon photoisomerization alter the chromophore–protein interactions, which trigger signaling. Phytochromes typically have a C-terminal histidine protein kinase domain.

Light-induced protein kinase activity was first reported in cyanobacteria. In Synechocystis, it has been shown that the pixJ1 gene which encodes a phytochrome-like photosensory protein is essential for phototaxis. Site-directed mutagenesis revealed that a Cys-His motif in the second GAF domain binds a linear tetrapyrrole chromophore characteristic for phytochromes. PixJ1 protein showed a photoreversible conversion between a blue light-absorbing form and a green light-absorbing form, which in the dark reverts to the blue light-absorbing form. In contrast to the plant phytochromes, red or far-red light irradiation produced no change in the spectra of PixJ1. Therefore, this and other cyanobacterial phytochromes have been named cyanobacteriochromes.

Cryptochromes

The power of Arabidopsis genetics led to the first isolation of a cryptochrome blue light photoreceptor protein. Arabidopsis seedlings grown under light have a shorter hypocotyl than seedlings grown in the dark. This response can be mediated by blue (420–500 nm), red (600–700 nm), or far-red (700–750 nm) light. Certain Arabidopsis mutants have lost the capacity to respond to blue light (cry 1−) or to red light (PhyB−), respectively. The Cry1 gene was isolated and shown to be a flavoprotein with sequence similarity to DNA photolyase, but it lacked detectable photolyase activity and it contained a distinct C-terminal extension (Figure 3(a)). On the basis of these properties, it was concluded that Cry was the long-sought blue light receptor and was named cryptochrome 1. Cryptochromes mediate various light responses, including the entrainment of circadian
The Evolutionary Origin of a Novel Gene Encoding Cryptochrome

One of the fundamental problems in evolutionary biology concerns the origin of genes with a novel function. The cryptochromes allow us to address this question in a profound way.\(^9^0\) There at least two strong evolutionary selective driving forces for the evolution of photosensory proteins; sunlight as an energy source, especially for autophototrophic organisms, and UV avoidance to prevent DNA damage. Geological studies show that there was little oxygen in the atmosphere at Precambrian times before the evolving autophototrophic plankton generated large amounts of oxygen as a by-product of photosynthesis. Therefore, there was no protective ozone layer, and primitive organisms were exposed to heavy doses of UV irradiation during daytime. UV irradiation causes DNA damage mainly by inducing cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone adds between adjacent pyrimidine bases on the same DNA strand which lead to mutations. Bacteria have evolved DNA repair enzymes called DNA photolyases that are capable of repairing these photoproducts. Photolyases are flavoproteins mediating DNA repair in a light-dependent manner.\(^9^2\) They contain a flavin adenine dinucleotide (FAD) as a catalytic chromophore and a second chromophore which is either methenyltetrahydrolfalen (MTHF) or deazaflavin (7,8-didemethyl-8-hydroxy-5-deazariboflavin; 8-HDF) which serves as a light-harvesting antenna. Only the fully reduced form (FADH\(^{−}\)) of flavin is catalytically active. During light activation, light energy is mainly absorbed by the light-harvesting chromophore. After light absorption by the light-harvesting chromophore, the excitation energy is transferred to the fully reduced flavin chromophore by resonance transfer. An electron is then transferred from high-energy FADH\(^{−}\) directly to the pyrimidine dimer, which splits the cyclobutane ring and restores the two pyrimidine monomers. There are two classes of photolyases (types I and II) which repair cyclobutane pyrimidine dimers (CPD) and another class involved in the repair of (6-4) photoproducts first identified in *Drosophila*.\(^9^3\) The fact that photolyases are activated by blue light may not be coincidental, because only blue light reaches substantial depth in water.

Photolyases and cryptochromes not only share extensive sequence similarity, but they also have very similar 3D structures,\(^9^1\) the only difference is the extended C-terminal part of cryptochromes (Figure 3(a)). Photolyases are found in almost all species of prokaryotes and eukaryotes, whereas cryptochromes are present in higher plants and most animals, but only in a few other eukaryotes and prokaryotes, suggesting that photolyases are ancestral to cryptochromes. The phylogenetic analysis (Figure 3(b)) indicates that the classic plant cryptochromes (green) which have lost photolyase activity have evolved from CPD-photolyases (classes I and III), whereas DASH (*Drosophila, Arabidopsis, Synechocystis, Homo*) cryptochromes are more closely related to class II CPD-photolyases. Interestingly, DASH cryptochromes have retained photolyase activity, but they can repair only single-stranded DNA, and not double-stranded DNA substrates, whereas classic plant and animal cryptochromes have lost DNA repair activity completely. Animal cryptochromes (pink and violet) are more closely related to 6-4 photolyases. The tripartite structure of the phylogenetic tree suggests that the transition from photolyases to cryptochromes has occurred three times during evolution and that DASH cryptochromes are in a transition stage because they function both as blue light receptor and a DNA repair enzyme, because they have partially retained DNA repair activity. Mammalian cryptochromes have lost their photoreceptor activity and retained only their function as transcriptional regulators. Whereas rhodopsin, the most common photoreceptor in animals, is localized in membranes at the periphery of the photoreceptor cells, cryptochromes are localized in the nucleus, because they are derived from DNA-repair enzymes, and they were converted to transcriptional regulators which have to be active in the nucleus. Therefore, a gene encoding a DNA-repair enzyme has evolved into a gene encoding a blue-light photoreceptor, and finally a transcription factor.

Magnetoreception by Cryptochromes

In addition to its function as a blue light/near UV photoreceptor in growth regulation, flowering, circadian clocks etc., there is some recent evidence that cryptochromes are also involved in magnetoreception in both animals and plants. A large variety of animals has the ability to sense the geomagnetic field and use it as a source of directional information, as a magnetic compass. The biophysical basis of magnetoreception is not known, but a specific model for magnetoreception in birds has been proposed.\(^9^4\) Behavioral experiments indicated that the magnetic compass of migratory birds is an inclination compass, which is sensitive to the axis, and not to the polarity of the magnetic field lines.\(^9^5,9^6\) Magnetic compass
**Figure 3** | Phylogenetic tree of the family of cryptochromes/photolyases. Upper panel: Domain structure of cryptochromes and photolyases from *Escherichia coli* and *Arabidopsis thaliana* (At), respectively. The N-terminal domain which binds two chromophores methenyltetrahydrofolate (MTHF) and flavin adenine dinucleotide (FAD) shown in yellow is highly conserved among all classes of cryptochromes and photolyases. By contrast, the C-terminal extension (black) is variable and not found in photolyases. Lower panel: Phylogenetic tree of cryptochromes/photolyases. Green: plant cryptochromes. Pink and violet: two groups of animal cryptochromes. Blue: DASH cryptochromes and some related proteins. Red, brown, and gray: CPD photolyases classes I, II, and III. An asterisk (*) marks dual-type belonging to either class I CPD or (6-4) photolyases capable of DNA repair, but they also possess photoreceptor activity. CPD, cyclobutane pyrimidine dimer; DASH, *Drosophila*, *Arabidopsis*, *Synechocystis*, Homo. (Reprinted with permission from Ref 91. Copyright 2011 Annual Reviews Inc)
orientation is dependent on the wavelength of ambient light. Orientation is possible in blue and green light, but under yellow-orange-red light the birds are disoriented. Neurophysiological experiments also indicate that magnetoreception depends on light and an intact retina. The model proposed by Schulten et al. assumes that magnetoreception involves radical-pair processes that are governed by anisotropic hyperfine coupling between (unpaired) electrons and nuclear spins. They show theoretically, that the Earth’s magnetic field strength (~0.5 G) can produce significantly different reaction yields for different alignments of the radical pairs with the magnetic field. However, to function as magnetic compass sensors absorption of blue light must produce radical pairs with lifetimes in the range of milliseconds. Using cryptochrome 1a from the migratory garden warbler, it can be shown that these flavoproteins indeed form radicals with lifetimes in the range of msec, when excited by blue light.

In Arabidopsis, it has been shown that an increase in intensity of the ambient magnetic field enhances hypocotyl growth inhibition under blue light when cryptochromes serve as photoreceptors, but not under red light when phytochromes are the active photoreceptors. In Cry− mutants lacking cryptochromes no increase in growth inhibition is observed.

In Drosophila cryptochromes are also implicated in magnetoreception. In a binary-choice T-maize behavioral assay in which a magnetic field is produced on one side, whereas the other side has no exogenous magnetic field, the flies can be entrained to find their food on one side of the maze under full-spectrum light (300–700 nm), but not if the UV A/blue parts of the spectrum (<420) are blocked. Cryptochromes (Cry−) deficient mutants do not show any responses to the magnetic field under full spectrum lights indicating that Cry+ is required for magnetoreception. These findings are supported by a separate line of evidence: In response to blue light Cry causes a slowing down of the circadian clock of the wake-sleep cycle, ultimately leading to arrhythmic behavior. Exposure of flies to magnetic fields, with a maximum effect at 300μT enhances the slowing down of the clock. This effect of the magnetic field was only present in blue light, but absent in red-light. No effects of the magnetic field were found in Cry− mutants. These genetic experiments clearly implicate cryptochromes in magnetoreception.

With respect to the biophysical basis of magnetoreception the widely held view that radical pairs generated by three tryptophane residues in cryptochromes mediate Cry’s ability to sense magnetic fields, does not seem to be correct. Mutating the tryptophane residues to phenylalanine did not affect the ability of transgenic flies to respond to the magnetic field. However, recent data indicate that magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor.

Rhodopsins

Rhodopsins are membrane proteins with seven helical transmembrane domains which form an internal pocket in which the chromophore retinal is bound. Retinal is bound to the ε-amino group of a specific lysine residue in the α-helix VII forming a protonated Schiff base. The action of light involves specific isomerization of a double bond in the chromophore leading from 11-cis retinal to all-trans retinal in mammalian photoreceptor proteins, and from all-trans to 13-cis retinal in bacteriorhodopsin. This isomerization induces a conformational change of the protein leading to signal transduction. Earlier work on retinylidene proteins has been comprehensively reviewed by Spudich et al. The extensive work on structure and function of rhodopsin by Hubbell et al. The Handbook of Photosensory Receptors is also a most valuable source of information.

Rhodopsins can be subdivided into two large categories, the microbial rhodopsins, a heterogeneous group which includes archaeal light-driven ion pumps and sensory rhodopsins and halorhodopsins, bacterial rhodopsins from uncultured marine bacteria, fungal opsins, rhodopsins from cyanobacteria and dinoflagellates, and channelopsins from green algae. The second large group comprises the animal rhodopsins which are G-protein-coupled receptors (GPCRs), which include mammalian rhodopsin, one of the most extensively studied photoreceptor proteins.

Microbial Rhodopsins

Microbial rhodopsins are phylogenetically and functionally diverse (Figures 4 and 5). An unrooted phylogenetic tree shown in (Figure 4) reflects this heterogeneity.

Archaeal Rhodopsins

Primarily archaeal rhodopsins are ion pumps, either proton pumps or Cl− pumps capable of converting light energy into chemical energy. The resulting proton gradient is used to drive ATP synthesis. These type I rhodopsins are found in all three domains of life suggesting that they existed before the separation of archaea, eubacteria, and eukaryotes. Therefore, light-driven proton transport as a means of procuring energy may have predated the evolution of photosynthesis in cyanobacteria. In fact, the phylogenetic
**FIGURE 4** | Unrooted phylogenetic tree of microbial rhodopsins. Source: Hiroshi Suga.

**FIGURE 5** | Structure and function of various microbial rhodopsins. Proteorhodopsin, halorhodopsin, sensory rhodopsin of *Anabaena* and the two channelopsins function as ion channels, whereas sensory rhodopsin I is coupled to a transducer complex, which transmits the light signal via a histidine kinase and phosphorylates a regulator of the flagellar motor. (Reprinted with permission from Ref 105. Copyright 2005 Wiley-VCH)
analysis suggests that cyanobacteria have acquired rhodopsins by horizontal gene transfer because *Nostoc* resides with archaeal rhodopsins and *Gloeobacter* with the Dinoflagellates which probably took up a cyanobacterium as an endosymbiont which became ‘domesticated’ and turned into a chloroplast (see Russian Doll Hypothesis). In *H. salinarum*, four types of rhodopsins have been characterized: Bacteriorhodopsin which is a light driven proton pump absorbing maximally at $\lambda_{\text{max}}$ 568 nm, which generates a positive outside membrane potential. Halorhodopsin is a chloride transporter which hyperpolarizes the membrane by chloride uptake rather than proton ejection. Sensory rhodopsins, SRI and SRII, are phototaxis receptors controlling the cells motility in response to changes in light intensity and wavelength. SRI has a $\lambda_{\text{max}}$ at 387 nm and induces positive phototaxis (attraction) to orange light and is repelled by near-UV wavelength. SRII appears to have only a repelling function. It absorbs maximally in the spectral peak of sunlight and, therefore, is optimally tuned to efficiently detect the light and guide the cells to darkness under conditions in which photo-oxidative damage might occur. Sensory rhodopsins interact with the HtrI and HtrII transducer molecules that control a histidine protein kinase cascade modulating the flagellar motors. In cells devoid of Htr transducer SRI is reconverted from a sensory rhodopsin into a light-driven proton pump.

**Eubacterial Proteorhodopsins**

More recently, rhodopsins were also discovered in proteobacteria, mainly in marine environments by shot gun random sequencing. In the $\gamma$-proteobacterium SAR86 from surface water of Monterey Bay a rhodopsin functioning as a proton pump was found. In contrast, in the cyanobacterium *Anabaena* (also called *Nostoc*) it has a photosensory function. Also in the deep-sea at 75 m depth a proteobacterial rhodopsin was found with a blue-shifted absorption spectrum ($\lambda_{\text{max}} = 490$ nm).

This blue-shift can be explained by the fact that only blue light penetrates deeply into the water. Recent studies indicate that *Anabaena* photosensory rhodopsin interacts with a tetrameric transducer ASRT (Anabaena Sensory Protein Transducer) that binds to the promotors of several genes related to the utilization of light energy.

**Fungal Rhodopsins**

Homologs of archael rhodopsins are also found in fungi; in ascomycetes like *Botrytis* and in basidiomycetes such as *Ustilago* and *Cryptococcus*. Asp-85, the counterion to the retinal Schiff base is conserved in all fungal rhodopsins homologs except for *Cryptococcus*, which contains an Ala at this position. Genomic sequencing of *Neurospora* revealed the first eukaryotic homolog of archaeal rhodopsin called NOP-1. It is probably serving a sensory function. Neurospora is non-motile, but in the motile fungus *Allomyces* phototaxis of the zoospores was shown to be retinal dependent. However, no animal-type rhodopsin II has been found in fungi.

**Channelrhodopsins in Green Algae**

Positive and negative phototaxis in the green alga *C. reinhardtii* is associated with electrical currents carried by $\text{Ca}^{2+}$ and $\text{H}^+$ which can be measured within $<30\mu$s after flash stimulation in the eye spot (Figure 2). Searching an EST (expressed sequence tag) data base, two cDNA sequences were discovered encoding microbial-type rhodopsins, Channelopsin-1 (Chop-1) and Channelopsin-2 (Chop-2). Expression of the respective RNAs in *Xenopus* oocytes and mammalian tissue culture cells produces light-gated conductance that is highly selective for protons in ChR1, whereas ChR2 generates a large permeability for monovalent and divalent cations. ChR1 is a directly light-gated proton channel, and ChR2 is a directly light-switched cation-selective ion channel. In contrast to the sensory rhodopsins of halorarchaea which are coupled to a transducer a histidine kinase signaling to the flagellar motor (Figure 5), the Channelrhodopsins are not coupled to any transducer, i.e. the Channelrhodopsins are directly light-gated ion channels which open in response to a conformational change of the protein after light-induced isomerization of all-$\text{trans}$ retinal to 13-$\text{cis}$ retinal. This makes them an important tool to activate excitable cells like neurons, by illumination. It is possible to trigger behavioral responses in the nematode *Caenorhabditis elegans* by Channelrhodopsin-2 simply by illumination. Both Channelrhodopsin-2 and the light-driven Cl-pump halorhodopsin (see Figure 5) can be used as optogenetic tools, as genetically encoded switches that enable neurons to be turned on by bursts of light. Both rhodopsins proved to be functional in model organisms like *C. elegans* and zebrafish, and even to reinstall rudimentary visual perception in mice. This raises great hopes for gene therapeutic approaches to overcome blindness.

**Rhodopsins in Dinoflagellates**

The phylogenetic tree shown in Figure 4 indicates that the three partially characterized rhodopsins from dinoflagellates are closely related to that of *Gloeobacter*, a cyanobacterium. As shown for
Erythropsis, the rhodopsin seems to be localized in the eye organelle which is thought to be derived from a secondary chloroplast (see Russian Doll Hypothesis). As chloroplasts are derived from cyanobacteria, it is not surprising that the Dinoflagellate rhodopsin may be derived from cyanobacteria. The functional analysis by expression in frog oocytes and mammalian tissue culture cells indicates that the rhodopsin gene of Oxyrrhis indeed encodes a green-light receptor (E. Bamberg, unpublished).

**Animal Rhodopsins**

Animal rhodopsin is the molecule of ultimate sensitivity because it allows the detection of a single light quantum. This may explain why this sensitivity because it allows the detection of a single light quantum.119–121 This may explain why this tissue culture cells indicates that the rhodopsin gene of Oxyrrhis indeed encodes a green-light receptor (E. Bamberg, unpublished).

High-resolution structures were reported for bovine122 and squid rhodopsin.123 The structure of bovine rhodopsin is shown schematically in Figure 6 which is based on X-ray crystallographic studies by Palczewski et al.122 and refined by Teller et al.124 and Okada et al.125 All known animal rhodopsins carry the same chromophore, 11-cis retinal (or its close analogs) covalently bound to a specific lysine in the 7th α-helix of its apoprotein (opsin) via a Schiff base linkage which isomerizes to all-trans retinal upon illumination. As shown by Palczewski et al.122 ca. 30 amino acid residues contribute to the binding pocket for 11-cis retinal. The Schiff base linkage to lysine (K296) is positively charged and the counterion is glutamate (E113) in α-helix 3, whose presence was predicted 30 years ago.126 A most impressive amount of work went into the identification of the function of the individual amino acids by generating site-directed mutations in a rhodopsin gene that was totally synthesized by Khorana and co-workers (see Hubbell et al.103), so that rhodopsin has become the best known membrane protein.

The seven transmembrane helices form a channel as in bacteriorhodopsin and in Channelrhodopsin, but in animal rhodopsins the signal from the light-activated rhodopsin is not transmitted directly, but rather amplified by a G-protein coupled enzyme cascade. Therefore, animal rhodopsins are members of the very large class of GPCRs (Figure 7).

There are two major kinds of animal rhodopsins with different G-protein coupled enzyme cascades which are illustrated in Figure 8. They are found in two different kinds of photoreceptor cells, the ciliary
and the rhabdomeric photoreceptor cells. The ciliary photoreceptor cells (Figure 8(a)) contain c-type rhodopsins and induce a phosphodiesterase which hydrolyzes c-GMP, and the decrease in c-GMP levels leads to the closure of cGMP-gated Na\(^{+}/Ca^{2+}\) channels in the plasma membrane of the photoreceptor cells and therefore, to a hyperpolarization which can be recorded in an electroretinogram. In contrast, rhabdomeric photoreceptor cells contain r-type rhodopsins\(^{123}\) and use a different G-protein coupled signaling cascade (Figure 8(b)) which activates phospholipase C leading to a rise in phosphoinositol and intracellular Ca\(^{2+}\) and resulting in the opening of TRP- or TRPL-channels and a depolarization of the membrane potential. It is frequently stated in the literature that ciliary photoreceptors are...
found in vertebrates, whereas invertebrates would have rhabdomeric receptors. This notion is incorrect; ciliary photoreceptors which have specializations of the plasma membrane covering the cilium are found not only in vertebrates, but also various invertebrate phyla like Cnidaria, Ctenophores, and Bryozoa, as well as in embryos of Arthropods and Cephalopods, and in larvae of Urochordates. Rhabdomeric photoreceptors are present in Arthropods, Echinoderms, and Sipunculans. However, both types, ciliary and rhabdomeric coexist in Annelids and Molluscs, Cephalochordates, Nemertean, Nematodes, Platyhelminthes, and rotifers. In fact, the eye of the scallop (Pecten) which has both a lens and reflecting mirror which project onto two retinae, one with ciliary photoreceptors which hyperpolarize, and the other with rhabdomeric photoreceptors which depolarize when illuminated. The hyperpolarizing photoreceptor expresses a different kind of rhodopsin which is coupled to the α subunit of a Gq type G-protein. The hyperpolarizing response in scallops is due to opening of a GMP sensitive potassium channel which is different from that of vertebrate cells, i.e. closing of cGMP sensitive cationic channel. Thus, it is most likely
that the scallop $G_\alpha_c$-mediated phototransduction cascade is probably coupled with a guanyl cyclase to elevate cytosolic cGMP concentration.

Opsin evolution has recently been reviewed\textsuperscript{135,136} The phylogenetic tree of the opsin genes (Figure 7) indicates that the $G_\alpha_c$-, $G_\alpha_c$-, and $G_\alpha_t$-rhodopsins diverged before the divergence of protostomes and deuterostomes. It is interesting to note that the mechanism of photo response in the scallop is shared by some vertebrates: the photoreceptor cells of the parietal eye of a lizard respond to light by increasing cytosolic cGMP and opening channels\textsuperscript{137} suggesting the presence of a similar $G_\alpha_c$-mediated phototransduction mechanism. The presence of the three types of photoreceptors in both proto- and deuterostome species suggests that they were already there in the last common ancestor. In the box jellyfish, the larva of *Tripedalia* seems to have the most primitive kind of single-celled rhabdomeric photoreceptors (see below), whereas the adult has elaborate lens eyes with ciliary photoreceptors. The electrophysiology and biochemistry of larval photoreceptor cells remains to be done, but the morphology strongly supports the argument\textsuperscript{81} that cnidarians, the most primitive animals with eyes, already possess both types of photoreceptors ciliary and rhabdomeric. The phylogenetic tree of animal rhodopsins (Figure 7) shows that two different subfamilies of opsins are found in ciliary and rhabdomeric photoreceptors, respectively, $c$-opsins and $r$-opsins. In addition, there is a third subfamily called $G_\alpha_t$-opsins (coupled to $G_\alpha_t$ proteins) and a more heterogeneous group including retinochrome, peropsin, and neuropsin which serve as photoisomerases.

The analysis of jellyfish opsins\textsuperscript{77} revealed a surprising diversity of sensory molecules reflecting an early evolutionary radiation. The hydrozoan *Cladonema* which possesses eyes expresses as many as 18 different opsin genes, whereas *Podocoryne* which lacks eyes still expresses two opsins. The conserved lysine to which the chromophore $11\text{-cis}$-retinal binds, is present in all of them suggesting that they are indeed used for photoreception. However, only 7 of the 18 opsins are expressed in the eyes of *Cladonema*, whereas as 2 genes are expressed ubiquitously, 4 on the tentacles only and 5 on the manubrium and later in the gonads. Phylogenetic analysis suggests that the manubrium-specific genes are derived from the eye-specific opsins and may have been recruited into the gonads for controlling the spawning process which is light-controlled.

In contrast to this, large diversity of opsins in cnidarians, no opsins have been found in marine and freshwater sponges which use cryptochromes for vision\textsuperscript{79,138} and also in choanoflagellates their putative protist ancestors.\textsuperscript{27}

Genome-scale analyses corroborate the notion that cnidarian opsins can be classified into three groups representing the orthologs of the bilaterian $R_-$, $C_-$, and $Go/RGR$ opsin subfamilies\textsuperscript{135} prior to the evolutionary divergence of bilateria. The closest outgroup among the functionally characterized GPCRs are melatonin receptors. X-ray crystallographic studies indicate GPCRs like the $\beta_2$ adrenergic receptor have a structure which is almost superimposable to bovine rhodopsin.\textsuperscript{139} Pisani et al.\textsuperscript{136} propose a maximally parsimonious scenario of metazoan opsin evolution leading from the duplication of the melatonin-opsin ancestor to the eumetazoan stem lineage. Two further gene duplication events in the eumetazoan stem lineage subsequently separated the $R$-opsin from the $C$-plus $Go/RGR$ lineage, and finally the $c$-opsins from the $Go/RGR$ opsins. Studies on the box jellyfish *Carybdea rastoni*\textsuperscript{140} have revealed a fourth class of $G$-protein coupled rhodopsins which are designated as $G_\alpha_t$ opsins and may be typical for the majority of cnidarian rhodopsins. The $G_\alpha_t$-type $G$-protein mediated phototransduction cascade is used in the highly evolved ciliary photoreceptor cells of *Carybdea* and signals through cAMP rather cGMP. Illumination results in cAMP levels in the photoreceptor cells. As both the vertebrate and the box jellyfish phototransduction cascades involve cyclic nucleotide signaling there are clear similarities between the two. In summary, we can distinguish five different kinds of $G$-protein coupling; $G_t$ (transducin) coupling in $c$-opsins, $G_\alpha_t$ coupling in box jellyfish, and $G_\alpha_t$ coupling in scallop ciliary photoreceptors, all of which signal through cyclic nucleotides. $G_t$ coupling in rhabdomeric photoreceptors in insects, cephalopods, and human melanopsin which signal through phosphoinositol. Finally, a fifth class of rhodopsin molecules which serve as photoisomerases and are found in humans, amphioxus, squid etc.

As far as the connection of metazoan rhodopsins to microbial rhodopsins is concerned, we favor a slightly different view. There is hardly any sequence conservation which would help to find the origin of animal rhodopsins. However, there are clearly similarities which may not be accidental. First, the seven transmembrane structure with retinal attached via a Schiff base linkage to a specific lysine residue in helix 7 is shared. If we compare the rhodopsins from *Cladonema* with those of the cyanobacterium *Gloeobacter* and the three rhodopsins from dinoflagellates, there may be traces of sequence conservation, which, of course, are very scarce considering that the split has occurred at Precambrian times. Besides the homologies in helix 7 (K296, A295,
and L293), some homologies can also be detected in helix 6: Y268 which forms part of the retinal binding pocket and W265, and in helix 4 there is a highly conserved G174, and in helix 3 a conserved P142. This indicates that the major evolutionary difference between microbial and animal opsins is the coupling to different G-proteins. This is reflected by sequence homology between the cyclic AMP chemoreceptor of Dictyostelium and animal rhodopsin. There is 22% amino acid sequence identity between the N-terminal regions of the two receptors and 32% sequence similarity, if one considers conservative replacements. This indicates that animal rhodopsins are clearly members of the large family of GPCRs, closely related to chemoreceptors. This is of particular interest because Pax6 is not only a master control gene for eye development but also for the nose or antenna, respectively, with their odor and gustatory receptors. It is difficult to decide whether animal rhodopsins are derived from microbial rhodopsins or whether they have evolved completely independently as G-protein coupled photoreceptors. As all rhodopsins use retinal as a ligand it seems reasonable to assume that rhodopsin evolved from a GPCR that acquired the ability to covalently bind its ligand retinal and thus allowing it to evolve to a photoreceptor molecule. However, it seems unlikely to me that the covalent linkage of retinal to a specific lysine residue in the 7th α-helix could have occurred several times independently in microbial rhodopsins and GPCRs. Also the conservation of tryptophane (W265) and tyrosin (Y268) in α-helix 6 reflect strong similarities between microbial and animal opsins. This raises the possibility that Exon shuffling caused by recombination events between microbial rhodopsin and different GPCRs led to the rapid evolution of the four types of metazoan opsins. The distal exons for the transmembrane domains (TM) 6 and 7, in particular, TM 7 with the retinal attachment site at lysine 296, may have originated from a microbial rhodopsin gene and the more proximal exons including the ERY motif in helix 3 and the loop between TM 5 and 6 which serve to bind the G-protein may be derived from the respective GPCR genes. As more data accumulate it may be possible to discriminate between these and other hypotheses.

In addition to its well-known function in vision, rhodopsin also has role in temperature discrimination. As shown in Drosophila larvae mutations in the nina A gene encoding rhodopsin, a GPCR, eliminates thermotactic discrimination in the comfortable temperature range from 19° to 24°C. The thermosensory signaling pathway includes a heterotrimeric guanine-nucleotide-binding protein (G protein), a phospholipase C, and the transient receptor potential TRPA1 ion channel, as for vision; however, thermotaxis toward 18°C is light-independent.

Another interesting nonvisual opsin-mediated phototransduction is the discharge of nematocytes in Hydra. Bright illumination inhibits nematocyte discharge. Non-nematocyte neurons located in battery complexes express opsin, cyclic nucleotide gated ion channels and arrestin which are all known components of phototransduction cascades. The origin of these phototransduction cascades might even have preceeded the evolution of eyes in cnidaria.

Color Vision

Vertebrates have basically two types of photoreceptors, rods and cones. The rods serve for highly sensitive dim-light vision, whereas the cones mediate color discrimination and high visual acuity at higher light intensities (see Yokoyama and Pichaud for review). The nocturnal Tokay Gecko (Gecko gecko) has a pure rod retina, whereas the diurnal American chameleon (Anolis carolinensis) has only cones in its retina. The respective opsins have a wide range of light absorption (λ_{max}) between 360 and 560 nm corresponding to the wide range of light wavelength available in the environment.

The rods that contain a single rhodopsin receptor with a λ_{max} of 500 nm are color blind, because they cannot discriminate between wavelength and light intensity. It makes no difference whether they have been hit by 10 light quanta of 500 nm wavelength or 20 quanta of a wavelength for which rhodopsin has half the sensitivity. A system for color detection must consist of at least two types of receptors with different absorption maxima (dichromatic color vision). Primates including humans have a trichromatic color detection system with three kinds of cone receptors having absorption maxima in the blue (420 nm), green (535 nm), and red (565 nm) range of the spectrum. All these light-sensitive receptor molecules are opsins, i.e. membrane proteins with seven transmembrane domains and 11-cis retinal attached to a specific lysine residue in transmembrane helix 7 via a Schiff base. The three types of color receptors differ only in a relatively small number of amino acid residues. The 11-cis retinal has a λ_{max} of 440 nm, but when it is bound to various opsins it detects a wide range of absorption maxima from 360 to 560 nm. The absorption maxima closely correspond to the wavelength to which the animal is naturally exposed. For example, the coelacanth fish which lives at a depth of 200 m in the ocean has only two visual pigments with a λ_{max} around 480 nm, since only blue light can penetrate into deep

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Polarization Vision

The study of polarization vision began with Santschi’s (1923)\textsuperscript{148} and Frisch’s (1949)\textsuperscript{149} observations that insects could use scattered sunlight for navigation, and that it was the polarization of scattered skylight which served as the most important compass cue. Mainly through the pioneering work of Wehner\textsuperscript{150,151} which combines behavioral studies in the field and sensory neurobiological approaches in the laboratory, robotics, and computer simulations, we gained deep insights into the problem of how insects, in this case the desert ant \textit{Cataglyphis}, detect the pattern of polarized light in the sky, process it neurally and use it for navigation. \textit{Cataglyphis} lives in the Sahara desert and stays inside its cooler nest until the outside surface temperature reaches values up to 50°C and its predators (lizards) are hiding underground. It leaves the nest and forages for its prey which are mainly small animals killed by the blazing sun. However, after 20 min of random foraging it has to return to its nest or it will be killed by the heat. It does so by homing back to its nest in a straight line, using optical landmarks only in the immediate vicinity of its nest. This puts an extremely strong selective pressure on their navigational skills, because they die when they do not find their nest in time. \textit{Cataglyphis} uses a compass for navigation based on the pattern of polarized light. The question of how a tiny little brain, as that of the ants, can solve such a complex navigational problem, has been solved by a multidisciplinary approach.\textsuperscript{150,151} The ‘hardware’ for a somewhat simplified polarization map is built into the large compound eyes on the head of the ant, into the geometrical order in which the UV-sensitive ommatidia are arranged. The specialized rhabdomeres of these ommatidia serve as analyzers for polarized light. The ant does not have to perform any calculations, it simply has to turn its head in the direction in which the sum of the signals from these special UV-receptors is maximal, and the animal is orientated along the sun-meridian. This navigational system can be considered as a matched-filter system. It deviates systematically from the real direction, but it is precise enough for the ant to find its nest. From the directional deviations the matched-filter system can be deduced. This is a paradigm for the evolution of behavioral traits under conditions of extreme selective pressure.

EYE PROTOTYPES

Unicellular Photoreceptor Organelles: The Russian Doll Hypothesis

Photoreceptor organelles range from a relatively simple eyespot as in cyanobacteria and in \textit{Chlamydomonas} (Figure 2) to the most elaborate structures resembling a human eye in some dinoflagellates like \textit{Erythropsis} and \textit{Warnovia}, but assembled in a single cell (Figure 9). They have been described by Greuet\textsuperscript{63,64} and consist of a cornea-like layer, a lens-like structure, a retina-like structure with stacked membranes (or microvilli) and a pigment cup, all in one cell (Figure 9).

Greuet proposed that these photoreceptor organelles are modified chloroplasts which have lost their capacity for photosynthesis. Therefore, \textit{Erythropsis} and \textit{Warnovia} are heterotrophic and use their photoreceptor organelles (ocelloids) for catching their prey. Recent work of Hayakawa et al. (Hayakawa S, Takaku Y, Hwang J S, Horiguchi T, Gehring W, Ikeo K, Gojobori T, unpubl. data). strongly supports Greuet’s hypothesis because it shows that these ocelloids contain DNA presumably a remnant of chloroplast DNA, and that they function as photoreceptor organelles. \textit{Erythropsis} and \textit{Warnovia} are difficult to culture and therefore, individual cells have to be collected in the ocean. My collaborator Hiroshi Suga succeeded, however, to establish a c-DNA library from a single cell, from which a fragment of a rhodopsin gene was sequenced. As shown in Figure 4 the \textit{Erythropsis} gene and two other dinoflagellate genes map close to the one of \textit{Gloeobacter}, a cyanobacterium indicating that they are of chloroplast origin. The retina-like structure (Figure 9(h)) consists of closely stacked membranes or microvilli which are strongly birefringent in polarized light (Figure 9(f)) indicating that the molecules are highly ordered in these membranes. \textit{In situ} hybridization of rhodopsin c-DNA to \textit{Erythropsis} (= \textit{Erythropsidinium}) shows...
that rhodopsin is present in the retina-like structure (Hayakawa S, Takaku Y, Hwang J, Horguchi T, Gehring W, Ikeo K, Gojobori T. unpubl.). Therefore, we can conclude that the ocelloid is virtually a camera-type eye-organelle of endsymbiotic origin. These findings led to the proposal of a Russian Doll Hypothesis which assumes that light sensitivity first arose in cyanobacteria (first Russian doll), the earliest known fossils on earth. These cyanobacteria were subsequently taken up by eukaryotic red algae (second doll) as primary chloroplasts, surrounded by an outer and an inner bacterial membrane, separated by a proteoglycan layer. Subsequently, the red algae were taken up by dinoflagellates (third doll) as secondary chloroplasts surrounded by an additional third membrane coming from the primary red algal host. In some species of dinoflagellates like Erythropsis and Warnovia, the secondary chloroplasts were transformed into elaborate photoreceptor organelles as explained above. Cryptoperidinium which has less elaborate eyespots, has taken up a third generation chloroplast from a diatome, and has become autotrophic again. Nevertheless, its eyespots contain lamellar bodies with stacked membranes which are highly reminiscent of human rod outer segments with stacked discs containing rhodopsin.

Although these first three Russian dolls are strongly supported by phylogenetic data, the fourth endosymbiotic transfer is highly speculative: Because dinoflagellates are commonly found as symbionts in cnidarians, they may have transferred some genes including some photoreceptor genes to cnidarians representing a fourth Russian doll. This is of course the most speculative step in the Russian doll model. However, there are some indications that some Endosymbiotic Gene Transfer (EGT) may have occurred between dinoflagellates and cnidarians. For example, dinoflagellates and cnidarians exclusively share nematocytes one of the most specialized cell-types which are not found in any other metazoan phylum. Furthermore, the genes encoding the lamellar body organelles, which closely resemble the outer segments of human rod cells, might also have been acquired by metazoans through EGT. The transfer of genes from mitochondria and chloroplasts to the host nucleus is well documented. In dinoflagellates, this transfer might have been facilitated by the fragmentation of the chloroplast DNA into gene-sized DNA-circles. This last step of the hypothesis is being tested by comparative sequencing the genomes of dinoflagellates and cnidarians with eyes and looking for genes that were possibly transferred. However, the sequencing of dinoflagellate genomes is hampered by the fact that they generally have very large genomes, some of which are considerably larger than the human genome.

A Unicellular Eye Prototype in Cnidarians
A priori the prototypic eye must have evolved from a single cell. However, most eyes contain at least two cell-types, sensory photoreceptor cells and pigment cells. This is also true for cnidaria the most ancestral animal phylum in which eyes are found. Some hydrozoan jellyfish like Cladonema have a lens eye at the base of each tentacle and some box jellyfish like Tripedalia have a battery of sensory organs on each of the four sides of the animal. These batteries called rhopalia contain lens eyes, slit eyes, and a statocyst. The lens eyes contain both pigment and photoreceptor cells. However, the eyes of their planula larvae are unicellular eye prototypes with pigment granules.

**FIGURE 9** | Eye organelles of the unicellular dinoflagellates *Erythropsis* and *Warnovia*. (a) *Erythropsis*, (b) eye organelle of *Erythropsis*, (c) *Warnovia*, (d) eye organelle of *Warnovia*, (e) nucleus and eye organelle of *Warnovia*, and (f) birefringence in the retina-like structure detected by polarized light in *Warnovia*. (a–f) Source: Makiko Seimiya and Jean and Colette Febvre. (g) Ultrastructure of the eye organelle of *Warnovia* and (h) ultrastructure of the retina-like structure with regularly stacked membranes and large pigment granules. (g and h: Reprinted with permission from Ref 64. Copyright 1969)
FIGURE 10 | Unicellular photoreceptors in the planula larva of the box jellyfish *Tripedalia*.\(^{81}\) (a) Planula larva and (b) unicellular photoreceptor with pigment granules, microvilli, and a flagellum. (Reprinted with permission from Ref 81. Copyright 2003 Royal Society Publishing)

microvilli which presumably contain rhodopsin or some other visual pigment, and a steering cilium for phototaxis (see above; Figure 10). It remains to be shown whether these unicellular eyes represent rhabdomeric photoreceptors as is strongly suggested by their morphology. By contrast, the adult eyes of *Tripedalia* and *Cladonema* contain ciliary-type photoreceptors and c- or s-rhodopsins.

The Darwinian Eye Prototype

In ‘The Origin of Species’ Charles Darwin had great difficulties to explain the evolution of organs of extreme perfection such as the eyes. *A priori* it seems absurd to try to explain an eagle’s eye simply by variation and selection. However, in the chapter on ‘Difficulties of the theory’ he found a way out of this dilemma and proposed that all the highly perfect eyes originated from a single prototypic photosensory organ consisting of two components only, a photoreceptor cell (which he called a nerve) and a pigment cell shielding the light from one side. Such an eye prototype would allow its carrier to determine the direction of the incoming light and confer a selective advantage over organisms which can only discriminate between light and dark. In fact, this prototype postulated by Darwin on theoretical grounds can be found in nature. The planarian *Polycelis auricularia* has multiple eyes in the head region, which consist of one photoreceptor and one pigment cell only (Figure 11). The two cells arise from a common precursor by cell differentiation\(^{153}\) (Watanabe et al., unpublished data).

The Darwinian prototype can also be found in larvae which often reflect a more ancestral stage of evolution. In the trochophora larva of *Platynereis*,\(^{84,155}\) a pair of larval eye spots are found, which are composed of one pigment cell and one photoreceptor cell only. As in *Drosophila* *Pax6*, *six1/2*, and *atonic (atoh)* are expressed in the larval *Platynereis* eye anlage. The single photoreceptor cell is of the rhabdomeric type with microvilli and expresses r-opsin which presumably interacts with the Gq-\(\alpha\) subunit canonical for r-opsins.

In contrast, the prototypic eye of the brachiopod *Terebratalia transversa*\(^{156}\) consists of two cells, both of which are putative photoreceptor cells deploying a modified enlarged cilium for light perception and have axonal connections to the larval brain. These cells express besides *Pax6* and *otx*, c-opsin confirming that these larval eyes deploy ciliary-type photoreceptors for directional light detection.

A most interesting kind of larval prototypic eye has been described in certain marine flatworms. The larvae of *Pseudoceros canadensis* have two different eye-types: The right eye is composed of one cup-shaped pigment cell and three sensory cells.\(^{157}\) Each sensory cell extends an array of straight, cylindrical, tightly packed microvilli (forming a rhabdomere) into the pigment cell cup. This clearly represents a rhabdomeric type of photoreceptor cell. However, the left eye is composed of one pigment cell and four sensory cells. Three of these photoreceptor cells are of the rhabdomeric type as for the right eye; however, the fourth central sensory cell sends unusually large arching cilia into the pigment cup between the three rhabdomeres. This indicates that ciliary and rhabdomeric (microvillar) characteristics can coexist in the same eye. This strongly supports the notion (see animal opsins) that both phototransduction mechanisms, ciliary and rhabdomeric, were present from the beginning of metazoan evolution.

The mechanisms underlying this first step of cellular differentiation leading from a common precursor cell to a photoreceptor and a pigment cell can be deduced from evolutionary developmental studies. The gene locus which serves as a pigment...
cell determinant was first identified more than 60 years ago as a mutation in mice by Hertwig and later cloned and designated as Microphthalmia transcription factor (Mitf). Mitf mutations affect several different cell types, but mainly pigment cells. Vertebrates have two kinds of pigment cells, the melanocytes of the skin, which are derived from the neural crest, and the retinal pigment epithelial (RPE) cells of the eye, which are derived from the optic cup, which represents an evagination of the brain. The Mitf gene has been cloned and shown to encode a basic helix-loop-helix zipper transcription factor. Arnheiter has independently proposed a common evolutionary origin of pigment cells and photoreceptor cells.

Several lines of evidence indicate that Mitf is a pigment cell determinant. Mitf is strongly expressed in pigment cells in the RPE and in loss-of-function mutants the RPE hyperproliferates, and in the dorsal part the RPE is transformed into a correctly layered, inverted retina. Tachibana et al. were able to transform fibroblasts into pigment cells (melanocytes) by stably transfecting NIH 3T3 cells with human Mitf c-DNA. In zebrafish, homozygous nacre mutants carrying a mutation in Mitf, can be rescued by injecting the wild-type Mitf gene into early embryos and inducing gene expression with a heat shock promoter. Mitf is positively regulated by Pax6 and Pax2. In mice, loss-of-function of either Pax6 or Pax2 alone does not reduce Mitf expression, but if both Pax6 and Pax2 are nonfunctional Mitf is no longer expressed. As a negative regulator CHX10, a paired-like homeodomain (HD) transcription factor, has been identified. These observations have led to the following concept of differentiation of the retina: Pax6 first induces the optic vesicle (see below). Subsequently, the differentiation of these neural cells into neuroretina and RPE is controlled by an interaction between Pax6 and Mitf. The neuroretina is specified by Pax6 and antagonized by Mitf. Mitf is a repressor which in turn is negatively regulated by CHX10. CHX10 is induced by fibroblast growth factor (FGF) in the neuroretina and prevents Mitf from transforming the neuroretina into a pigment epithelium. Interestingly, Mitf is highly conserved in evolution and has been found in ascidians, Caenorhabditis, Drosophila and even in Tripedalia jellyfish. Therefore, we propose that the first step of cell differentiation in eye evolution leading to the formation of photoreceptor versus pigment cells is based on an interaction between Pax6 and Mitf (see Figure 12).
FIGURE 12 | General scheme of eye evolution. The first step in eye evolution is the evolution of a light receptor molecule which in all metazoa is rhodopsin. In the most ancestral metazoa, the sponges, a single Pax gene, but no opsin gene has been found. In the larva of the box jellyfish Tripedalia, a unicellular photoreceptor has been described. The adult jellyfish forms complex lens eye with ciliary photoreceptor cells, which form under control of PaxB, a putative precursor of Pax6. However, the eyes of the hydrozoan jellyfish Cladonema are controlled by PaxA. We propose that the prototypic eye consisting of just two cells a photoreceptor cell and a pigment cell originated from a unicellular photoreceptor by a first step of cell differentiation. This cellular differentiation led to formation of a photoreceptor cell and a pigment cell under the genetic control by Pax6 and Mitf, respectively. As true innovations are rare in evolution all the more complex eye-types arose monophyletically from one of these Darwinian prototypes leading to a large diversity of eye-types by divergence, parallel evolution, and convergence.

DEVELOPMENT AND EVOLUTION OF THE DIFFERENT EYE TYPES

Pax6 and the Monophyletic Origin of the Various Eye Types

The leading neo-Darwinists like Ernst Mayr assumed that the various eye types found in the different animal phyla evolved independently 40–60 times in the course of evolution. This concept of a polyphyletic origin of the eyes was mainly based on morphological and embryological arguments. The compound eye of insects has an entirely different structure from the camera-type eye of vertebrates. The camera-type eyes of cephalopods and vertebrates were taken as the perfect example of convergent evolution, because they are similar in structure, but their embryological
development is different; the vertebrate eye develops from an evagination of the brain, whereas the cephalopod eye forms as an invagination of the skin. However, this notion of a polyphyletic origin of the various eye types was hardly compatible with Darwin’s idea of an ancestral prototype. As he pointed out in ‘The Origin of Species’ this prototype cannot be explained by selection as an accelerating driving force. Selection can only become effective once a minimal function is established. The prototypic eye has to function at least to a small extent before selection can set in. Thus, the Darwinian prototype must have arisen as a purely stochastic event. Therefore, the probability of this happening 40–60 times is extremely low.

More recent developmental genetic experiments open up an entirely different perspective on eye evolution (Figure 12) and argue strongly for a monophyletic origin of the various eye types. It began with the discovery of the Drosophila homolog of the mammalian Pax6 gene. Pax6 was first cloned in the mouse and shown to correspond to the mutation Small eye (Sey) in mice and to Aniridia in humans. Surprisingly, the homolog of Pax6 of Drosophila turned out to encode the eyeless (ey) gene, which had been discovered as a mutation as early as 1915 by Hoge. The fact that both the mammalian and the insect Pax6 genes are essential for eye development, suggested that the generally accepted hypothesis of a polyphyletic origin of the various eye types might not be correct. This led to the idea that Pax6 might be a master control gene for eye development in both insects and vertebrates. The term master control gene was proposed by Weintraub et al. for the muscle determining gene MyoD and extended by Lewis to the homeotic genes which in a combinatorial fashion determine segmental identity along the anteroposterior axis in Drosophila and mice. One way of demonstrating the function of a master control gene is to construct an inducible gain-of-function mutation and express the master control gene ectopically. For the homeotic Antennapedia (Antp) gene, the ectopic expression leads to a homeotic transformation of the antenna into a leg. The decisive experiment for ey is shown in Figure 13. Embryonic eyeless c-DNA was ectopically expressed in the antennal, wing and leg discs by means of the yeast gal4 system. By using different enhancer lines, ectopic eyes can be induced on the legs, wings, halteres, and antennae of the transgenic flies. This clearly demonstrated the role ey as a master control gene in switching on the entire cascade of target genes required for eye morphogenesis. These ectopic eyes are functional in that the photoreceptor cells can generate a normal electroretinogram and establish functional synapses. Of course, eye morphogenesis cannot be induced in any tissue of the fly at any stage of development, but eye induction is possible in all imaginal discs up to a certain stage of differentiation. The master control gene first has to interact with subordinate control genes to repress the resident developmental program and to install the eye program. If the cells have proceeded too far along their developmental pathway they become locked in (fully determined) and ectopic ey expression has no longer any effect.

While mammals have a single Pax6 gene, Drosophila and other holometabolous insects have two Pax6 paralogs, which were designated as eyeless (ey) and twin of eyeless (toy). The Toy protein shares 91% sequence identity in the paired domain (PD) and 90% in the HD with human and murine Pax6 proteins, as compared to 95 and 90% for EY. Outside of these highly conserved domains Toy is more similar to the mammalian protein, particularly in its overall length and at the C-terminus where it shares a transcriptional activation domain with other Pax6 proteins that is absent in EY. In Drosophila, it is more widely expressed than Ey and the loss-of-function phenotype is headless rather than eyeless. It is often assumed that a master control gene for eye development should be expressed specifically in the eyes, but this notion is incorrect. The master control gene not only has to induce eye formation, but the eye has to be properly located in the body plan (see below) and connected to the brain. Therefore, Pax6 is also expressed in most of the head region including parts of the brain where the proper neuronal connections have to be made. Despite the fact that toy has a more mammalian-like expression pattern, it is not the more ancestral gene, because ey and toy arose by gene duplication at exactly the same time. As shown in Figure 12 toy can also induce ectopic eyes, but it does so by first activating ey which in the course evolution became intercalated underneath toy into the eye developmental program. It requires a functional ey gene for inducing eyes. Gene duplication, diversification, and intercalation into a developmental pathway are an important evolutionary mechanism for generating biodiversity. Therefore, toy became the master control gene on the top of the hierarchy. This is in line with the observation that toy is expressed before ey, at the blastoderm stage when the body plan is laid down, whereas ey is expressed only later during germband extension. Obviously, the gene on the top of hierarchy has to be expressed first in development.
The mammalian Pax6 gene can functionally substitute for the *Drosophila* eyeless gene: The ectopic expression of mouse Pax6 in *Drosophila* induces ectopic compound eyes\(^{173}\) and the reciprocal experiment of injecting *ey* and *toy* mRNA from *Drosophila* into Xenopus embryos at the two cell stage also leads to the induction of ectopic eye structures.\(^{185}\) Of course, these are frog (vertebrate) eyes since we have only injected the master control gene of *Drosophila* into frog embryos and not the entire gene cascade necessary to form a *Drosophila* compound eye.

So far, we have been considering only the compound eyes of *Drosophila* for which a single master control gene is sufficient for ectopic induction. However, for the induction of the ocelli on the top of the fly head the combinatorial interaction between at least two master control genes, *toy* and *orthodenticle* (*otd*)\(^{166,186}\) is required as found for homeotic genes. These gene regulatory pathways for compound eyes and ocelli differ significantly (Figure 14).

The genetic cascade specifying the eye developmental pathway has been elucidated in some detail. The master control gene *toy* first activates *ey* and together they activate *sine oculis* (*so*) which encodes a homeobox protein,\(^{187,188}\) which forms a dimer with *eyes absent* (*eya*).\(^{189,190}\) *Eya* has dual function and acts as a transcription factor in the nucleus and as a protein phosphatase in the cytoplasm.\(^{191}\) The gene *dachshund* (*dac*) encodes a nuclear protein which is required for differentiation of ommatidia, but it is also required for leg development.\(^{192,193}\) *Toy* also activates *optix* (*opt*) another member of the *sine oculis* (*six*) gene family and the Pax gene *eyegone* (*eyg*) which is also required for eye development and functions neither upstream nor downstream of *toy* and *ey*, but it cooperates with these two genes in eye morphogenesis.\(^{194}\) A regulatory scheme for the top of eye developmental pathway is presented in Figure 14(a). It involves a large number of positive and autoregulatory feed back loops.
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FIGURE 14 | Initiation of the developmental pathway for compound eye and ocelli development. Pax6 homologs toy and ey are on top of the hierarchy, so and eya as well as dac are second-order transcription factors connected by various feedback loops to the master control genes ey and toy. In ocellar development, an additional master control gene otd is required. toy, twin of eyeless; ey, eyeless; eyg, eyegone; opix; so, sine oculis; eya, eyes absent; dac, dachshund; otd, othodenticle; hh, hedgehog.

In order to verify the results obtained by gain-of-function mutants, the corresponding loss-of-function mutants have to be examined. Knocking out ey or toy does not block the development of compound eyes completely, and even the ey−, toy− double mutant which is essentially headless still forms a couple of red ‘balls’ of ommatidia in the thorax. Only in triple mutants lacking all three Pax genes ey, toy, and eyg eye structures are completely lacking.166

Pax6 has been shown to be involved in eye development in all bilaterian phyla that were analyzed so far and in all the major eye-types including the camera-type eye of vertebrates, the compound eye of insects, the camera-type eye of cephalopods, the mirror-eye of Pecten, and most importantly the primitive larval eyes of annelids, the trochophora larva of Platynereis,84 the brachiopod larva,156 and the ascidian (urochordate) larva.195 Originating from a common prototype under the control of Pax6 and a set of second-order transcription factors like sine oculis (so/six), eyes absent (eya), and dachshund (dac) which are also highly conserved in evolution, the various eye-types have evolved. There are no functional constraints associated with a given transcription factor to control eye development. A transcription factor can control any gene which has the appropriate cis-regulatory sequences. If a gene like Pax6 is always involved in eye development, this is for evolutionary historical reasons, because it was previously involved in the genetic control of eye development in the last common ancestor and continues to be involved during further evolution. The fact that transcription factors can control any set of target genes or any developmental programme, is clearly shown by the three subclasses of six genes all of which contain a homeobox: The six1 and six3 subclasses (blue) both regulate eye development in Drosophila as well as in vertebrates, whereas the six4 subclass (red) controls muscle development (Figure 15). This indicates that with the same tool box of transcription factors, a large variety of eye-types can be made depending on the downstream target genes which are selected.

There appeared to be some exceptions to the rule that Pax6 is consistently expressed in the eye primordia, in particular, in C. elegans and in sea urchins which do not have image forming eyes. C. elegans has retained the Pax6 gene even though it does not have any eyes, but eyes are found in some ancestral marine nematodes. However, C. elegans has a nose and a small brain which are under Pax6 control, so that the selective pressure to retain the Pax6 gene is still maintained. By contrast, the rhodopsin genes have been lost, because their main function is photoreception, and as C. elegans lives underground or inside rotting fruits there is no selective pressure to maintain vision, which is generally true for animals living in the dark, e.g. in caves.
An interesting case is provided by sea urchins which do not have image forming eyes. Soon after the discovery of Pax6 as a master control gene for eye development, a Pax6 gene was found in *Para-centrotus lividus* which seemed to be an exception to the rule. The Pax6 gene was also found to be expressed in the tube feet which are extended between the spines and serve for locomotion. It has been known that sea urchins which lack both image-forming eyes as well as eyespots are negatively phototactic and respond to both visible (\(\lambda_{\text{max}}\) at 450 nm) and ultraviolet components of sunlight. Two recent studies on *Strongylocentrotus purpuratus* and *Strongylocentrotus droebachiensis* clearly show that sea urchins have their photosensory organs in their tube feet and their photoreceptor cells express a rhabdomeric-type opsin (r-opsin) indicating that the two major types of opsins, r- and c-opsins, were present prior to the split of proto- and deuterostomes (see above). These findings also show that you can use Pax6 expression to detect previously unknown photosensory organs.

For *Drosophila*, the transcriptome of eye development has been deciphered. In the larval eye imaginal disc mostly a set of transcription factors are activated which are high up in the gene cascade specifying eye development and include about 100 genes that are expressed specifically in the eye imaginal disc as compared to a leg imaginal disc which serves as a reference (Figure 16). During pupal development approximately 400 genes become active mostly encoding structural molecules, muscle proteins, proteins involved in neurotransmission and pigmentation. In the adult fly approximately 500 genes involved in signaling pathways, organelle biogenesis,
neurotransmission, and vision are transcribed. In total, we estimate about 1000 genes to be involved in the eye developmental pathway after subtraction of all the genes which are also expressed in other imaginal discs, in this case the leg disc.

Similar studies are being carried out in the mouse and it is becoming increasingly clear that starting from a highly conserved set of transcription factors including Pax6, six, eya, dac etc. the regulatory cascade diverges rapidly indicating that the various eye-types are made with the same tool box, but different overlapping downstream target genes. This is compatible with the hypothesis of intercalary evolution presented below.

The evolutionary origin of Pax6 is still largely unknown. So far Pax genes characterized by a paired box which encodes a bipartite DNA binding domain consisting of a PAI and a RED subdomain have only been found in multicellular metazoans and exclusively in bilateria. Both in mammals and insects there are approximately 9 or 10 Pax genes. They nicely illustrate an evolutionary principle which Jacob has called ‘tinkering’. Pax genes may contain two DNA binding domains a PD and HD and an octapeptide sequence to which an auxiliary factor may bind. These three domains occur in all possible combinations in the various Pax genes: Only a PD, a PD plus a HD as in Pax6, a PD plus a HD plus an octapeptide, a PD and a partial HD, a partial PD and a complete HD. Obviously, some recombination and deletion events have generated this diversity. With respect to Pax6 in Drosophila, it has been shown experimentally that the HD is dispensable for eye induction. As the HD is likely to be required for some other function, selective pressure and fulfil a function other than eye induction. Since ey and toy are highly pleiotropic, the HD is likely to be required for some other function.

In sponges no opsin genes have been found, but a single Pax gene is present. In cnidarians only five Pax genes Pax A, B, C, D, and E have been found and no true Pax6 homolog, even though some hydrozoan and box jellyfish evolved advanced eye-types. PaxA and PaxC correspond to pax neuro in Drosophila, PaxB to Pax2/5/8, PaxD to Pax3/7, and PaxE to Drosophila eyegone (eyg). In the box jellyfish Tripedalia, Kozmic et al. have provided evidence that PaxB an ortholog of vertebrate Pax2/5/8 is a regulator of eye development capable of inducing ectopic eyes in transgenic flies. PaxB may be interpreted as a precursor of Pax6. However, in the hydrozoan Cladonema PaxA is strongly expressed in the photoreceptor cells, whereas PaxB and PaxE are transcribed in the manubrium, the feeding organ in which also the gonads develop. Cladonema PaxA induces ectopic eyes in Drosophila imaginal discs, whereas PaxB and PaxE do not. Furthermore, the PD of PaxA protein binds directly to the 5' upstream region of eye-specific opsin genes, whereas PaxB does not. These data show an involvement of Pax genes in hydrozoan eye development as in bilaterians, support the monophyletic evolutionary origin of all animal eyes. The distinct classes of Pax genes, which may have played redundant roles in early metazoan evolution, were flexibly deployed for eye development in different animal lineages. In bilateria this role was completely taken over by Pax6.

The camera-type eye of cephalopods (octopus, squid, cuttle-fish etc.) has previously been considered to represent a paradigm for convergent evolution. However, this notion has to be modified in the light of the discovery of a highly conserved Pax6 gene in squids which shares 67% amino acid sequence identity with vertebrates. In the PD, squid Pax6 shows 91–95% sequence identity with its homologs in vertebrates, Drosophila, Nemertine, and sea urchins, whereas the HD shows 90–98% identity with Pax6 from these species. In situ hybrization to squid embryos reveals Pax6 in the developing eyes, the optic lobes of the brain, the arms, and the mantle. The hybrization to the eyes and optic lobes is expected, expression in the arm primordia may reflect the presence of other sensory organs, and the expression in the mantle is of particular interest, because this is the site of eye formation in other molluscs like scallops and ark clams. Ectopic expression of squid Pax6 cDNA in Drosophila induces the formation of ectopic eyes.

These findings indicate that the cephalopod eye like all other bilaterian eyes is specified by the Pax6 gene and has originated monophyletically and subsequently evolved by divergent, parallel, and convergent evolution. It should be emphasized that bivalves and snails have lense, mirror, and compound eyes, and more primitive cephalopods like Nautilus have pin-hole eyes, whereas only the squids and octopuses have the most complex camera-type eyes. Therefore, the molluscs cover the entire spectrum of eye-types, all going back to a successful Darwinian prototype.

Comparative genomic between the camera-type eyes of octopuses and humans show that of 1052 nonredundant genes which are expressed in the octopus eye 729 (=69%) are in common with the human eye, which is a surprisingly large fraction. Of these 1052 genes, 1019 are already found in the last common ancestor of bilateria, and 875 out of 1052 are conserved between humans and octopus. By comparing three species of cephalopods with camera-type eyes with molluscs with pin-hole eyes (Nautilus) and mirror-eyes (scallops); 5707 cephalopod camera eye-specific candidate genes (5707) were selected.
for further analysis. From the 1571 genes found in common between cephalopod and vertebrate camera-type eyes, 156 genes were identified which were under positive selection. These studies suggest that both changes in gene expression and in the primary structure of the respective proteins (through positive selection) have contributed to the evolution of the cephalopod camera-type eye.

Localization of the Eyes in the Body Plan
The criterion that homologous organs have to occupy the same position in the body plan was disproven by our experiments on the ectopic induction of compound eyes all over the body plan. Furthermore, the notion that the master control gene for eye development has to be eye-specific proved to be wrong. Pax6 has a highly pleiotropic expression pattern; it is expressed in the entire head region, in the compound eyes, the ocelli, in the nervous system, a large area of the brain and in the spinal cord or the ventral nerve cord, and also in the nose and antenna, respectively. This is similar to Hox genes which in a combinatorial fashion specify entire body segments. Therefore, the eye not only has to be built, but it has to be positioned in the body plan and properly connected to the nervous system.

The positioning of the eye primordia in the Drosophila embryo was analyzed by Blanco and Gehring and is summarized in Figure 17. The toy gene is activated by the maternal-effect genes bicoid (bcd) and torso (tor) in the anterior portion of the embryo and its expression domain is defined by repression from all sides by hunchback (hb), knirps (kni), and decapentaplegic (dpp). The repressor defining the posterior border of repression remains to be identified. It is important to note that the same genes which specify the body plan are also involved in positioning the eye.

As mentioned above, the ocelli are positioned on the head of the fly by orthodenticle (otd). Otd/otx genes are also involved in positioning of the eyes in many different phyla like vertebrates, annelids and planarians.

In recently discovered Precambrian Lobopodia fossils which are considered to be among the ancestors of the Arthropods, a species called Microdictyon sinicum has been described which seems to have a pair of compound eyes in every segment, whereas the eyes are confined to the head region in Cardio-dictyon catarulum. Therefore, the eyes may have been present in the prototypic body segment and in the course of cephalization confined to the head region where they are most useful for controlling locomotion. The original formation of a pair of eyes in every body segment may still be reflected by Pax6 expression in the ventral nerve cord in every thoracic and abdominal segment in the Drosophila embryo.

Development and Evolution of the Lens
The eye lens of vertebrates is a classical model of embryonic induction (see Gunhaga for review). A comparison of the genetic control of lens development between Drosophila and vertebrates reveals a number of molecular commonalities which were unsuspected by classical studies (see review by Charlton-Perkins et al.).

Conservation of Pax6 Target Sequences
If Pax6 is highly conserved in evolution, the cis-regulatory elements to which it binds should also be conserved. This prediction was tested analyzing the cis-regulatory element of the chicken δ crystalline gene encoding a lens-specific protein that is found in birds and reptiles only, due to their evolutionary relatedness. Previous studies have shown the lens-specific expression of the δ1-crystallin gene is under the control of the D5 enhancer which is only 30 bp long and contains both a Pax6 and a Sox2 binding site. To test the idea that Pax6 and Sox2 together with the DC5 enhancer could form a regulatory circuit in a very distantly related animal, the DC5 enhancer was fused to GFP and introduced into Drosophila, to study its pattern of expression. The results show that the DC5 enhancer is not only active in the compound eye but, remarkably, specifically active in those cells which are responsible for secreting the liquid lens, i.e. the cone cells. However, the regulation of the DC5 enhancer is carried out by Pax2 rather than Pax6 in

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**FIGURE 17** | Positioning of the eye in head region of the Drosophila embryo. Proposed model for the onset of toy expression. toy is activated (arrows) by the maternal effect genes bicoid (bcd) which forms a protein gradient and torso (tor). It is repressed from all sides by hunchback (hb), knirps (kni), and decapentaplegic (dpp). The posterior repressor remains to be identified.
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combination with SoxN the homolog of Sox2. Both proteins (D-Pax2 and SoxN) bind cooperatively to the DC5 enhancer and activate the target gene synergistically. As Pax6 and Pax2 proteins derive from the same ancestor, we can assume that during evolution Pax6 function in vertebrate lens development was retained by Pax2 in *Drosophila*. These findings demonstrate a remarkable evolutionary conservation of regulatory circuits between chicken and fly.

**Intercalary Evolution of Morphogenetic Pathways**

For the evolution of more complex eyes originating from the Darwinian prototype, we have proposed a mechanism of intercalation of new genes into the eye developmental pathway.\(^4,166\) The prototypic eye already possesses the master control Pax6 on the top of the hierarchy and the genes required for vision like opsins, G-protein signaling, arrestins etc., but it lacks, for example, lens protein genes and other genes required for morphogenesis of more complex eyes. At least two genetic mechanisms for the recruitment of new genes into a developmental pathway are known: Gene duplication and subsequent divergence and enhancer fusion. Both have been found in eye development in *Drosophila*. Gene duplication, for example, has occurred in *Drosophila* Pax6 giving rise to *ey* and *toy* followed by functional divergence. The *toy* gene retains most of the original master control function in the head including the compound eyes, the ocelli, the antennae etc. and in the ventral nerve chord, whereas *ey* has undergone subfunctionalization and is mostly required for the formation of compound eyes. The original positive control over the second-order target gene *sine oculis* (*so*) is retained by both genes and the *so* enhancer contains binding sites that are either recognized by *toy* alone or by both *ey* and *toy*.\(^217\) The *ey* gene has become inserted downstream of *toy* into the eye morphogenetic pathway.

In vertebrates, another case of gene duplication and divergence has been described for *Mitf* in zebrafish. As mentioned above, vertebrate pigment cells arise from two embryonic sources; the neural crest provides the pigment cells of the skin, whereas the retinal pigment cells originate from the optic cup, i.e. an evagination of the brain. The *nacre* mutant is a loss-of-function of *Mitf* (or *Mitfa*) and lacks the neural crest derived melanophores, although it is expressed in both the neural crest precursors and in the eye.\(^218\) Subsequently, Lister et al.\(^219\) have identified a second *Mitf* gene, *Mitfb* which is coexpressed with *Mitfa* in the RPE, where it complements the *nacre* mutation, but it is not expressed in neural crest melanoblasts leading to a loss of neural-crest-derived pigment cells. This loss of neural-crest-derived pigment cells can be rescued by expressing *mitfb* the neural-crest-derived melanoblasts under the *mitfa* promoter. Therefore, *Mitfa* and *Mitfb* together recapitulate the function of a single ancestral gene.

The second known genetic mechanism is enhancer fusion and has been demonstrated by Piatigorsky and Wistow\(^220\) for various lens proteins. A variety of lens proteins originated from various enzymes or small heat shock proteins. By transposing them in the vicinity of an enhancer which leads to the expression in the lens, such genes can be recruited into the lens morphogenetic pathway. If such proteins are transparent to visible light and have the right refractive index and stability, they can serve as a constituent of the lens. Similarly, one of the major constituents of the cuticular lens of *Drosophila*, Drosocrystallin, was cloned\(^221\) and the analysis of its amino acid sequence clearly indicates that it belongs to a large family of cuticle proteins and was recruited into the lens.

By intercalation of additional genes into the eye developmental pathway increasingly complex eyes can evolve from the prototype supporting Darwin’s theory of eye evolution from a simple prototype to highly complex eyes.

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