Casting a Genetic Light on the Evolution of Eyes

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REVIEW

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Light has been exploited for information by organisms through the evolution of photoreceptors and, ultimately, eyes in animals. Only a handful of eye types exist because the physics of light constrains photodetection. In the past few years, genetic tools have revealed several parallel pathways through which light guides behavior and have provided insights into the convergent evolution of eyes. The gene encoding opsin (the primary phototransduction protein) and some developmental genes had very early origins and were recruited repeatedly during eye evolution. Eye lens proteins arose separately and make up a diverse group, many of which were co-opted from other functions. A major challenge now is understanding how newly discovered pathways for processing light evolved and how they collaborate with eyes to harvest information from light.

Understanding how eyes evolved into what Darwin called an "organ of extreme perfection" (*1*) requires analysis of evolutionary constraints, key selective forces, and possible origins. The evolution of photodetection, giving rise to eyes, offers a kaleidoscopic view of selection acting at both the organ and molecular levels. The repeated exploitation of some regulatory gene sequences in eye development and lens formation raises questions about why certain transcription factors have been regularly recruited to build eyes. The ease with which we can now analyze the evolution of structural gene sequences across species belies the difficulties in tracing the selective forces that shaped regulation of gene expression.

Evolutionary Constraints and Functional Adaptations

Although the variety of eyes in the animal kingdom seems astonishing, physical laws have constrained solutions for collecting and focusing light to just eight types of eye optics (Fig. 1) (*2*). Animal eyes are not simple photon detectors, but organs that produce an image by comparing light from different directions. Biological pinholes, lenses, or mirrors are used to focus an image on photoreceptors (*2*). Light travels in straight lines, and information is carried by wavelength, intensity, and/or polarization, which set limits on eye dimensions and detection systems. Of around 33 animal phyla, about one-third have no specialized organ for detecting light, one-third have light-sensitive organs, and the rest are animals with what we would consider eyes. Image-forming eyes appeared in 6 of the 33 extant metazoan phyla (Cnidaria, Mollusca, Annelida, Onychophora, Arthropoda, and Chordata), and these six contribute about 96% of the known species alive today (*2*).

As earliest evolution occurred in water, which transmits only a limited range of wavelengths, the mechanisms for photon response converged on biochemical solutions that set the course for subsequent evolution (*3*). The evolution of eyes very likely proceeded in stages. First were simple eyespots (early Cambrian period, 570 to 500 million years ago), with a small number of receptors in an open cup of screening pigment. Eyespots would distinguish light from dark but could not represent complex light patterns. Invagination of this eyespot into a pit would add the capacity to detect the direction of incident light. Addition of receptors may then have led to a chambered eye, whereas duplication of an existing pit may have led to a compound eye (*2*). Adding an optical system that could increase light collection and produce an image would later dramatically increase the usefulness of an eye. Whereas primitive eyes can provide information about light intensity and direction, advanced eyes deliver more sophisticated information about wavelength, contrast, and polarization of light.

How many genes might it take to make an eye and how many are expressed exclusively in eye development? Two preliminary answers to the first question from *Drosophila* and mice differ greatly in their estimates. UCLA undergraduates (n = 138) each screened 10 mutant *Drosophila* for eye defects and identified 501 eye-related genes (*4*) or about 3.5% of the *Drosophila* genome. These mutations were distributed among 19 different functional categories (*5*). The largest categories included genes used for signal transduction or regulation of transcription or that were novel. In mice, Williams *et al*. (*6*) reported an expressed sequence tag (EST) library of 15,000 transcripts from ~10,000 genes; ~7500 transcripts were expressed in the retina, regulating both retinal development and function. The hard question is how many genes are used only in development and then play no role in function, and this is completely unknown. Assuming half are associated with development, ~3750 genes are involved, which is 18 times the number in *Drosophila*. However, these estimates are hard to compare for two reasons. First, they are based on quite different techniques. Second, *Drosophila* eyes consist of identical repeated units of photoreceptors, whereas vertebrate retinas are markedly more complex and include photoreceptors and five additional types of processing cells.

Functional constraints have produced nearly identical optical designs in distinctly unrelated animals, most notably fishes and cephalopods. In both lineages, the chambered or camera-like eyes in which an image falls onto a two-dimensional array of photoreceptors are similar in a large number of functional details, despite their great phylogenetic distance (*7*). Invertebrate and vertebrate photoreceptors are distinctly different, most likely arose independently, and are located at the very back of the retina in fish (and all vertebrates) but at the front in cephalopods. Although these eye types are not homologous and the animals carrying them are from distinctly different lineages, there are some homologies among structural and developmental molecules. Both eyes use phylogenetically related forms of opsin as their primary photodetection molecule, and an important regulatory gene, *pax6*, has been found in both vertebrates and some cephalopods, although not in octopus. The use of homologous genes to build nonhomologous structures may lie at the heart of understanding eye evolution and evolutionary processes more generally.

Shared genes may suggest homologous evolutionary paths but may also underlie convergent evolutionary outcomes. For example, the octopus eye arose ~480 million years ago (Mya) and the vertebrate eye 640 to 490 Mya, long after their common ancestor (~750 Mya). Comparing ESTs from octopus eye tissue with those from human eyes revealed ~70% that are commonly expressed, and 97% of these genes are estimated to have existed in the common ancestor of bilaterians (*8*). Overall, about 875 genes have been conserved between humans and octopuses, which may have provided the substrate for the convergent evolution of the camera eye in cephalopods and vertebrates. Among these genes might be a common gene regulatory network recruited at least twice for constructing chambered eyes.

Capturing Photons

The transduction of photons into cellular signals uses seven transmembrane–spanning opsin proteins (30 to 50 kD) that combine with a vitamin A–derived, nonprotein retinal chromophore. Opsins, which control sensitivity to light of different wavelengths, appeared before eyes did (*2*) and evolved into seven [or possibly more (*9*)] distinct families (*10*) (Fig. 2). Opsin was present before deuterostomes split from protostomes

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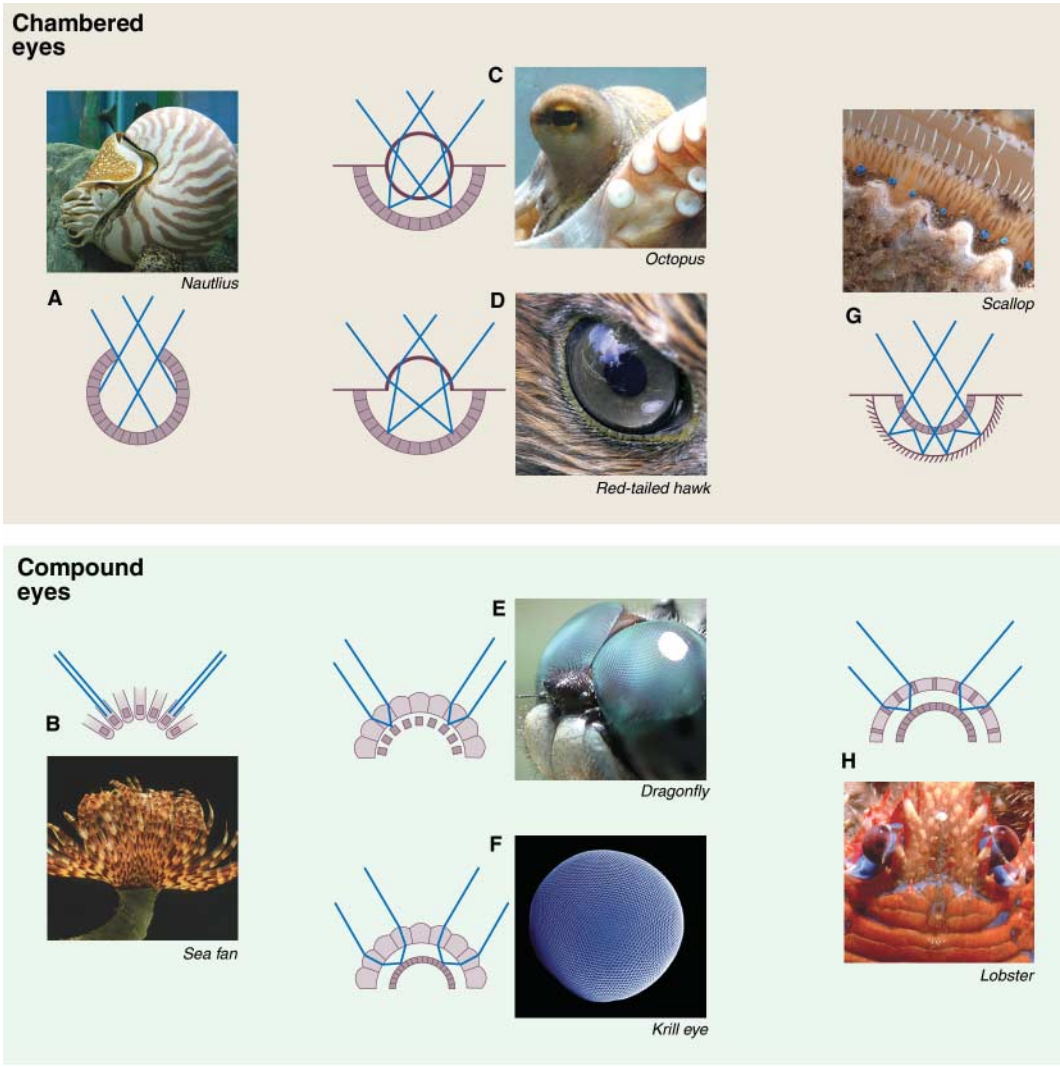

Fig. 1. Eight major types of optics in animal eyes. Both chambered eyes (top) and compound eyes (bottom) form images using shadows (**A** and **B**), refraction (**C** to **F**), or reflection (**G** and **H**). Light rays shown in blue, photoreceptive structures are shaded. The simple pit eye (A) (chambered nautilus) led to the lensed eyes in fish and cephalopods (C) (octopus) and terrestrial animals (D) (red-tailed hawk). Scallop eyes (G) (bay scallop) are chambered but use concave mirror optics to produce an image. The simplest compound eye (B) (sea fan) found in bivalve molluscs led to the apposition compound eye (E) (dragonfly) found in bees, crabs, and fruit flies; the refracting superposition compound eye (F) (Antarctic krill) of moths and krill; and the reflecting superposition eye (H) (lobster) found in decapod shrimps and lobsters. Diagrams modified by permission from (*2*). [Sources: (A) Wikipedia; (B) Robert Pickett/CORBIS; (C) Russell Fernald/Stanford University; (D) Steve Jurvetson/Wikipedia; (E) David L. Green/Wikipedia; (F) Gerd Alberti, Uwe Kils/Wikipedia; (G) Bill Capman/Augsburg College; (H) Lawson Wood/CORBIS]

(*11*). The size of each opsin family is growing rapidly as investigators look at nontraditional organisms and in unexpected places. Multiple new opsin genes, as well as new genes for other phototransduction-specific families [e.g., heterotrimeric guanine nucleotide–binding proteins (G proteins) and nucleotide-gated channels], arose early in vertebrate evolution during extensive chromosome duplications and very likely facilitated retinal specializations (*12*). For example, opsin gene duplication was responsible for the independent evolution of three-color (trichromatic) vision in old and new world primates (*13*). Similarly, opsin gene duplications in Lepidoptera, followed by an increased rate of evolution, produced a diversity of pigments sensitive to visual spectra important for specific species (*14*). Photoreceptor wavelength absorption spectra are exquisitely modulated by a small collection of amino acid side groups adjacent to the chromophore-binding site in the seventh transmembrane domain of opsins, where the effects of natural selection are now most evident (*15*).

An example of how color vision shapes cone opsin evolution is in the visual systems of cichlid fishes in the East African lakes. In one riverine species, ancestral to the lake species, seven cone opsin genes are present as the result of gene duplications. Although only four cone opsins are found in the adult retina and, hence, can contribute to wavelength discrimination by the animal, the rest are expressed at various points during ontogeny. This preservation of opsin genes may offer a substrate for rapid selection of different visual chromatic sensitivities in response to selective pressures (*16*). Another mechanism for modifying the spectral sensitivity is found in bluefin killifish. Animals living in murky swamps have different color sensitivities from those living in clear springs, and the difference is produced through differential expression of cone opsin genes within individual photoreceptors, although how this is regulated is unknown (*17*).

The two best-known photoreceptor types use distinct families of opsins packed in quite different membrane specializations and require different transduction mechanisms (Fig. 3). Vertebrate photoreceptors use members of the ciliary opsin (c-opsin) family incorporated into specialized cilia, whereas invertebrate photoreceptors use members of the rhabdomeric opsins (r-opsin) that are typically formed into rhabdoms. Each receptor type uses different G proteins: transducin in vertebrates and the G_q family in invertebrates. Vertebrate photoreceptors produce hyperpolarizing potentials via a phosphodiesterase cascade; invertebrate photoreceptors are depolarizing and use a phospholipase C cascade. The site of biochemical signal amplification is different between these receptor types, as are the mechanisms for terminating the response. Moreover, opsins in invertebrates are fixed to their membranes (*18*), which allows polarization detection, whereas those in vertebrates are not. It now seems clear that these photoreceptor types arose independently and coexisted in urbilaterians before bilaterians arose (see below).

In using vision to extract information about the environment, all animals exploit the same properties of light: intensity differences to produce contrast and wavelength differences to produce hue. However, no unique solutions exist, and specializations that evolved to process intensity and wavelength differ among species; these differences reflect how similar problems are solved via diverse

mechanisms through natural selection. For example, mammals and bees use long wavelength photoreceptors for intensity and color vision, whereas flies and birds have evolved separate sets of photoreceptors for these two purposes (*19*). The genetic substrates that supported such different evolutionary paths are unknown. Even though blowfly and monkey photoreceptors evolved independently and use different molecular mechanisms, signal processing, and other physiological steps, the information about the world delivered to the nervous system is nearly identical (*20*). These few examples reveal the different routes natural selection has taken during the evolution of eyes in response to the information available in light.

Parallel Universe?

The visual pigments described above are called type 2 opsins to distinguish them from microbial, or type 1, opsins, which are much older and are used for collecting energy and information from photons found in archaea and eukaryotic microbes. Thanks to new techniques for genetic sequencing of samples from fresh and sea water, salt flats, and glacial seas, the number of known type 1 opsins is increasing quickly (currently >800) (*21*). There are striking similarities between opsin types 1 and 2: Both are seven transmembrane–spanning domain proteins, both use an associated retinal moiety to capture light, and, in both, retinal is attached in a Schiff base linkage via a lysine residue in the seventh helix (*21*). However, type 1 opsins differ in physical size and in the distribution of their intramembrane domains, which reflects the differences in their signaling cascades. Type 1 opsins function within the membrane to pump ions or to signal other integral membrane proteins, as opposed to signaling via intracellular G proteins. Finally, the two retinal molecules are photoisomerized quite differently. Researchers were astonished to discover that despite remarkable convergence in molecular details of their function, there is no phylogenetic relationship between them (*21*). So the fundamental mechanism for detecting light using an "opsinlike" protein, associated with retinal, has been discovered and exploited twice independently. Progenitors of the type 1 opsins probably existed in earliest evolution before the divergence of archaea, eubacteria, and eukaryotes, which means that a light-driven ion transport mechanism for deriving energy used in association with opsin 1 preceded the evolution of photosynthesis as a means for using the Sun's energy (*21*).

Lenses

Simple eyes don't have pupils or even lenses, so they can provide only coarse information about the distribution of light in the environment. Lenses allow eyes to collect and concentrate light, which leads to increased sensitivity and allows information contained by that light to be spatially resolved. Advanced eyes collect light through an aperture and focus it with a lens onto photoreceptor cells. As lenses are made from proteins, could the molecular phylogeny of lens proteins instruct us about eye evolution?

Vertebrate lenses are formed from concentric layers of highly elongated fiber cells that differentiate from a peripheral anterior layer of epithelial cells. These contain high concentrations of soluble proteins called crystallins because they maintain transparency. In contrast, the lens proteins of most invertebrate eyes are secreted by specialized cells. A very unusual case is that of a parasite (*Neoheterocotyle rhinobatidis*) in which the lenses are of mitochondrial origin (*22*).

There are three major gene families of crystallins widely expressed in vertebrate lenses that account for most of the protein in aquatic and terrestrial vertebrates: α-crystallins (2 to 3 members), β-crystallins (6+ members), and γ-crystallins (2 to 16 members). It was originally thought that these proteins had uniquely evolved to function as lenses, but some are found expressed in heart, brain, and other tissues of the eye. Recent data reveal that a precursor to βγ-crystallin exists in a urochordate (*Ciona intestinalis*), and functional tests suggest that co-option of ancient regulatory circuits may account for its role in vertebrate lenses (*23*). The remaining vertebrate lens proteins are a diverse, nonconserved group, several of which serve as enzymes elsewhere in the body. Many of these taxon-specific lens proteins have been co-opted from other functions, typically as enzymes, and usually the same gene encodes both the enzyme and lens protein, a process termed "gene sharing" (*24*).

Two taxon-specific lens crystallins, ε (birds and crocodilians) and τ (birds, fish, and reptiles), are active glycolytic enzymes encoded by one gene and demonstrated to be bifunctional (*24*). Such sharing is thought to precede duplication of a structural protein gene, typically followed by specialization of the paralogous genes into different functions. In both duck (*25*) and ostrich (*26*), δ-crystallin genes are bifunctional; they act as metabolic enzymes (argininosuccinate lyase) and lens proteins. In contrast, in chicken, the one (δ1) expressed in the lens has no enzyme activity, and the other (δ2) is enzymatically active (*25*). Similarly, the glycolytic enzyme, lactate dehydrogenase, is a crystalline in crocodilians, elephant shrews, and some birds and is expressed in lenses of various invertebrates. This kind of molecular opportunism is so effective that it has also occurred in both cephalopods (*27*) and *Drosophila* (*28*). One possibility is that because lenses require production of a relatively large amount of protein, genes that have been strongly up-regulated in other tissues might be selected for lens function. Such gene sharing has also been seen to a lesser extent in corneal epithelial tissue, which suggests that certain proteins might be chosen because of a possible role in protecting transparent tissue from ultraviolet radiation (*29*). The common strategy of assembling lenses from diverse proteins seems to be a convergent evolutionary solution that has occurred independently many times in vertebrates. Co-option of taxon-specific ζ-crystallins is thought to have occurred at least three times independently (*30*).

Functionally, the exquisite gradient of refractive index necessary to allow spherical lenses to focus light (*31*) is a convergent solution that has evolved in water-dwelling vertebrates and invertebrates alike. What remains unknown is how genetic programs assemble differing amounts of diverse proteins to preserve the essential functional properties of lenses and whether there is

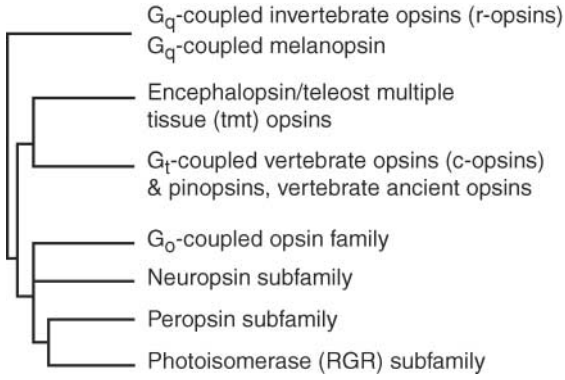

Fig. 2. A simplified schematic molecular phylogenetic tree inferred by the neighbor-joining method showing the seven known opsin subfamilies. Three families transduce light using G protein–coupled mechanisms (G_q, G_t, G_o); the best known are G_q or r-opsins found in invertebrate photoreceptors and G_t or c-opsins found in vertebrate photoreceptors. Encephalopsin and its teleost homolog tmt are found in multiple tissues with unknown function. Pinopsins, closely related to c-opsins, are expressed in the pineal organ of several vertebrates, and vertebrate ancient opsins are expressed in nonphotoreceptor retinal cells, including amacrine and horizontal neurons in teleost fish retinas. Similarly, neuropsins are found in eye, brain, testes, and spinal cord in mouse and human, but little is known about them. Peropsins and the photoisomerase family of opsins bind *all-trans*-retinal, and light isomerizes it to the 11-*cis* form, which suggests a role in photopigment renewal. These are expressed in tissues adjacent to photoreceptors, consistent with this role. Recent data suggest that some cold-blooded vertebrates have an additional opsin type, named parietopsin because it is found only in parietal eye photoreceptors (*9*). [Redrawn with permission from (*11*).]

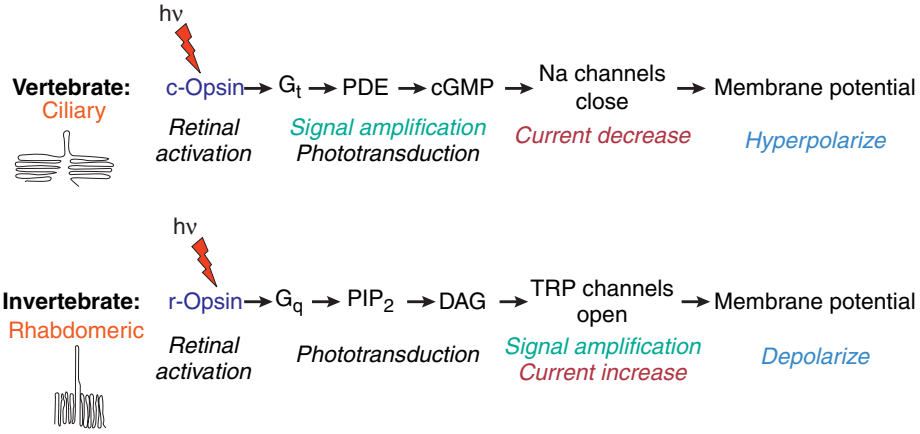

Fig. 3. Schematic illustration showing the key differences between simplified representations of (top) canonical vertebrate ciliary phototransduction and (bottom) invertebrate rhabdomeric phototransduction, where hν represents incident photon energy. The two different opsin types (c-opsin and r-opsin) are contained in distinctly different membrane types, ciliary and rhabdomeric. The opsins are coupled to different families of G proteins that act via different types of transduction cascades. Amplification occurs during phototransduction in ciliary receptors and during channel opening in rhabdomeric receptors. These cascades produce signals of different sign. G_t, transducin; PDE, phosphodiesterase; cGMP, cyclic guanosine monophosphate; G_q, guanine nucleotide–binding protein α15; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol.

any rhyme or reason to which specific proteins are used in particular taxa.

Origins of Eyes

Historical views on eye evolution have flip-flopped, alternately favoring one or many origins. Because members of the opsin gene family are needed for phototransduction in all animal eyes, a single origin was first proposed. But subsequent morphological comparisons suggested that eyes evolved 40 or more times independently (*32*); this finding is based on, among other things, the distinct ontogenetic origins of eyes in different species (*33*). For example, the vertebrate retina arises from neural ectoderm and induces head ectoderm to form the lens, whereas cephalopod retinas result from invaginations of lateral head ectoderm, ultimately producing an eye without a cornea. Multiple origins were also supported by an elegant simulation model. Starting from a patch of light-sensitive epithelium, the simulation, under selection for improved visual acuity, produced a focused camera-type eye in less than 4×10^5 generations. For animals with generation times less than a year, this would be less than a half million years (*34*).

The idea that eyes arose multiple times independently was challenged by the discovery that a single developmental gene, *pax6*, can initiate eye construction in diverse species (*35*). However, subsequent work has shown that *pax6* does not act alone and that building an eye requires suites of interacting genes. Discussion about the evolutionary origins of eyes was invigorated by the discovery that homologous genes can trigger construction of paralogous systems for photodetection, just as homologous *hox* genes do for paralogous body parts across phyla (*36*).

Eye development proceeds via morphological transformations of newly generated tissue that are regulated by multiple genes with expression patterns that overlap in time and space. Functions for at least 15 transcription factors and several signaling molecules have been described for human and mouse eye development, many of which are also widely expressed in other tissues. For *Drosophila* photoreceptor arrays, it is now known that seven genes [*eyeless* (*ey*), *twin of eyeless* (*toy*) (both of which are *pax6* homologs), *sine oculus* (*so*), *eyes absent* (*eya*), *dachshund* (*dac*), *eye gone* (*eyg*), and *optix*] collaborate (*37*). These genes, in combination with the Notch and receptor tyrosine kinase pathways and other signaling systems, act via a complex regulatory network (*37*).

Deletion of any one of the seven genes causes radical reduction or complete loss of the *Drosophila* eye. Yet in collaboration with certain signaling molecules, any one of them, except *sine oculus*, can cause ectopic expression of an eye. Like other developmental cascades, a network of genes is required for organogenesis. *Six1*, *Dach*, and *Eya* are important in the formation of the kidney, muscle, and inner ear, as well as eyes, which suggests that this suite of genetically interacting gene products may have been recruited repeatedly during evolution for formation of a variety of structures (*38*).

Appearance of photodetection systems probably happened many (possibly hundreds of) times, until selection produced at least the two independent, main types of photoreceptor types known today—ciliary and rhabdomeric (Fig. 3). The other opsin families likely also have photodetection capacities, mediated by structures still unknown. Although the two main photoreceptor types were thought to be strictly segregated into vertebrates (ciliary) and invertebrates (rhabdomeric), recent studies show that elements of both photoreceptor types probably coexist in most organisms.

An overlooked hint about the existence of multiple photodetection systems came from the discovery of both depolarizing and hyperpolarizing responses to light stimuli from cells located in different layers of a scallop retina (*Pecten irradians*). Depolarizing potentials, characteristic of invertebrate photoreception, arise from the proximal layer, and hyperpolarizing potentials, characteristic of vertebrate photoreception, arise from the distal layer (*39*). In 2004, Arendt and colleagues (*40*) found that the polychete ragworm (*Platynereis dumerilii*) had ciliary photoreceptors in the brain in addition to rhabdomeric photoreceptors in its eyes. The canonical opsins associated with each photoreceptor type were localized only with its type (e.g., vertebrate c-opsin with ciliary receptors in the brain and invertebrate r-opsin with rhabdomeric receptors in the eye). Thus, both main types of "eyes" exist in a worm. Correspondingly, in vertebrates, Berson and colleagues (*41*) had found that a small population of intrinsically photosensitive retinal ganglion cells (the neural output of the retina) use melanopsin, a member of the r-opsin family. Melanopsin in these neurons functions via transduction pathways like those in invertebrates and signals presence or absence of light in parallel to and collaboration with the well-known image-forming visual system (*42*).

Arendt (*43*) proposed that rhabdomeric photoreceptors might be the evolutionary ancestors of vertebrate ganglion cells because of their use of r-opsin and the expression of a constellation of transcription factors including pax6, Math5, Brn3, and BarH. Further, he suggested that other retinal processing neurons, horizontal and amacrine cells, might also share in this rhabdomeric photoreceptor ancestry, but have lost photosensitivity. Taken together, these data show that at least two kinds of photoreception existed in the Urbilateria, before the split into three Bilateria branches at the Cambrian. Moreover, each branch of the family tree still carries versions of both of these photoreceptor types, along with other opsin-dependent photodetection systems yet to be fully described. In the course of evolution, vertebrate vision favored ciliary photodetection for the pathway that delivers images, whereas invertebrates favored rhabdomeric photodetection for their main eyes, although why this might be remains unknown. Along both evolutionary paths, secondary photodetection systems remained to give additional information about light, possibly to instruct

circadian rhythms, phototaxis, or other light-dependent behaviors. But, if vertebrates are an example, these two photodetection systems functioned together, rather than remaining separate. Although the remaining five families of opsins have not been fully characterized, it seems probable that they also respond to light, and organisms use the information they provide.

Genomics and Eye Evolution

For decades, scientists have given considerable attention to the primary imaging system in vertebrates, myopically focused on the function of rod and cone photoreceptors and the visual information they deliver. The discovery that animals have multiple parallel pathways to extract information from light and that these coexist in invertebrates, as well as in the eyes of vertebrates, offers new vistas for discovery in development, function, and evolution of eyes and these other novel systems. Genomics could now be used to identify gene regulatory network kernels, similar to those proposed for body plans, for eyes and their parallel systems. Development in a broader phyletic sample of invertebrate eyes could be instructive in helping identify such developmental networks and also for locating other photosensitive systems. Genetic methods have been used to reveal how photoreceptive ganglion cells interact with conventional photoreceptors functionally in mice, and these techniques could now be extended to identify the functions of the other opsin-based systems. Finally, there are abundant evolutionary questions that might be resolved through genomic approaches. Are the inner retinal neurons actually derived from photosensitive precursors? Are there other convergent optical systems like that of cephalopods and vertebrates with common genetic substrates that could be identified and compared? Is the unusual new opsin identified in the parietal eye (*9*) widespread and will its novel phototransduction system shed light on evolution? Light has been such an important source of information that evolution has exploited it in many ways that remain to be discovered and understood.

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REVIEW

Genomic Evolution of Hox Gene Clusters

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The family of Hox genes, which number 4 to 48 per genome depending on the animal, control morphologies on the main body axis of nearly all metazoans. The conventional wisdom is that Hox genes are arranged in chromosomal clusters in colinear order with their expression patterns on the body axis. However, recent evidence has shown that Hox gene clusters are fragmented, reduced, or expanded in many animals—findings that correlate with interesting morphological changes in evolution. Hox gene clusters also contain many noncoding RNAs, such as intergenic regulatory transcripts and evolutionarily conserved microRNAs, some of whose developmental functions have recently been explored.

Hox genes encode a large family of closely related transcription factors with similar DNA binding preferences. They have not been found in sponges, protozoa, or plants but are present in multiple copies in cnidarians and all bilaterian animals. As a distinct branch of the homeobox gene superfamily, Hox genes have been a source of fascination since their discovery because of their powerful functions in diversifying morphology on the head-tail axis of animal embryos. This power is revealed by dramatic duplications of head-tail axial body structures, called homeotic transformations, that can form when one or more of the Hox genes are activated in inappropriate axial positions in developing animals (*1*). The different HOX transcription factors are expressed in distinct, often overlapping, domains on the head-tail body axis of animal embryos (Fig. 1A), and assign different regional fates to these axial domains. As development proceeds, "head" HOX proteins specify the cell arrangements and structures that result in (for example) chewing organs, "thoracic" HOX proteins specify (for example) locomotory organs, and "abdominal" HOX proteins specify (for example) genital and excretory organs. Not surprisingly, extreme homeotic transformations are lethal at early stages of development. Hox genes are also of great interest because there is abundant correlative evidence that changes in Hox expression patterns and protein functions contributed to

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