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The Evolution of Eyes

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Abstract
Eyes are the preeminent source of sensory information for the brain in most species, and many features of eyes reflect evolutionary solutions to particular selective pressures, both from the nonbiological environment and from other animals. As a result, the evolution of eyes, among all the sense organs, has attracted considerable attention from scientists. Paired eyes in the three major phyla, vertebrates, arthropods and mollusks, have long been considered to be classic examples of evolutionary convergence. At the macroscopic level, this must be true since they arise from different tissues and have evolved radically different solutions to the common problem of collecting and focusing light. However, opsin, the light-absorbing receptor protein, has a significant amount of shared DNA sequence homology across the phyla, and recently it has been discovered that some part of ocular development in different phyla is coordinated by a homologous gene, *Pax-6*. So, although eyes from diverse phyla are clearly not homologous, neither can they be viewed as resulting solely from convergence. Instead, this shows that homology at the molecular level of organization does not predict homology at the organ or organismic level. The presence of homologous constituent molecules in nonhomologous structures reminds us that molecules are not eyes.

Introduction

Since the beginnings of biological evolution over 5 billion years ago, sunlight has fueled life and defined biological time on Earth. Light and the light/dark cycle were probably the most important selective forces ever to have acted on biological organisms, and they remain so. One of the most remarkable consequences of light on earth has been the evolution of vision. Based on paleontological evidence, eyes are thought to have evolved independently in different organisms at least 40 times and probably as many as 65 times [Salvini-Plawen and Mayr, 1977], confirming their importance to animals. Image-forming eyes arose 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 and Fernald, 1992]. Extant eyes have many shapes and sizes, reflecting the diverse solutions to the problem of obtaining an image.

Rather remarkably, however, homologous opsin proteins, essential for catching photons, are present in eye forms from widely divergent phyla. Recently it has become clear that homologues of another molecule, *Pax 6*, play a role in the development of arthropod, mollusk, and vertebrate eyes and share an evolutionary history evident at the molecular level [Halder et al., 1995b]. That is, structural and developmental aspects of distinctly polyphyletic ocular structures can be shown to be monophyletic using molecular sequence comparisons as well as specific developmental tests. How is this possible? Darwin [18959] penned a famous passage, anticipating that the evolution of the eye would become a target for attacks on his theory because

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eyes are such 'organs of extreme perfection and complication'. He wrote: '... that the eye ... could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree.' More than a century later, with new insights that span the molecular to the macroscopic, new mysteries reinforce Darwin's prescient writing. We still have much to learn from the evolution of eyes, both about eyes and about the process of evolution itself.

Why are Most Eyes Sensitive to the Same Range of Electromagnetic Radiation?

In all species in which vision is important, eyes have a spectrum of specialized adaptations that permit vision under a wide variety of circumstances. There are two premier selective pressures responsible for these adaptations: the physical properties of light *per se* and the natural variation of light. Physical laws define how light can be collected, focused and represented as an image, setting fundamental limits on the optical features of eyes [Fernald, 1988]. The daily and yearly variation of light intensity and spectral content have also been important selective pressures on eye evolution and set limits on the usefulness of vision.

One common feature of visual systems is their sensitivity to only a narrow range of wavelengths relative to the broad spectrum of energy produced by sunlight. An explanation for this limit is that eyes first evolved in animals living in water, which strongly filters all but two narrow ranges of electromagnetic (EM) radiation [Fernald, 1988]. As shown in figure 1, the range of EM radiation 'visible' for most organisms is a narrow, sharply defined band. This limited range of wavelengths has an attenuation six orders of magnitude lower than its flanking wavelengths. This means that only EM in this narrow band penetrates water and hence must have been the central selective pressure on early organisms, selecting for those that evolved biochemical mechanisms sensitive within this range. Interestingly, though many animal species have long since moved onto land where they are exposed to the broader spectrum of EM radiation from the sun, animal eyes remain limited to this narrow band.

Many insects can see well into the ultraviolet end of the visible spectrum, and recently it has been shown that some species of fish and birds also have receptors in this region [e.g., Viltala et al., 1995]. The other range of EM radiation that might allow transmission through water is at the very low frequency end of the spectrum ($<10^3$ Hz). Though there is no radiation from the sun at this low frequency, several groups of animals have evolved mechanisms to exploit this

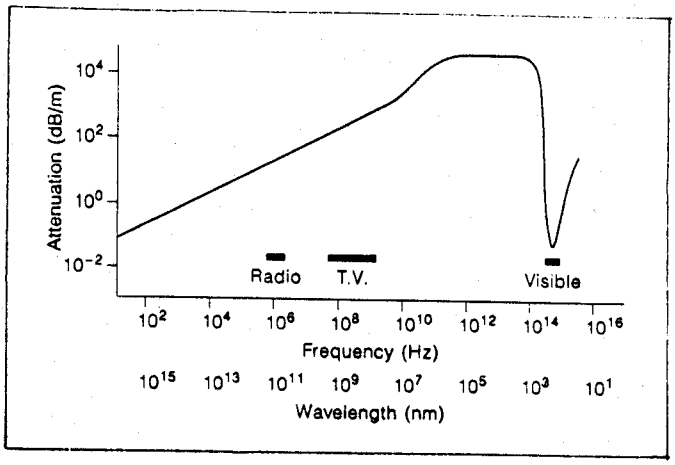

Fig. 1. Attenuation (dB/m) of electromagnetic radiation (EM) in sea water plotted as a function of frequency (Hz) and wavelength (nm) of that radiation. The narrow band of electromagnetic energy which corresponds to visible light is shown, as are the bands used for radio and television transmission. The band of EM radiation we now consider visible light is transmitted through water with an attenuation 65 orders of magnitude less than that of adjacent wavelengths [Redrawn from Fernald, 1988].

region of the spectrum. For example, weakly electric fish evolved independently in both Africa and South America, and species from both groups exploit the low frequency end of the EM spectrum for signaling conspecifics [Hopkins, 1988]. This signaling window is available in murky water and is used effectively by the small group of electric fish species. The EM radiation of such low frequencies, however, means that the wavelengths are very long (fig. 1), making image formation impossible, since physical laws dictate that any image-forming receptor must be large relative to the wavelengths detected. Consequently, electric fish have an array of small receptors distributed along their bodies, and 'images' of the world must be computed from the collective responses of these organs [Hopkins, 1995].

Thus the relatively narrow range of wavelength sensitivity is a residual reflection of our aquatic origins and illustrates how evolutionary choices persist.

Diversity in Extant Eyes

To be useful to their owners, eyes must capture light and, in image-forming eyes, resolve it into images. Eye design is fundamentally limited by the physical properties of light: it travels in straight lines, can be reflected, and varies in both wavelength (subjective hue or color) and intensity (subjective brightness). Many of the principles of de-

and even apparent errors we find in existing eyes reflect constraints arising directly from these physical properties of light. An obvious difficulty in analyzing the evolution of different eye types is deciding whether structural similarity has resulted from evolutionary convergence or common descent. Because of the physical laws governing the behavior of light, there are only a small number of ways to produce an eye with a usable image. Hence, similar structures have arisen in distinctly unrelated animals. Most frequently cited in this regard are the eyes of squids and fish, which are similar in a very large number of details, despite the fact that the organisms carrying these eyes are phylogenetically distant [Packard, 1972]. The demands of seeing underwater using a chambered- or camera-style eye have resulted in great similarities amongst eyes of these two groups including evolution of a spherical lens, even though the inverted retinal layers of the fish retina are distinctly different from the noniverted, simpler retina of squid. Thus, macroscopically, these eye types and the animals bearing them are nonhomologous, even though there are homologies at the molecular and developmental levels (see below).

Evolution of ocular structures has proceeded in two stages [Land and Fernald, 1992]. The first step, production of simple eye spots, is found in nearly all the major animal groups. Such eye spots are comprised of a small number of receptors in an open cup of screening pigment [see Land and Fernald, 1992]. Though useful for informing an animal about the distribution of light and dark, such eye spots can play no role in pattern recognition or control of locomotion. The second stage in eye evolution is the addition of an optical system that provides an image. As noted above, such eyes occur in 96% of known species distributed among six phyla [Land and Fernald, 1992]. At least eleven distinct optical methods of producing images have evolved; the most recently described is a telephoto lens, which was identified in 1995 [Ott and Schaefel, 1995]. Indeed, it is remarkable that 5 or six of the optical mechanisms have been discovered only in the past 25 years. The puzzle is why all animals do not have camera-type eyes which are demonstrably superior in several respects [Nilsson, 1989]. Perhaps conversion of one eye type to another requires intermediates that are either useless or much worse than the existing design, making such a switch essentially lethal to animals that depend on sight. Though this argument makes a certain amount of sense, some existing cases of novel optical combinations suggest this is probably not the whole story.

Textbooks tend to group animal eyes into two groups, the camera-style or 'simple' eyes and the compound eyes. Although this dichotomy reflects a real and fundamental difference in optical mechanisms, it conceals a remarkable diversity of optical systems subsumed under each heading [e.g., Nilsson, 1989; Land and Fernald, 1992]. There is not space here to enumerate the differences and possible evolutionary sources of each, but several discoveries suggest that the evolutionary paths to each kind of eye may not be as divergent as once thought.

Nilsson and Modlin [1994] have recently described a mysid shrimp *(Dioptromysis paucispinous)* that has a combined simple and compound eye. This eye is partly compound, with multiple facets eyactly 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. The shrimp are about 5 mm long, and the eyes, which sit at the ends of eyestalks, are roughly spherical. The surface of the eye is largely faceted, with the characteristic honeycomb of hexagonal facets (ca. 800–900), but at the back there is a single giant facet peering toward the shrimp's tail. Although these giant facets normally point backwards, the shrimp frequently rotates them forward, probably to get a better look at something since the giant facet has ca. five times the acuity (but much lower sensitivity) than the remainder of the eye. It is as if the shrimp is carrying a pair of binoculars for the occasional detailed look at something ahead of it. One serious problem with this combination eye is that the optics of compound eyes produce an upright image while the optics of simple eyes produce an inverted image. This discrepancy has been solved optically by this animal, so that the visual images produced by the different parts of the eye are continuous. The discovery that simple and compound eye types can be found in a single animal raise a question of how a developmental program could produce this outcome.

Eye Development: Evidence for Monophyletic Eye Origins?

Recently, Quiring et al. [1994] have demonstrated that the *eyeless* gene in the fruitfly *(Drosophila)* is homologous to the gene *Small eye* or *Pax-6* in mice. When this gene is missing in a fruit fly, no eyes develop, and when it is missing in mice, the result is also a reduced or absent eye. Thus *Pax-6* appears to play a role in eye development in organisms from widely different phyla. More remarkably, Halder et al. [1995a] showed that the *eyeless* gene of *Drosphila* could be used to induce ectopic eyes in *Drosophila* and also could rescue the *Small eye* defect in mice. Moreover, the *Pax-6* gene of mice also induces ectopic *Drosphila* eyes in flies, not mouse eyes. This is a particularly stunning result, because the coumpound eyes of fruit flies and chambered eyes of mice are radically different as described above.

The discovery of homologous genes guiding the development of decidedly nonhomologous eyes suggests that eye development itself might be a homologous process. Perhaps this explains why even the most pessimistic estimate of the time required for an eye to evolve is remarkably short. Even with generous assumptions. Nilsson and Pelger [1994] estimate that it would take less than 364,000 years for a camera eye to evolve from a light-sensitive patch, meaning that there would have been time enough for eyes to evolve more than 1,500 times since they first appeared in the Cambrian [Land and Fernald, 1992]. This implies that eyes can be expected to respond very rapidly to evolutionary changes in the lifestyle of a species. Indeed, eye design of a species may say less about its phylogenetic relationships than about its need for vision. Eye structure thus reflects primarily the animal's present requirement.

What does it mean that an important developmental gene, *Pax-6*, appears to be conserved across phylogeny. First, it is important to note that *Pax-6* also plays a role in the development of other tissue in the brain and elsewhere. Thus is it not exclusively an 'eye development gene' but rather a patterning gene co-opted for a variety of developmental functions. Second, the ancestral *Pax-6* genes are not promising. Both nematodes and sea urchins express *Pax-6* homologues, and neither has eyes. So although Halder et al. [1995b] argue that the discovery of conservation of *Pax-6* requires that eyes now be declared monophyletic, it is not so. We are perfectly comfortable with the notion that nonhomologous structures can contain homologous molecules. Consider similar or identical mitochondria in vastly different cells. Certainly one wouldn't argue for homology of the cells based on the fact that they contain homologous organelles much less homologous molecules. Indeed, we can assume that many more homologous molecules associated with development should be found in organogenesis. The challenge will be to discover how they result in such distinct end products.

Capturing Photons: Opsins are Conserved

Visual information from the environment is ultimately detected by specialized cells, the photoreceptors located in a thin layer of cells in the retina at the back of the eye. The photons containing light energy are collected by the lens and focused onto the photoreceptors where they are captured by two molecules acting together: opsin in close association with a visual pigment or chromophore. The fundamental transduction event is a change in the length of the chromophore in response to excitation by photons. This transformation of photon energy into a change in atomic dimension results in the opsin becoming enzymatically active, ultimately causing a decrease in current flow across the outer segment membrane. The main result of this transduction is that the photon energy becomes electrical energy that is interpretable by the nervous system.

The opsins, which are the protein components of the visual pigments, have a history that precedes eyes. Goldsmith [1990] and others have compared the opsins' evolutionary lineages in detail. The opsins consist of seven transmembrane helices with short loops on both sides of the membrane. The chromophore is attached covalently to opsin at a site in the seventh transmembrane domain. These features are common to all metazoan opsins, and, based on comparison of the DNA sequences, they must share a common ancestry. In particular, several regions of the molecule show close similarity in opsins from vertebrates, insects and *Octopus*, whose ancestries diverged in the Cambrian [Land and Fernald, 1992]. This homology suggests that the molecule responsible for the initial absorption of photons has been exquisitely tuned over evolutionary time. In addition, the high level of conservation has allowed relatively easy recovery of the substances that encode opsin from the eyes of many different species, giving us a remarkable amount of information about its evolutionary history (e.g., Chang et al., 1995).

Although metazoan opsins appear to have evolved along several separate lines from a common ancient ancestor [Fryxell and Meyerowitz, 1991], what happened earlier is not clear. Bacteriorhodopsin, from *Halobacterium* does not show significant amino acid similarity with cattle rhodopsin [reviewed in Maden, 1995]. Moreover, it is the double bond 13 of the chromophore, rather than 11, that is altered by light. Nonetheless, like metazoan opsins, bacteriorhodopsin belongs to a large superfamily of proteins, all of which have seven transmembrane helices and operate by activation second-messenger cascades [e.g., Hall, 1987]. These proteins include neurotransmitter and peptide receptors as well as the family of odorant receptor molecules. Whether these similarities suggest a very ancient common ancestry or a more recent recruitment is not clear. An excellent review of the opsin phylogenies is that of Goldsmith [1990].

Photoreceptors with a peak sensitivity to a particular wavelength differ in certain characteristics of the opsin molecule they contain [e.g., Nathans et al., 1986; Nathans, 1987]. Indeed, it seems that the subtle differences in the amino acid identities at particular sites 'tune' the retina to a particular peak wavelength [e.g., Nakayama and Khorana, 1990, 1991]. Recent work has related specific amino acids in the sequence of the opsin molecule to the peak wavelength absorbance [e.g., Neitz et al., 1991; Chang et al.,

1995]. These analyses have used the evolutionary experiments to define how wavelength varies as a function of opsin sequence details. The discovery and clarification of a direct causal link between a molecular structure and its consequences for a perceptual process is remarkable in its own right but also because we features described are common to all metazoan opsins.

The selective pressures for evolution of color vision must be manifold. Improved detection of food [e.g., Nagle and Osorio, 1993; Osorio and Vorobyev, 1996], mates and enemies all could be enhanced with the added dimension of chromatic detail. This is supported by detailed phylogenetic analyses of the relationships among vertebrate visual pigments. These suggest that vertebrate visual pigments have envolved along at least five lines and that these origins diverged from an ancestral type before teleost fish diverged from other vertebrates [e.g., Hisatomi et al., 1994]. Though there are some differences in interpretation [Okano et al., 1992], the general notion that visual pigments evolved along parallel lines following an ancient divergence seems correct. More recent evolutionary change can be seen in primate photopigments. Old world monkeys, apes and humans have trichromatic vision, while New World monkeys are quite polymorphic, having dichromatic or trichromatic color vision [Jacobs, 1996]. Humans seem unique in their polymorphism of trichromacy [e.g., Neitz et al., 1996], which might reflect the absence of potent selective pressure.

Focusing Light: Some Lens Proteins Are Conserved Among Vertebrates, Others Are Not

Vertebrate eyes develop from a diverse collection of embryonic sources through a complex set of inductive events [reviewed in Graw, 1996]. Whereas the neural retina is derived from the diencephalon, the lens comes from surface ectoderm, and the iris and ciliary body arise primarily from the neural crest. Mapping the genes known to play a role in mouse eye development, for example, shows that some of these genes are present on every chromosome [Graw, 1996; fig.2]. The apparent patchwork assembly of the eye makes it all the more surprising that common developmental programs seem to produce comparable outcomes across a broad phylogenetic divide [Halder et al., 1995a]. Can we use the phylogeny of conserved lens proteins to gain insight into eye evolution?

Vertebrate lenses are formed from modified epithelial cells, which contain high concentrations of soluble proteins known as crystallins [e.g., Bloemendal, 1981]. This nomenclature arose not because the proteins exist in a crystalline state but rather because they are packed in a highly organized fashion [Bloemendal and Spector, 1989]. The change in relative concentration of these proteins from the periphery to the center of lense produces the refractive index gradient necessary for a lens to be useful to an animal. For aquatic vertebrates, a precise gradient is essential for the production of a usable lens, since the lens must have an extremely high equivalent refractive power [Fernald and Wright, 1983; Kroeger et al., 1994]. It should be noted that, in principle, the identity of the proteins seems not to be important, since the crystallin proteins are not more transparent than others. Indeed, most vertebrate lenses are comprised of a collection of two or more crystallin proteins in various amounts [Hendriks et al., 1987]. Rather, the distribution of protein concentration as a function of radius is the key to a successful lens. Thus the challenge in understanding lens evolution lies in discovering how the distribution of proteins within a lens is established and maintained.

Of the eleven lens crystallins now known, α, β, γ-crystallins are ubiquitous among vertebrates. Once described as 'dull proteins' [de Jong et al., 1988], the production and deployment of proteins used in lenses has provided an interesting view of evolution in action. Phylogenetic trees based on DNA sequence comparisons of α-crystallin show that there is strong directional selection in vertebrates that require flexible lenses. These are the land-living vertebrates who have air as the substance adjacent to their lenses and can count on the air/lens interface as the major refractive contribution to the dioptric power of the lens [Fernald, 1989]. When selection is relaxed, as in the blind subterranean rodent *Spalax ehrenbergi*, however, selection becomes quite evident. The gene sequence for α-crystallin changes four times as fast as in sighted rodent relatives, but at only one-fifth the rate for neutral evolution in pseudo genes [Hendriks et al., 1987]. Why has α-crystallin been retained at all? Because *Spalax* does respond to light/dark cycles for thermoregulation [Haim et al., 1983]; α-crystallin has been proposed as a possible regulatory element required for the proper formation of a lens [Hendriks et al., 1987]. This proposal seems reasonable, given the remarkable disparity among other proteins implicated in eye development. This developmental role may include the establishment of a protein concentration gradient appropriate for that specific lens.

Until relatively recently, all crystallins were thought to be unique to lens tissue and to have evolved for this special function. However, despite their apparently specialist role, most of the crystallins are neither structural proteins nor lens specific [Wistow, 1993]. There seem to be two major groups of lens crystallins, those present in all vertebrates

and others that are taxon specific. The α, β, γ-crystallins are common to all vertebrates and are related to stress proteins. The α-crystallin is identical to the ubiquitous small heat shock proteins, while β, γ-crystallins are relatives of a bacterial spore coat calcium-binding protein [Wistow and Piatigorski, 1988]. The α-crystallins have non-lens expression which serves diverse other functions, whereas β, γ-crystallins otherwise act only in bacterial spore formation [Wistow, 1993]. Thus the genes for these three lens proteins seem to form a preferred group whose functions may extend beyond simply providing some of the protein used in all vertebrate lenses. The remaining proteins used in lenses are taxon-specific proteins that, surprisingly, also serve as metabolic enzymes in that lineage. For example, in crocodiles and some bird species, the glycolytic enzyme lactate dehydrogenase B is a major protein in the lens [Wistow et al., 1988]. Indeed, four of the eight taxon-specific crystallins are identical to metabolic enzymes and products of the same genes. One gene coding for a protein with two entirely different functions has been termed 'gene sharing' [Piatigorski et al., 1988; Wistow et al., 1990] and is proposed as an evolutionary strategy that preceded gene duplication and subsequent specialization [Piatigorski and Wistow, 1989]. In this scenario, a protein is recruited for a second function resulting in gene recruitment, so that one gene codes for a protein with two (or more) functions. The two functions may place competing demands on a single protein that could drive gene duplication and subsequent specialization of each gene for a specific function [Wistow, 1993]. The possible resolution of adaptive conflict arising from gene sharing has been carefully examined by Wistow [1993] whose discoveries led to understanding this novel evolutionary process.

Why might enzymes be recruited as vertebrate lens tissue? It has been suggested that the robust regulation of enzyme production might be advantageous for producing sufficient protein for a lens [Piatigorski et al., 1988], but there is not much beyond this speculation to support this notion. There may be some deeper reason, however, because this molecular opportunism seemed such a good idea; mollusks independently evolved the same strategy [Doolittle, 1988]. Squid eyes, described above because of their convergence with fish eyes at the organ level, have lenses whose protein content is nearly entirely the enzyme glutathione S-transferase [Tomarev and Zinovieva, 1988]. This convergence of molecular strategy suggests that enzymes as lenses may have a functional meaning, or that it is easy to get lens cells to make a lot of enzyme, or that there or other reasons not yet understood.

Edwards and Meyer [1990] reported that a constituent of crystalline cones exists throughout the insects and crustacea, perhaps mirroring the monophyletic line of α-crystallins in vertebrates. Thus, even though arthropods have every known type of optical structure at the organ level, their eyes, like those of vertebrates may contain homologous molecules in their refractile lenses. Whether this is true awaits molecular characterization of lens proteins in key arthropod species, but it is interesting to speculate how common building blocks responsible for the diverse eye functions of phototransduction and photon focusing might be assembled into diverse structures.

As yet, there is no evidence that any of the proteins used in lenses in vertebrates are related to those used in invertebrates, in parallel to the ubiquitous use of opsin for phototransduction. However, there is an interesting parallel in the appearance of *Pax-6* in the development of lenses. Recent reports show that several crystallin genes are targets of *Pax-6* in the lens [Cvekl et al., 1994, 1995; Richardson et al., 1995]. Possibly, the recruitment to the lens may have resulted from the acquisition of *Pax-6* responsivity [Richardson et al., 1995]. As noted above, *Pax-6* appears to play a role in many other tissues besides the eye, so its appearance alone is not sufficient to suggest possible monophyletic notions about eyes. Nonetheless, this hints that there may be more surprises in store as the genes responsible for eye specific development are discovered.

Conclusions

Despite new insights yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, with the caveat that they contain homologous molecules responsible for many structural, functional, and even developmental features. Given a growing list of homologous gene sequences amongst molecules in the eye across vast phylogenetic distances, the challenge now is to discover what makes eyes of arthropods, mollusks and vertebrates so different. Since strictly homologous development processes must produce homologous structures, key elements responsible for the development of nonhomologous eyes remain missing. Understanding what makes eyes different may be a bigger challenge than finding what they have in common.

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