

Homology and Convergence in the Evolution of the Eye

Introduction

The evolution of the eye has been an important topic in biology since the field's inception. Charles Darwin saw the highly complex organ as the ultimate test for his new theory of natural selection (Darwin, 1859). Though contemporary critics as well as modern proponents of the Intelligent Design hypothesis have seized upon Darwin's reverence for the complexity of the vertebrate eye, Darwin himself proposed a sequence for the gradual evolution of the eye that has aged respectably in the face of subsequent research on the topic (Salvini-Plawen and Mayr, 1977). Many studies have been conducted focusing on the physiology, ontogeny, and phylogeny of eyes in their many diverse forms throughout the animal kingdom, and modern techniques have allowed for molecular and genetic contributions to the evolutionary narrative. Contrary to the claims of critics, modeling of eye evolution indicates that a complex eye could evolve from a rudimentary photoreceptor/pigment arrangement in as few as several hundred thousand years, with a pessimistic upper limit of about 1 million years (Nilsson and Pelger, 1994). Accordingly, most researchers posit that the vertebrate eye evolved over a period of 100 million years, beginning about 600 MYA and progressing during the Cambrian Explosion (Lamb et al., 2007).

That there exists such a diverse range of eye structures in the animal kingdom, and that the myths of the barriers to eye evolution have been debunked, have raised the question of how many times eyes have independently evolved through history. In many ways, this question is one of semantics, in that it depends on how you define an eye. Phototaxis, which is "positive or negative displacement along a light gradient or vector" (Jekely, 2009), ranges in organisms from single photoreceptor cells coupled with a pigment, to the compound eyes of invertebrates, to the

vertebrate or cephalopod camera eye. Photosensitive cells are even found in non vision-related roles, such as photoreceptors in the pineal gland of non-mammalian vertebrates (Kusakabe et al., 2009). This review will define an eye as any structure that contains photoreceptive cells and pigments arranged to facilitate vision. The question of independent evolution also depends on how you define common origin, i.e., whether you're discussing the origin of molecules, cells, or tissue (Lamb et al., 2009). In all cases, homologies among animal eyes are apparent. They all feature a photoreceptor cell containing photosensitive molecules, called opsins, connected to a phototransduction cascade that promotes an eventual response to light stimuli (Vopalensky and Kozmik, 2009). Thus, homologies could exist at the level of opsins, pigments, other structural proteins, phototransduction pathways, and transcriptional regulation of eye development. This review attempts to synthesize the current state of the literature as it relates to the degree of homology and convergence in extant eye phylogeny. Put more simply, this review attempts to answer the question: how many times has the eye evolved?

Comparison of Photoreceptors

A simple way to begin answering this question is to examine photoreceptors. A photoreceptor is a type of cell with embedded light-sensitive structures called rhodopsins, which consist of an opsin transmembrane protein bound to retinal (Arendt and Wittbrodt, 2001). Retinal changes conformation when struck by a photon, transducing the energy into an electrical signal. Two types of photoreceptor cells exist: rhabdomeric and ciliary. In rhabdomeric photoreceptors, the apical cell membrane folds into microvilli or lamellae. Rhabdomeric photoreceptors are associated with sensing light for the purpose of sight, and are found primarily in the eyes of

protostomes, though they also appear in non-random clades of deuterostomes (Lamb et al., 2007). In the case of ciliary opsins, the ciliary membrane folds into inner discs or tubules (Arendt and Wittbrodt, 2001). Ciliary photoreceptors are found only in deuterostomes, and are mainly associated with detecting light for seasonal or circadian rhythms, though vertebrate eyes use ciliary opsins for vision (Lamb et al., 2007). Interestingly, though vertebrate eyes do not contain rhabdomeric photoreceptors, they are thought to contain rhabdomeric sister cells which function as retinal ganglia, as determined by closely-related transcription factors (Arendt, 2003). Analysis of ciliary opsins shows that an ancestral mutation led to a highly stable light-activated form of the rhodopsin (Terakita et al., 2004). Lamb et al. (2007) propose that for pelagic vertebrate ancestors, this mutation would have necessitated a biochemical pathway to isomerize the ciliary rhodopsin back to its inactivated form, as opposed to using a second photon to complete this exchange. This alteration would have conferred an evolutionary advantage to organisms that ventured into deep sea niches with low light, rendering rhabdomeric photoreceptors obsolete and allowing for their conversion into retinal ganglia nerve cells.

In a classic review, Salvini-Plawen and Mayr (1977) attempt to account for every independent emergence of eyes or proto-eyes in the animal kingdom. They document the arrangement and cell differentiation of eyes in many different phyla. Although the authors concede that rhodopsins appear to be highly conserved across all groups, they argue that eyes may not be as ancestral as rhodopsins, and they report that some intermediate rhodopsin forms had been discovered in some echinoderms (Brandenburger and Eakin, 1973). On the basis of the different arrangement and cell differentiation patterns, Salvini-Plawen and Mayr (1977) conclude that eyes appear to have emerged independently in at least 40 phyletic groups, perhaps up to 65, and possibly even more pending the discovery of unpigmented photoreceptors or new species.

This study has since been the subject of criticism. Several years later, Eakin (1979) reversed his opinion on the purported intermediate rhodopsin forms, stating that echinoderm rhodopsins were truly rhabdomeric (Arendt and Wittbrodt, 2001). Further, molecular, genetic, and regulatory evidence shows surprising conservation among animal eyes (Arendt and Wittbrodt, 2001; Vopalensky and Kozmik, 2009; Ranade et al., 2008), casting serious doubt on the claims that eyes arose independently so many times. Few review authors since Salvini-Plawen and Mayr have been so bold as to provide such a large and definitive prediction.

A definitive conclusion regarding the evolutionary relationship of rhabdomeric and ciliary rhodopsins can, however, be given. Both rhodopsins are seven-transmembrane proteins that bind to retinal with a Schiff base linkage to a lysine on the seventh transmembrane domain (Larusso and Ruttenberg, 2008). Combined with various functional similarities, the evidence suggests potential homology between the photoreceptors. This has turned out not to be the case. Peptide sequence analysis performed by Larusso and Ruttenberg shows that the rhodopsins are not related by an ancient gene duplication event, i.e. they do not share a common ancestral precursor or gene. Instead, ciliary rhodopsins are members of the family of G-protein coupled receptors (GPCRs) and signal via heterotrimeric G-proteins (Jekely, 2009). These rhodopsins are posited to have evolved from non-opsin GPCRs, which predate eukaryotes. Therefore, the analogous rhodopsins represent a remarkable example of molecular convergence.

Investigating Urbilateria

Gene and transcription factor homologues are found to be involved in the development of both vertebrate and invertebrate eyes (Arendt and Wittbrodt, 2001). This suggests that the

ancestor to all bilaterians, known as Urbilateria, had some measure of primitive vision and central processing capability. The study of both the precursors to and descendants of Urbilateria is important for determining the ancestry of the bilaterian eye.

Phototaxis before Urbilateria

It is instructive to look at primitive phototaxis for clues as to how more complex eyes evolved. The simplest form of phototaxis is two-dimensional “biased random walk” (McCain et al., 1987). A prokaryotic cell with a primitive sensory rhodopsin combined with a photopigment detects only a gradient of light, and randomly changes the direction of its beating flagella until it is oriented towards a light source. According to McCain et al. (1987), the function of sensory rhodopsins in prokaryotes is analogous to that of eukaryotes. Eukaryotes are the first cells to exhibit three-dimensional phototaxis (Jekely, 2009). All phototactic eukaryotes have a fixed shape, polarized cell(s), swim in a spiral, and orient themselves with ciliary beating. Each of these features likely evolved independently many different times, and three-dimensional phototaxis is found in five of six major eukaryotic groups.

Rhodopsins can also be traced back to before Urbilaterians. As noted previously, rhodopsins are highly conserved throughout history, though they arose at least twice. Cnidarians possess rhodopsins, though they do not participate in phototaxis (Plachetzki et al., 2007). Poriferans and choanoflagellates do not possess rhodopsin. Therefore, it is postulated that rhodopsins were first present in the cnidarian-bilaterian common ancestor (Jekely, 2009). Rhodopsins are not, however, correlated to the specific photopigment utilized by the photoreceptor cell (Vopalensky and Kozmik, 2009). There are generally three types of

photopigments – melanins, ommochromes, and pterins – and their random distribution through phylogeny indicates that photopigments and photoreceptors assembled indiscriminately and independent of the ancestral state. Less likely, it has been hypothesized that these pigments coexisted in the ancestral photoreceptor and were lost after divergence (Vopalensky and Kozmik, 2009).

Characteristics of Urbilateria

There are numerous homologies found among bilaterian eyes that indicate what features the common ancestor very likely possessed. For example, the signaling cascade at the beginning of phototransduction appears to be conserved throughout bilaterians (Arendt and Wittbrodt, 2001). At the first step, the retinoid is isomerized by a photon, activating a G-protein and ultimately polarizing or depolarizing the photoreceptor cell. To quench this cascade, the activated rhodopsin is phosphorylated by rhodopsin kinase and bound by arrestin, which outcompetes the G-protein. The presence of factors related to rhodopsins, a G-protein, rhodopsin kinase, and arrestin-like molecules in many different phyla indicate a primitive form of this cascade was likely present in Urbilateria.

The most convincing evidence for eye homology is the tightly conserved set of transcription factors that control eye development across many phyla. According to Vopalensky and Kozmik (2009), “Given the enormous diversity of animal eyes, it came as a surprise that certain transcription factors are redeployed for visual system development far more often than others.” For example, *Pax* genes code for transcription factors and are defined by a highly conserved paired DNA binding domain, and additionally a second homeodomain in some genes.

A *PaxB* homologue has been found in poriferans as well as placozoans (the simplest metazoans), meaning *Pax* genes predate the origin of a central visual or nervous system, and indeed all extant metazoans. Studies of multiple phyla show that, although *Pax* genes are not at the top of the genetic developmental cascade, they are critical for the development of normal eyes, and some have proposed considering *Pax6* a ‘master control gene’ (Gehring and Ikeo, 1999). *PaxB* homologues are conserved in both invertebrate and vertebrate eyes (Arendt and Wittbrodt, 2001). *Pax* genes are involved in tight, highly interactive networks with other ancient genes, such as *Six* and *Eya* (Vopalensky and Kozmik, 2009). The origin of both the *Six* and *Eya* gene families predates the evolution of the eye, and orthologues of both are involved in eye development in all bilaterians. This homologous network, therefore, must have existed in the ancestors of Urbilateria, and was recruited for eye development sometime before metazoans diverged.

Transcription factor homology is also found in the development of rhodopsins. In both ciliary and rhabdomeric photoreceptors, the gene *Otx* is critical for the development of photoreceptors and the regulation of opsins and the phototransduction cascade (Ranade et. al, 2008; Vopalensky and Kozmik, 2009; Arendt and Wittbrodt, 2001). One of the targets of *Otx* is *Crx*, which is highly expressed in differentiating photoreceptor cells, and regulates genes involved in the phototransduction cascade common to all animals. That *Otx* is critical in both photoreceptors, points to its ancient role in photoreceptor cell differentiation. Finally, the transcription factor *Mitf* is expressed in both bilaterians and cnidarians, and interacts with both *Otx* and *Pax* genes. Therefore, these three transcription factors likely interacted in an ancestral photoreceptor cell.

To summarize, all known descendents of Urbilateria share one of two rhodopsins, a fundamental phototransduction cascade, and a small conserved set of transcription factors.

Additionally, Arendt and Wittbrodt (2001) found that paired two-cell cerebral eyes innervated by cerebral ganglia are common across many of the protostomes and even some lower deuterostomes. Thus, it has been proposed that the eyes of protostomes and possibly deuterostomes are descended from an Urbilaterian with rhabdomeric photoreceptors, a pair of two-celled cerebral eyes connected to cerebral ganglia, and a well developed central processing system (Ranade et al., 2008; Arendt and Wittbrodt, 2001).

Divergence and Convergence after Urbilateria

The descendants of Urbilateria predictably show a vast range of divergence and convergence at all levels of eye development, structure, and function. For example, a broad range of organisms exhibit lens-containing eyes (Vopalensky and Kozmik, 2009). The water-soluble proteins found expressed in high concentration and contributing to the optical properties of the lens are known as crystallins. Crystallins are rarely lens-specific proteins, but are instead often found at low levels in other tissue and appear to have been recruited from a different original function. In stark contrast to opsins, a surprisingly diverse range of proteins are found functioning as crystallins in animal lenses, though there appears to be some logic behind which proteins are recruited. For example, vertebrate lenses contain crystallins related to heat-shock or stress proteins. These proteins originally evolved to withstand high stress, and thus they are sensible choices to remain functional and soluble for the duration of the organism's life. Crystallins exemplify the phenomenon of 'gene sharing,' in which proteins evolve new functions simply through a change in gene expression (Piatigorsky and Wistow, 1989). Thus, while

crystallins are not conserved through evolutionary history, they show a reliable convergence on basic eye structure observed in extant species.

Another example of divergence and convergence is visual cycling in vertebrates and invertebrates. Visual cycling is the conversion of the isomerized retinoid back to its inactive state, which occurs via a biochemical pathway in ciliary photoreceptors (Kusakabe et al., 2009). Different pathways for visual cycling have evolved in vertebrates and invertebrates. Kusakabe and Tsuda (2007) chose to study a larval tunicate as a model vertebrate due to its similarity and close evolutionary relationship with vertebrate larva. They found that different pathways exist for retinoid cycling in rods, cones, and non-visual photoreceptor cells. The authors conclude that the visual cycle was present in the last common ancestor of tunicates and vertebrates (Kusakabe et al., 2009). Since divergence, invertebrates and vertebrates have refined and altered this biochemical pathway, though the fundamental function of retinoid cycling has been conserved.

Conclusion

Neat answers to questions of evolutionary origins are hard to come by, especially in literature treating the evolution of the eye. Since Salvini-Plawen and Mayr (1977) estimated the number of independent phyletic origins of eyes, few authors have been so explicit in giving an answer to the same question. Experts on eye evolution lack recourse to fossilized evidence of intermediate or extinct proto-eyes, since soft tissue rarely mineralizes (Lamb et al., 2007). This nuance in the literature has also been in response to the uncovering of deep homologies among animal eyes (including highly conserved transcription factors, phototransduction cascades, and fundamental arrangement) which suggest the prominence of common ancestry in animal eyes.

We now have a detailed understanding of the billion-year-old ancestor to all bilaterians, Urbilateria, which possessed rhabdomeric rhodopsins, a pair of cerebral eyes, and a central processing system. The narrative of the evolution of the eye has been further complicated by the discovery that ciliary and rhabdomeric rhodopsins, found in near-complete phyletic isolation, do not share a common origin. Instead, ciliary rhodopsins predominated in metazoans due to an ancient mutation, leaving sister cells of rhabdomeric rhodopsins to assume the role of retinal ganglia. As Lamb et al. (2009) have written, after considering ancestral homology, it is difficult to distinguish between truly independent subsequent improvements on the eye and which developments “might better be construed as variations on a theme.” Thus, while it can be confidently asserted that rhodopsins independently evolved at least two times, the broader question of independent eye evolution requires an involved answer and further research.

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