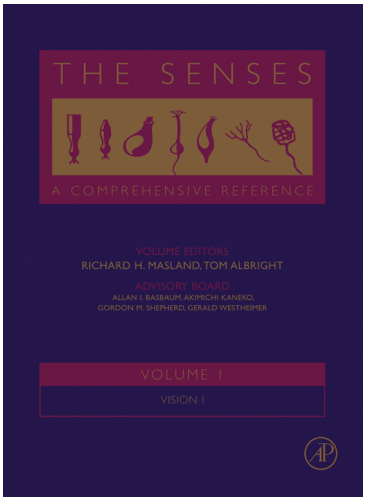

R D Fernald, Evolution of Vertebrate Eyes. In: Allan I. Basbaum, Akimichi Kaneko, Gordon M. Shepherd and Gerald Westheimer, editors The Senses: A Comprehensive Reference, Vol 1, Vision I, Richard Masland and Thomas D. Albright. San Diego: Academic Press; 2008. p. 9-24.

1.02 Evolution of Vertebrate Eyes

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Glossary

monophyletic A group of organisms or traits arising from a single, inferred common ancestor or ancestral trait.

polyphyletic A trait which evolved independently in different organisms.

phylogeny The evolutionary relatedness amongst organisms.

photoreceptor Specialized type of neuron found in the eye capable of converting light into a neural signal.

phototransduction The process of converting light energy into neural energy.

1.02.1 Introduction

Sunlight provides energy essential for all life on earth and is a profoundly important selective force, which has driven the evolution of processes that harvest the sun's energy. However, equally important, light is the premier source of information for many species driving evolution of light-sensing organs, including eyes that harvest information. So since the beginning of biological evolution on our planet over 5 billion years ago, sunlight has both fueled and informed life. Light, and the light/dark cycle from the rotating earth may be second only to sex as the most important selective force that has acted on biological organisms. One of the most remarkable evolutionary consequences of sunlight has been the evolution of mechanisms that convert photons into signals useful to organisms. Understanding the evolutionary history of eyes, however, has been vexed, because their fossil remains give limited information about their function and origins. So in understanding the genetic, biochemical, and structural remnants of eye evolution, Ernst Mayr's dictum: "evolution is an affair of phenotypes" provides a guide. This is particularly true when trying to uncover commonalities amongst the varieties of eyes and mechanisms to convert photons into energy useful to their owners.

How did eyes evolve? Darwin knew that eyes offered a special challenge to evolutionary thinking stating "... that the eye ... could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree" (Darwin, C., 1859). This is the most frequently cited quote of Darwin about eyes but he also wrote: "Reason tells me, that if

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numerous gradations from a simple and imperfect eye to one complex and perfect can be shown to exist, each grade being useful to its possessor, as is certainly the case; if further, the eye ever varies and the variations be inherited, as is likewise certainly the case; and if such variations should be useful to any animal under changing conditions of life, then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, should not be considered as subversive of the theory" (Darwin, C., 1859). Though understanding eye evolution challenges the imagination, several new findings have changed our fundamental understanding about the origins of eyes.

New insights about eye evolution at the molecular level are the discovery of clusters of genes implicated in eye development that are conserved in eyes across large phylogenetic divides. Moreover, it is now clear now that vertebrate genomes contain nearly twice as many genes encoding light-transducing opsin proteins as previously known. But most importantly, physiologists have identified two fundamentally different kinds of photodetection systems in single organisms. In fact, within the eye of vertebrates, there are now known to be two fundamentally different kinds of phototransduction cascades, apparently collaborating to interpret information from light signals. Taken together, these new insights provide a much clearer story about how and how often eyes arose during evolution.

1.02.2 Structural and Functional Adaptations in Eyes

1.02.2.1 General Constraints

Walls G. L. (1942), in his monumental book provided remarkable insights about the many features of the vertebrate eye with detailed drawings showing the range and variety of vertebrate eye phenotypic characteristics. The variety of adaptations in eyes produced by selective pressures for vision in different habitats is truly astonishing. But eye structures depend critically on the physical properties of light which sets limits on how light can be collected and focused. For example, eyes have evolved to be sensitive to only a narrow range of wavelengths, relative to the broad spectrum of energy produced by sunlight (see Figure 1). This is probably because early evolution occurred in water that reduces light dramatically as a function of wavelength (Fernald, R. D., 1988). Selection favored biochemical mechanisms sensitive in this limited range of wavelengths and set the sensitivity for subsequent evolution of light detection. Many species have long lived on land where they are exposed to the broader spectrum of electromagnetic radiation from the sun, yet most animal eyes transduce light only within the original narrow band dictated by water. Some insects and species of fish and birds later evolved additional receptor types for ultraviolet light (e.g., Viltala, J. *et al.*, 1995) in response to the terrestrial

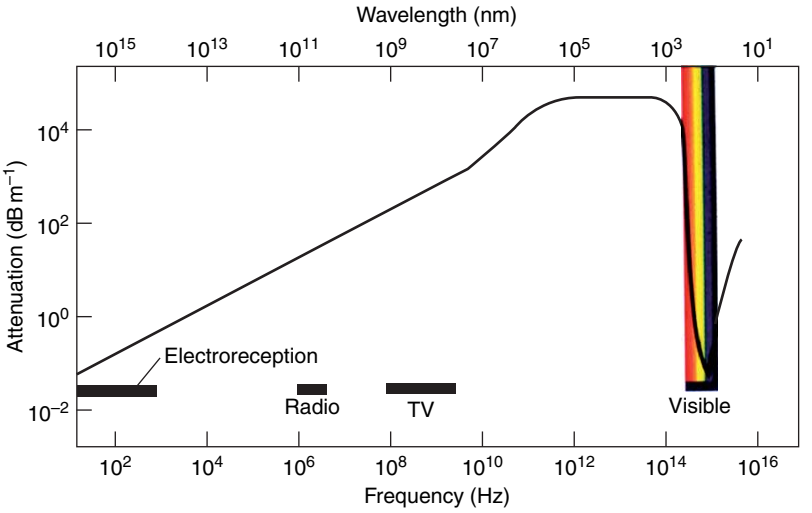

Figure 1 The attenuation (decibels per meter) of electromagnetic (EM) radiation by water as a function of wavelength (nm; top) and frequency (Hz; bottom). Significant amounts of EM radiation cannot pass through water except in two ranges: under 10^3 Hz and from 10^{14} to 10^{15} Hz. Animals have exploited these two ranges for communication: weakly electric fish communicate using the low range and visible light used by all eyes is in the upper range. Adapted from Fernald, R. D. 1988. Aquatic Adaptations in Fish Eyes. In: Sensory Biology of Aquatic Animals (*eds.* J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga), pp. 185–208. Springer.

environment. Thus the narrow range of wavelength sensitivity is a residual reflection of our aquatic origins and illustrates how early evolutionary solutions persist in the evolved organs.

Among animals, of the ~33 animal phyla, about one-third have no specialized light-detecting organ, one-third have light sensitive organs, and the remaining third have eyes (Land, M. F. and Nilsson, D. E., 2002). Image-forming eyes have appeared in six 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 (Land, M. F. and Fernald, R. D., 1992), suggesting that eyes contribute to evolutionary success. Existing eyes range in size from a fraction of a millimeter to tens of centimeters in diameter and the range of types and locations suggests that they can evolve relatively easily (see below).

1.02.2.2 Optical Constraints

Only three types of image forming optical systems have evolved in eyes (Figure 2): images formed by shadows, by refraction (e.g., lens and/or cornea), or by reflection as first systematically studied by Land M. F. (1981). Since physical laws governing light fundamentally limit how an eye can function, similar structures have evolved in distinctly unrelated animals such as fishes and cephalopods. The chambered or camera eyes in these two lineages are very similar in a large number of details, despite the fact that their owners are phylogenetically quite distant (Packard, A., 1972; Fernald, R. D., 2006). Both evolved spherical lenses to achieve sufficient refractive power for focusing light underwater, but the inverted retinal layers of fishes and all vertebrates are distinctly different from the noninverted, somewhat simpler retinae of cephalopods. Moreover, each group uses a different family of opsin molecules and different transduction cascades to process photons. Macroscopically, these eye types and the animals bearing them are not homologous, even though there are striking similarities and even some homologies at the molecular and developmental levels. This seeming contradiction lies at the heart of understanding eye evolution.

The greatest variety of eyes has been found in invertebrates, which have both camera eyes (e.g., cephalopods) and compound eyes (e.g., *Drosophila*). Moreover, invertebrates also have the greatest variety in eye number and location in particular species. Whereas vertebrates have paired, chambered, lensed eyes on the head, invertebrates species may have multiple, nonpaired eyes and eyes in remarkable locations. For example, certain butterflies have light-detecting organs located such that darkness signals successful copulation (Arikawa, K. *et al.*, 1996a; 1996b). In addition, Nordström K. *et al.* (2003) described a visual system in the planula of a box

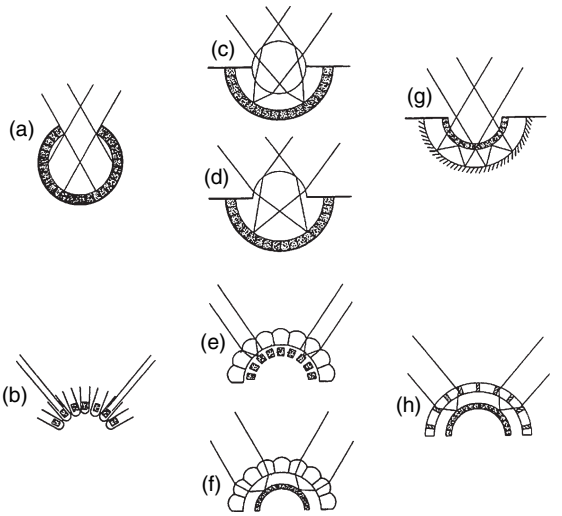

Figure 2 Major eye optical types found in animals. Chambered eyes (top: (a), (c), (d), and (g)) and compound eyes (bottom: (b), (e), (f), and (h)). Eyes form images using shadows (a, b), refraction (c–f), and reflection (g, h). The simple pit eye (a) led to the lensed eyes in fish and cephalopods (c) and terrestrial animals (d). Scallop eyes (g) are chambered but use concave mirror optics to produce an image. The simplest compound eye (b) found in bivalve molluscs led to the apposition compound eye (e) found in bees and crabs, the refracting superposition compound eye (f) of moths and krill and the reflecting superposition eye (h) found in decapod shrimps and lobsters. Adapted from Land, M. F. and Nilsson, D. E. 2002. Animal Eyes. Oxford University Press.

jellyfish *Tripedalia cystophora*, with eyecups directly connected to motor cilium meaning no nervous system processes visual information, making the eyes a complete sensory–motor system.

There is great variation in the capacities of eyes depending ultimately on their structure. For example, resolution of an image, as measured in degrees subtended differs by about 13-fold among vertebrates and even more between vertebrates and invertebrates. Eagles have the greatest acuity that is around 10 000-fold greater than that found in planaria (Land, M. F. and Nilsson, D. E., 2002). Similarly, a comparison of relative sensitivities among vertebrates reveals a range of 4×10^5 between highly sensitive deep sea animal vision and human foveal vision (Land, M. F. and Nilsson, D. E., 2002).

Another remarkable adaptation is differential wavelength sensitivity of photoreceptor types producing what we call hue or color discrimination. The selective pressures for evolution of wavelength discrimination appear to have been quite pervasive. Probably, the added value of better contrast detection, which increases the likelihood of recognizing food, mates, and predators, would have been enhanced with chromatic information (e.g., Nagel, M. G. and Osorio, D., 1993; Osorio, D. and Vorobyev, M., 1996). Indeed, recent work comparing eight primate taxa suggests that trichromatic vision evolved where leaf consumption was critical (Lucas, P. W. *et al.*, 2003). In support of this idea, many species of diurnal reptiles and birds have colored retinal filters, made of oil droplets, which appear to have evolved to increase the number of colors that can be discriminated, suggesting selective pressure for improved color vision (Vorobyev, M., 2003).

1.02.2.3 Lenses: Multiple Protein Types and Gene Sharing

Eyes collect light through an aperture, and focus it with a lens onto photoreceptor cells specialized to convert photons into neural signals. Some eyes exist without pupils and even without lenses (e.g., *Nautilus*), but eyes that evolved to give their owners a clear view of the environment over a short timescale do have lenses. Since lenses are constructed of tightly packed proteins, their evolutionary relationships might provide some insight into eye evolution.

In vertebrates, lenses are formed from modified epithelial cells which have high concentrations of soluble proteins, known as 'crystallins' because they are packed into arrays. In contrast, in most invertebrates, lens proteins are secreted by specialized cells in the eye. Interestingly, lenses of mitochondrial origin have been found in the two pairs of eyes of the parasite *Neoheterocotyle rhinobatidis* (Rohde, K. *et al.*, 1999). Despite very different cellular origins, to function optically lens proteins must be distributed to produce a radial gradient of refractive index that is low at the edge of the lens and high in the center (see Kroeger, R. H. H. *et al.*, 1999). An exact gradient of refractive index is essential for vision in animals living in water but such gradients are also found in lenses of terrestrial vertebrates and invertebrates. Perhaps most remarkably, cephalopods assemble their spherical lens from two distinct embryological sources, yet manage to produce the obligatory gradient of refractive index (Jagger, W. S. and Sands, P. J., 1999).

Until quite recently, the 10 or so crystallin proteins found in lenses were thought to be unique to lens tissue, presumed to have evolved for this function and formed a closely related phylogenetic family. Of the large number of crystallins, alpha and beta–gamma crystallins are indeed specialized lens proteins in vertebrates, related to heat shock protein and schistosome egg antigen, respectively. However, the many remaining vertebrate lens proteins are a not conserved but rather are a diverse group, many of which are used as enzymes elsewhere in the body. Surprisingly, most of these taxon-specific lens proteins are actually products of the same genes as the enzymes, a double use that has been termed 'gene sharing' by Wistow G. (1993a; 1993b). For example, a crystallin protein in the duck lens was shown to be similar to a metabolic enzyme, argininosuccinate lyase. Both the lens protein and the metabolic enzyme are encoded by the same gene, not from duplicated genes and such sharing might be a prelude to gene duplication. Such molecular opportunism is so effective that it has also occurred both in cephalopods (Tomarev, S. I. and Zinovieva, R. D., 1988) and in *Drosophila* (Janssens, H. and Gehring, W. J., 1999). One possibility is that since lenses need the production of a relatively large amount of protein, genes that have been successfully upregulated in other tissues might be preferentially selected.

Remarkably, the brittlestar (*Ophiocoma wendtii*), form crystal lenses as a part of their skeletal armor from calcite crystals. The crystals, oriented to bring light onto the photoreceptive surfaces in the body, focus the light much as corrective lenses might and effectively concentrate the light by ~50 times (Aizenberg, J. *et al.*, 2001).

The common cellular strategy of assembling lenses from diverse, phylogenetically unrelated proteins seems to be a convergent evolutionary solution that has occurred in many vertebrates independently. The exquisite gradient of the refractive index, which evolved in vertebrates and invertebrates alike resulted because it is the only way known to make an optically useful lens. What remains unknown is how such diverse protein species are assembled and folded to preserve key properties of transparency and refractive index gradient along the axis of the lens. The challenge for understanding lens development is to identify mechanisms responsible for organizing diverse proteins into a functioning lens. However, since lenses appear to have evolved along independent lines, their phylogenetic relationships do not provide a useful window into eye evolution.

1.02.2.4 Capturing Photons: The Opsin/Retinal Solution

Transducing light is the essential function of visual systems and an ancient molecule, opsin, in association with other key players has a long evolutionary history. Vertebrate opsins, also called visual pigments, appeared before eyes (Land, M. F. and Fernald, R. D., 1992) and evolved along at least seven lines, diverging from an ancestral type, before teleost fish diverged from other vertebrates (e.g., Hisatomi, O. *et al*, 1994) and indeed before deuterostomes split from the protostomes (Terakita, A., 2005). This suggests that a common ancestor had multiple opsin genes, which has been recently confirmed (see below). The exact sequence along which opsins evolved is still open to interpretation (e.g., Okano, H. *et al*., 1992), but it is clear that they evolved in parallel.

Opsins are seven transmembrane proteins (30–50 kDa) that associate with a nonprotein moiety, the chromophore, retinal. Among the ~1000 opsin forms that have been described to date, the phylogenetic differences among the seven major groups correspond to specific functional classifications (Figure 3). These classes differ in several ways but perhaps most importantly in their transduction via different G proteins. For example, although both vertebrate and invertebrate photosensitive opsin receptors are G-coupled (e.g., heterotrimeric guanine nucleotide-binding protein-coupled), and both use 11-*cis*-retinal or a close variant as their chromophore, vertebrate rod-and-cone opsins signal through photoreceptor-specific, G proteins called transducins. In contrast, invertebrate opsins signal through the G_q family of G proteins. In addition, photic responses are terminated differently. In vertebrates, excitation is followed by a combination of phosphorylation of the excited opsin, the binding of arrestin proteins which is then followed by regeneration of the active chromophore form needed for photosensitivity (Figure 4). The process in invertebrates is quite different where the G protein is inactivated by its target, phospholipase C.

Opsin function is very well understood (e.g., Menon, S. T. *et al*., 2001) and the adaptive radiation of pigment types due to natural selection for particular wavelength responses has been described for some special cases (e.g., east African cichlids, Sugawara, T. *et al*., 2002; squirrelfish, Yokoyama, S. and Takenaka, N., 2004). Moreover, the evolutionary relationships among rhodopsin molecules is well-known and some

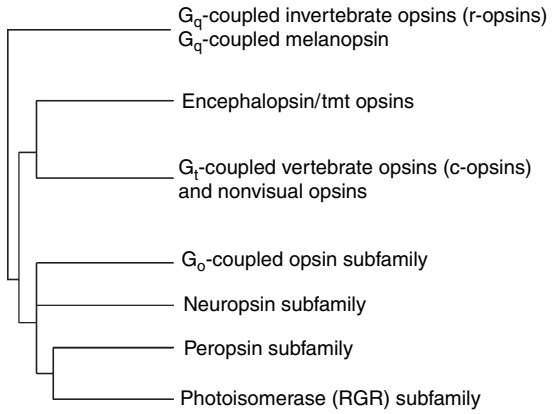

Figure 3 A simplified schematic molecular phylogenetic tree inferred by the neighbor-joining method showing the seven known opsin subfamilies. RGR, retinal G-protein-coupled receptor; TMT, teleost multiple tissue. Adapted from Terakita, A. 2005. The opsins. Genome Biol. 6, 213.

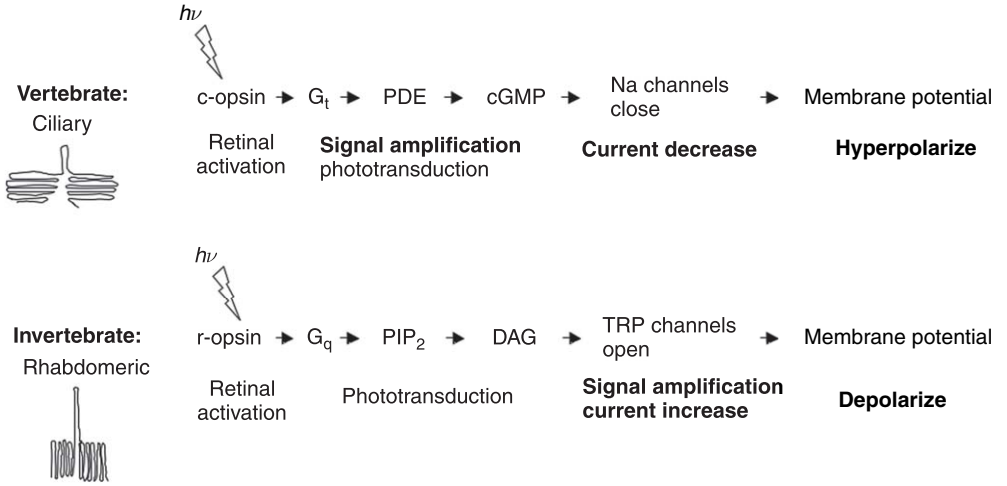

Figure 4 Schematic illustration showing the key differences between a simplified representation of (top) canonical vertebrate ciliary phototransduction and (bottom) invertebrate rhabdomeric phototransduction where $h\nu$ represents incident photon energy. The two different opsin types (c-opsin and r-opsin) 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. cGMP, cyclic guanosylmophosphate; DAG, diacylglycerol; G_q, guanine nucleotide-binding protein α15; G_t, transducin; PDE, phosphodiesterase; PIP_2, phosphatidylinositol-4,5-biphosphate.

of this is based on understanding the interaction between retinal and opsin (Marsh, L. and Griffiths, C. S., 2005). However, there has been considerable variance in spectral sensitivities that likely resulted from specific selective advantages for one solution over another. Detailed comparisons between terrestrial vertebrates and insects, for example, reveal that there are not unique solutions to encoding both spatial and spectral information. Mammals and bees use long-wavelength receptors for luminance and color vision while flies and birds have evolved separate sets of photoreceptors for the two purposes (Osorio, D. and Vorobyev, M., 2005).

Primate photopigments also offer examples of relatively recent evolutionary change in these important molecules. For example, Old World monkeys, apes, and humans have trichromatic vision, while New World monkeys are polymorphic, having dichromatic or trichromatic color vision (Jacobs, G. H., 1996). In this context, *Homo sapiens* may be unique in the polymorphism found in our color vision system (e.g., Neitz, M. *et al*, 1996). The variance in number and kinds of photopigments in the human retina might reflect the reduced selective pressure on color vision. The subtlety of selective pressures on chromatic detection is clear in many species. For example, in the bluefin killifish, the relative abundance of cone types depends on whether the animals live in springs or swamps (Fuller, R. C. *et al*., 2003). The novel differential spectral sensitivity in these populations is produced epigenetically through differential expression of cone classes in the retina, rather than via modification of the spectral tuning of opsin molecules, showing that there are different ways to achieve different kinds of chromatic sensitivity.

Another kind of mechanism modifying wavelength sensitivity in cone photoreceptors that depends on life history changes has been described in Pacific salmon (*Oncorhynchus gorbuscha*) and in winter flounder (*Pseudopleuronectes americanus*). As salmon grow and move from being planctivores living in surface waters where ultraviolet (UV) light is abundant to fish-eating predators in deeper waters where blue-green light prevails, they remodel their UV-sensitive cones with insertion of an opsin that is tuned to blue wavelengths (Cheng, C. L. and Flamarique, I. N., 2004). A similar mechanism has been previously reported in winter flounder (*Pseudopleuronectes americanus*) in which a single opsin type in juveniles, located in hexagonally arranged single cones is replaced by three different opsin types in photoreceptors arranged in a square array after the animal has metamorphosed into an adult (Evans, B. I. and Fernald, R. D., 1993; Evans, B. I. *et al*., 1993).

These examples and abundant others show that animals have evolved eyes with resolution, sensitivity

and wavelength detection to match their needs. Specifically, wavelength sensitivity can change with such life history changes and be quite different in closely related species. The best understood visual transduction mechanisms are those used for the main visual input in both vertebrates and invertebrates. The role of the five other opsin families and their mechanisms of visual transduction are beginning to be understood although a great deal remains mysterious.

1.02.3 Evolutionary Origins

1.02.3.1 Developmental Evidence about Eye Evolution

Logically, eyes might be monophyletic, having evolved from a single progenitor, or polyphyletic, having arisen more than once during evolution. Once it was understood that opsin played a role in phototransduction in all eyes that had been investigated, eyes were thought to have a single, monophyletic origin. The notion was that a phylogenetic tree of one important functional protein, opsin would link all eye types together. However, Salvini-Plawen L. V. and Mayr E. (1977) took a completely different perspective and compared overall structure, photoreceptor types, developmental origins of eye tissue, position of receptor axons, and other anatomical markers among eyes using existing fauna. Based on this analysis, they came to the conclusion that eyes evolved not once but at least 40 different times, and possibly many more (reviewed in Land, M. F. and Fernald, R. D., 1992). This 'multiple-origins' hypothesis, based on morphological evidence was unchallenged for 20 years until results comparing developmental pathways at the molecular level revealed a major surprise. Specifically, Gehrig and co-workers showed that *pax6* could induce ectopic eyes in fruit flies, leading them to dub this a 'master regulatory gene' (Halder, G. *et al*, 1995). Based on these results, Gehring W. J. and Ikeo K. (1999) proposed that because a single, well-conserved gene, *pax6*, could initiate eye construction in mice and flies, eyes must have arisen from a single ancestor. Did eyes appear many times in the course of evolution making them polyphyletic, as claimed by Salvini-Plawen and Mayr based on phenotype or have all eyes 'descended' directly from a common, primitive form, making them monophyletic, as claimed by Gehring *et al.* based on genes controlling development? Since this original debate erupted, there have been several important discoveries showing that eyes must have arisen more than once and, that we carry the evidence in our own eyes!

By the Cambrian period (570–500 million years ago), eyes were present in the form of very simple eyecups, useful for detecting light but not for processing directional information. Although the causes are unknown, explosive speciation, or the 'Big Bang' of animal evolution happened during the Cambrian (Conway-Morris, S., 1998). Existing eye types improved radically, coincident with the appearance of carnivory and predation. The evolution of ocular structures has proceeded in two stages (see Figure 2; Land, M. F. and Fernald, R. D., 1992). First was the production of simple eye spots which are found in nearly all the major animal groups and contain a small number of receptors in an open cup of screening pigment (Land, M. F. and Fernald, R. D., 1992). This kind of detector cannot play a role in recognizing patterns but rather for distinguishing light from dark. The second stage in eye evolution is the addition of an optical system that can produce an image. As noted above, image-forming eyes occur in 96% of known species distributed among the six phyla. Although there are basically only three methods of forming an image, among the known eye types are at least 11 distinct optical systems producing images, the most recently described is a telephoto lens, identified in the chameleon in 1995. Indeed, six of the optical systems have only been discovered in the past 25 years.

Since camera-type eyes are demonstrably superior in several respects (Nilsson, D. E., 1989), why do not all animals have them? Camera-type eyes require big heads and bodies to hold them, which likely restricted the number of animals that have followed this evolutionary path. Also, it is probable that, having evolved one eye type, conversion to another type requires intermediate stages that are much worse or useless when compared with the existing design. This would make a switch essentially lethal to animals that depend on seeing. Although this argument makes sense intuitively, some existing cases of novel optical combinations suggest this is probably not the whole story.

For teaching purposes, textbooks tend to group animal eyes into two groups, the camera-type or 'simple' eyes and the compound eyes, which does reflect a real and fundamental difference in visual systems. However, such a dichotomy conceals a remarkable diversity of optical systems subsumed under each heading.

For example, Nilsson D. E. and Modlin R. (1994) described a mysid shrimp (*Dioptromysis paucispinous*) that has a combined simple and compound eye: partly compound with multiple facets exactly like the eye of an insect, and partly simple with a single lens focusing an image on a sheet of receptors like that of a human. These shrimp are about 5 mm long with nearly spherical eyes at the ends of stalks. In addition to the facets (~800–900) there is a single giant facet facing the shrimp's tail, which the shrimp frequently rotates forward probably to get a better look at something since that facet has ~5 times the acuity (but much lower sensitivity) than the rest of the eye. It is as if the shrimp were carrying a pair of binoculars for the occasional detailed look at something ahead of it. The discovery that simple and compound eye types can be found in a single animal raises the question of how a developmental program could produce this outcome.

1.02.3.2 Developmental Evidence about Eye Evolution

Classical experimentation directed at understanding ocular development focused on vertebrate eyes, a specialized extension of the brain. Experimental models were primarily limited to mice and chicks due to their extensive prior exploitation as model organisms. The beautiful images available today make the often subtle but distinctive morphological changes during eye development seem much more obvious than they were when first observed since it is now possible, with scanning electron microscopy and sophisticated methods of controlling the state of tissue development, to watch unfolding of the production of an eye.

Eyes develop from the prospective forebrain, beginning in the eyefields, which are made up of cells of the anterior neural plate. As the prosencephalon grows, this region moves forward until the optic groove forms, and the neuroectoderm of the groove locally contacts the surface ectoderm, inducing the lens placode. As the placode invaginates to form the lens vesicle, the optic vesicle forms the bilayered optic cup, which ultimately becomes the eye. The interaction between the optic vesicle and the lens placode was identified as the 'organizer of the lens' by Spemann H. (1924). The presumptive lens arises from the lens placode, a thickening of the ectoderm in contact with the optic vesicle. Coincident with this change is the onset of expression of proteins that will form the lens. Other structures of the eye are formed by large- and small-scale tissue movements, caused and accompanied by the expression of tissue-specific genes at that site. The cornea arises from the surface ectoderm over the lens and from migrating mesenchyme derived from the neural crest. Many of the original observations about the role of specific tissue bits in these processes resulted from exquisite embryonic manipulations related to transplantation experiments. For example, Nieuwkoop P. D. (1963) identified, among other things, the source tissue essential for the induction of eye production.

With macroscopic changes described, the next challenge has been to synthesize the phenomenological, macroscopic morphological observations with molecular explanations of eye development and understand what this tells us about evolution. The morphological process of eye development has been viewed as a set of steps toward a final tissue arrangement but underlying this apparently straightforward sequence of large-scale events are distributions of gene expression with substantial overlap in both time and space. Gene expression is closely regulated, and we know that specific genes and gene products are used repeatedly, making the causal relationships among the players difficult to conceptualize. Nonetheless, progress in characterizing the genes responsible for particular steps in eye development has been reasonably rapid, as shown in several recent reviews (Harland, R., 2000; Chow, R. L. and Lang, R. A., 2001; Graw, J., 2003). Functions for at least 15 transcription factors and several signaling molecules have been described in human and mice eyes, based on developmental disorders and/or molecular manipulations (e.g., Graw, J., 2003). As with other molecular actors, the transcription factors and signaling molecules are expressed during ocular development are also essential for normal development in a wide range of other tissues. This suggests that a particular combination of expression patterns and their timing is important for the proper functioning of these genes in eye development.

It is known that the paired box gene 6 (*pax6*), a member of the family of genes that encode transcription factors with a homeodomain and a paired domain, appears to be important in eye formation across many species. The remarkable demonstration that *pax6* can induce eyes where they should not be ('ectopic') in *Drosophila* (Halder, G. *et al.*, 1995), and similar subsequent demonstration in vertebrates (Chow, R. L. *et al.*, 1999), led to the suggestion that there might be 'master control genes' responsible for

development and differentiation of ocular tissue in many species. Subsequent work has shown, however, that 'master control gene' is a misnomer since a suite of genes are required, collectively, to initiate eye development, all of which are essential. Moreover, as noted above, the genes in question actually have dynamic spatial and temporal expression during many stages of eye development, in addition to expression for essential purposes in other tissues and organs in the brain and elsewhere. Nonetheless, it is remarkable that some of the same genes appear in the context of eye development, despite great evolutionary distance among the owners of the eyes. How this might have occurred is discussed below.

For *Drosophila* eyes, the story has become considerably more complex. It seems that not one but a collection of seven genes, encoding transcription factors and two signaling molecules collaborate to make eyes (reviewed in Kumar, J. P., 2001). These nuclear factors (*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*) and signaling systems, including the Notch and receptor tyrosine kinase pathways, act via a complex regulatory network that is reasonably well understood (see Kumar, J. P., 2001; Figure 1). The master gene hypothesis is not supported, because deletion of any of these genes causes loss or radical reduction in the *Drosophila* compound eye and, surprisingly, any gene except *sine oculus,* in collaboration with certain signaling molecules, can cause ectopic expression of an eye in a limited set of imaginal disks. This means that the whole troupe is needed to produce a reasonable eye. Why this might be so is suggested by recent work showing that the *eya* gene products are phosphatases, the first case in which a transcription factor can itself dephosphorylate other proteins to fine tune gene expression (Li, X. *et al*., 2003). This elegant work demonstrated the details of interactions among *Six1*, *Dach*, and *Eya* in the formation of the kidney, muscle, and inner ear, as well as eyes, suggesting that this suite of genetically interacting proteins has been recruited repeatedly during evolution for organogenesis of different structures.

It is difficult to abandon the heuristic of hierarchical regulatory processes in development originally proposed by Lewis to characterize homeotic properties of bithorax and antennapedia genes but molecular analysis of eye development shows that this concept may not be useful. Instead, eye development appears to need new ways of thinking about how complex tissues are made and how such organs arose in evolution. The widespread and redundant activities of specific genes during ocular development (e.g., Chauhan, B. K. *et al*., 2002; Baumer, N. *et al*., 2003) suggest that hierarchies, if they exist, are unknown and the more likely scenario is the orchestrated activity of a suite of molecular actors.

As described above, the diversity of eyes confirms their dynamic evolutionary past. Explosive speciation, or the 'Big Bang' of animal evolution happened during the Cambrian (Conway-Morris, S., 1998), when existing eye types appear to have improved radically, coincident with the onset of carnivory and predation. Many selective forces were likely at work (Fernald, R. D., 2000), including perhaps the first instances where light enabled behavioral signals (Parker, A. R., 1998) so no predominant selective force can be claimed. The rapidity of eye evolution has always been a question, but, using a simulation, Nilsson D. E. and Pelger S. (1994) suggested that about 2000 sequential changes could produce a typical image-forming eye from a light sensitive patch. With reasonable estimates, this suggests that an eye could evolve in less than half a million years making the virtual explosion of eyes during the Cambrian seem quite reasonable (Land, M. F. and Nilsson, D. E., 2002). After the Cambrian, three phyla emerged: arthropods, mollusks, and chordates. Although these groups all use the opsin molecule to capture light, details of the structure and function of their eyes differ considerably.

One of the most interesting developmental differences among extant eyes is the embryonic origin of the different structures comparing the camera eyes in vertebrate and cephalopod eyes (summarized in Nilsson, D. E., 1996). Cephalopod eyes form from an epidermal placode through successive infoldings, whereas vertebrate eyes emerge from the neural plate and induce the overlying epidermis to form the lens as described above. It is also noteworthy that the cephalopod eyes lack a cornea, which is present in all vertebrates whether aquatic or not. In addition to the differences in embryonic origin, photoreceptor cells divide into either ciliary or microvillar structures to provide the membrane surface for the opsin molecule (Salvini-Plawen, L. V. and Mayr, E., 1977). Microvilli predominate in invertebrates, whereas vertebrate photoreceptors are ciliary. Physiological responses are also quite different, with the microvillous receptors of arthropods and mollusks depolarizing to light, and the ciliary receptors of vertebrates hyperpolarizing to light. In phototransduction, vertebrate photoreceptors exploit

cyclic guanosine 5′-monophosphate (GMP) as a second messenger system, while invertebrates use inositol trisphosphate (Fernald, R. D., 2000). And, even though opsin is the key molecule for detecting light, mechanisms for regeneration (e.g., reisomerization) of the chromophore/opsin system are dramatically different among phyla (Gonzalez-Fernandez, F., 2003).

1.02.3.3 Functional Evidence about Eye Evolution

Until recently, the photo detection systems we understood well were localized primarily to eyes and pineal glands and a few other sites in the body such as the skin. For each of these, a canonical opsin and related transduction cascade were known. Specifically, ciliary structures associated with specific G proteins are known from vertebrate eyes and microvilli associated with inositol phosphate signaling cascades are known from invertebrate eyes (see above). Then, in several laboratories, each of these phototransduction cascades was found in unexpected organisms. Arendt D. *et al.* (2004) found that the polychete ragworm (*Platynereis dumerilii*) in addition to the rhadomeric photoreceptors in its eyes, had ciliary photoreceptors in the brain. This group also showed that the typical types of opsins associated with each photoreceptor type were both expressed in the ragworm and localized only with that type (e.g., vertebrate c-opsin in the brain and invertebrate r-opsin in the eye). This means that the two main types of 'eyes' exist in a worm.

The idea that two kinds of photoreceptors might exist in an invertebrate was first suggested by the pioneering work of Gorman who, with co-workers showed physiological and morphological data suggesting both types of photoreceptors exist in a scallop, *Pecten irradians* (Gorman, A. L. F. and McReynolds, J. S., 1969; Gorman, A. L. F. and McReynolds, J. S., 1971). These investigators found depolarizing and hyperpolarizing responses to light stimuli from cells located in different layers of the scallop retina, with depolarizing potentials arising from the proximal layer and hyperpolarizing potentials form the distal layer. The investigators interpreted their data solely with respect to the various kinds of selective advantages each response type might have but did not consider the evolutionary implications though their data support the existence of the two canonical receptor types in one organism.

Somewhat earlier, in vertebrates, a parallel set of results appeared. A small population of intrinsically photosensitive retinal ganglion cells were been discovered that play key roles in the regulation of nonvisual photic responses. Surprisingly, these rely on melanopsin (see Figure 3), an opsin first identified in vertebrate melanophores, brain and eyes by Provencio I. *et al.* (1998). The melanopsin in the retina was found to underlie photosensitive ganglion cells discovered by Berson D. M. *et al.* (2002), shown to be required for normal light-induced circadian phase shifting (Panda, S. *et al.*, 2002) and yet could not function without the presence of normal rods and cones (Ruby, N. F. *et al.*, 2002). Taken together, this meant that signals from the photosensitive ganglion cells were being combined with those from rods and cones somewhere in the visual system.

Photosensitive ganglion cells were then thought to comprise a nonimage-forming system that can detect the presence or absence of light but not much more. Subsequent functional analyses showed that retinal melanopsin functions via a phototransduction cascade that resembles invertebrate opsins and, in another similarity to invertebrates has intrinsic photoisomerase activity (Panda, S. *et al.*, 2005; Qiu, X. *et al.*, 2005). Adding to the remarkable set of discoveries, melanopsin-expressing ganglion cells in the primate retina have been shown to signal color and radiance levels to the lateral geniculate nucleus (Dacey, D. M. *et al.*, 2005). So, not only do vertebrates carry a version of the invertebrate visual transduction system with them, but it is used in a variety of ways, including to provide information to the 'image-forming' visual system.

There are several remarkable conclusions to be drawn from this work. First, these findings show that at least two kinds of photoreception existed in the Urbilateria, before the split into three Bilateria branches at the Cambrian (Figure 5), and, importantly, each of these branches still carry versions of these two systems. It is noteworthy that cryptochromes, also discovered very recently (Cashmore, A. R. *et al.*, 1999) are another photoreceptive system that is not based on opsin, has no molecular amplification and is found in both plants and animals. To date, cryptochromes have been shown to play a role in circadian rhythms (Green, B. C., 2004) and control of the iris muscle in birds (Tu, D. D. *et al.*, 2004) as well as many functions in plants.

Second, the two independently evolved light transduction pathways that coexist in both vertebrates and invertebrates now collaborate in collecting and processing information from photons. Although the evolutionary statement, 'survival of the

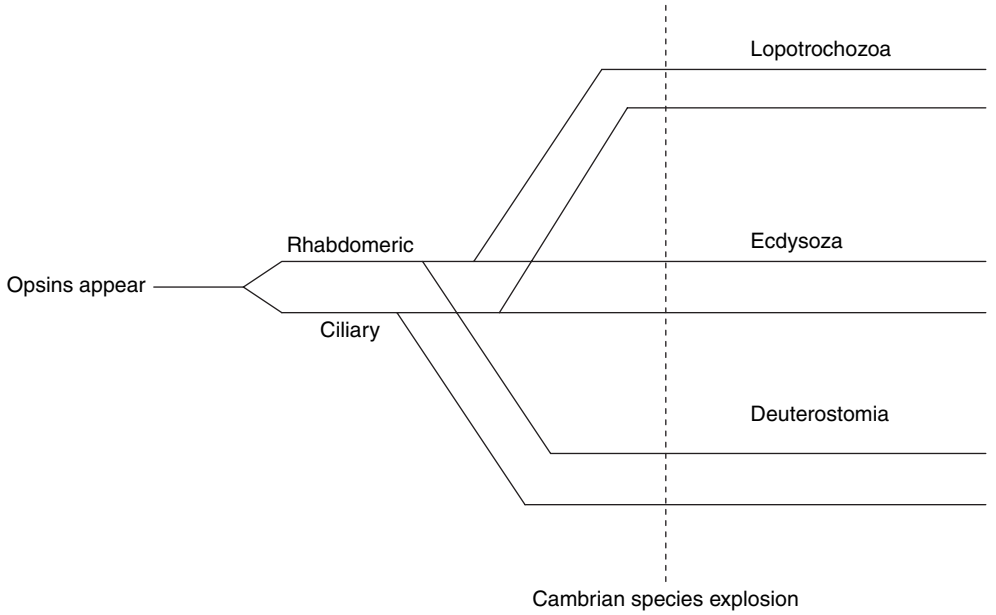

Figure 5 Schematic phylogeny of the Bilateria showing that the distinct rhabdomeric and ciliary organizaton of opsins preceded the split of the urbilateria. Adapted from Nilsson, D.-E. 2005. Photoreceptor evolution: ancient siblings serve different tasks. Curr. Biol. 15, R94–R96.

fittest' suggests a single survivor in an evolutionary race, here we see two contenders coexisting and even working together to inform adult organisms about information contained in light.

Third, since seven families of opsin have been described in vertebrates, including humans (see Figure 3 above), we can expect more surprises in how animals detect and use light. The additional opsins discovered recently have not yet been functionally characterized but the evidence suggests that there are no more opsins to be discovered (Kumbalasiri, T. and Provencio, I., 2005). Even so, figuring out how existing opsins might work together is an important challenge.

1.02.3.4 Other Solutions to Capturing Photons

As described, a persistent issues in the evolution of eyes is whether eyes evolved once or many times. Though it seems quite clear now that there were at least two kinds of phototransduction (e.g., ciliary and rhabdomeric) before the urbilateria split into three families (see Figure 4), energy and information are harvested in archaea and eukaryotic microbes using a system that clearly arose independently, via convergent evolution. Microbial, or type 1 rhodopsins, named to distinguish them from the visual pigments or type 2 rhodopsins, function to harvest light for energy, to guide phototaxis and probably many yet undiscovered functions (Spudich, J. L. *et al.*, 2000). While the number of known type 2 ('visual') rhodopsins has increased as described over the past several years (see above), the number of known type 1 rhodopsins has rapidly increased with the harvesting and genetic sequencing of ocean samples from a handful to over 800 (Spudich, J. L. and Jung, K. H., 2005). These type 1 rhodopsins are widely dispersed on the planet, found in organisms living in both fresh and sea water, salt flats, and glacial seas among others.

There are several fundamental differences between types 1 and 2 rhodopsins. First, there is no evident phylogenetic relationship between the genetic sequences of type 1 and type 2 rhodopsins. As more type 1 opsins are discovered, a connection may become apparent, but given the current state of knowledge, this seems unlikely. Second, the type 1 rhodopsins reveal convergent solutions to the mechanisms for converting photon energy. Both rhodopsin types consist of seven transmembrane domain proteins and, in each, retinal is attached in a Schiff base linkage via a lysine residue in the seventh helix (Spudich, J. L. *et al.*, 2000). However, type 1 rhodopsin (25–30 kDa) has a different organization of its intramembrane domains from type 2 rhodopsin (35 kDa), which reflects the fundamental difference in their signaling cascades.

Whereas type 1 rhodopsins function within the membrane to pump ions or signal to other integral membrane proteins, type 2 rhodopsins signal via G proteins, receptor kinases via the cytoplasmic loops (see above and Spudich, J. L. *et al*., 2000). Retinal is used in association with both apoproteins but these are photoisomerized quite differently. In the familiar, type 2 rhodopsins, 11-*cis*-retinal is transformed to all trans upon absorbing light while in type 1 rhodopsins, all-*trans*-retinal is transformed to 13-*cis* when absorbing light.

Taken together, the remarkable convergence of type 1 and 2 rhodopsins suggests that in the course of evolution, an opsin apoprotein, associated with retinal has been discovered and exploited twice. Clearly, when the seven transmembrane protein is appropriately solvated with retinal, it is useful for transforming the energy of photons into more useful forms. This also suggests that progenitors of the type 1 opsins may have existed in earliest evolution before the divergence of archaea, eubacteria, and eukaryotes. This means that the light-driven ion transport mechanism for deriving energy used in association with retinal 1 preceded the evolution of photosynthesis as a means for using the sun's energy (Spudich, J. L. and Jung, K. H., 2005). We can now wonder whether a proto eye-like structure using rhodopsin 1 remains to be found that would allow a comparison of an additional independent solution to extracting information from light.

1.02.4 How Did Eyes Evolve?

Eyes exist in a variety of shapes, sizes, optical designs, and locations on the body, but they all provide similar information about wavelength and intensity of light to their owners. Different tissues have been recruited to build lenses and retinas across the phyla (Fernald, R. D., 2006). In contrast, all eyes share the same mechanism of absorbing photons since the opsin–chromophore combination has been conserved across phylogeny. Despite new findings yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, particularly since the discovery that two photodetection systems had evolved prior to the split of the urbilateria into three families. Clearly, eye as we know them contain homologous molecules responsible for many structural, functional and even developmental features. Given a growing list of homologous gene sequences among molecules in the eye across vast phylogenetic distances, the challenge is now to discover what makes the eyes of *Drosophila*, squid, and mouse so different. Understanding what makes eyes different may be a bigger challenge than finding what they have in common.

It seems increasingly evident that as eyes evolved, different functional mechanisms have been generated by recruiting existing gene programs. From genome sequencing, we know that there are far fewer genes in organisms than previously thought, so the use and reuse of genes and their products in combinatorial assemblies as reported for known genomes make sense. In the development of eyes, this seems to be the rule not the exception. Specifically, in the evolution of eyes, it seems likely that light sensitivity evolved early in the Cambrian in the form of a proto-opsin molecule in association with the chromophore, retinal. This molecular combination, sensitive to light, became associated with the genes *pax6* (Sheng, G. *et al*., 1997), and possibly, *eya* (based on its phosphatase activity (Li, X. *et al*., 2003)). One can imagine that this combination was recruited and worked well in early evolved eyespots and other light-sensing organs. It would not be surprising, for example, to find these genetic players in the recently described eye without a nervous system (Nordström, K. *et al*., 2003).

Important insights about how regulatory gene networks might have evolved comes from what is called the 'hox paradox' (Wray, G. A., 2002). During development, orthologous genes are expressed in superficially similar domains during embryonic development of very different organisms (e.g., *Drosophila*; mouse) yet these embryos produce adults that are anatomically quite distinct having very few structures with common ancestors. Though not completely resolved, one resolution of this paradox is that there has been evolutionary convergence in the use of some genes and hence apparent homology (Wray, G. A., 2003). It seems that this is the likely scenario for the evolution of eyes. Some genes have been recruited into regulatory gene networks repeatedly, possibly committed early in evolutionary history and kept because they simply work well.

As different eye types evolved over time, there was probably repeated recruitment of particular gene groups, not unlike improvisational groups of actors, interacting to produce candidates for selection. The evolutionary fiddling through which various combinations or routines were tried could have led to numerous parallel evolutionary paths for eyes as we now envisage.

From this, two different mechanisms for transmitting the photic information to surrounding cells were

selected for, one in ciliary and one in rhadomeric photoreceptors. These two systems are likely present in all organisms as described above for worms and mice. The big surprise is that both of these transduction systems persisted with each selected as the primary visual system for a major branch of animals. So, the answer to the question of whether eyes evolved from a single prototypical eye (monophyletic), or whether they evolved repeatedly (polyphyletic), appears to be that quite evidently eyes arose at least twice and probably many times. And, as described above, given the vast number of organisms using rhodopsin 1, we should not be surprised if additional eyes appear in the biological world in the future.

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