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ORIGINAL SCIENTIFIC ARTICLE

A Genetic Perspective on Eye Evolution: Gene Sharing, Convergence and Parallelism

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Abstract Did the diversity of lens-containing eyes evolve from one ancestral eye (monophyletic evolution) or from multiple, independently derived eyes (polyphyletic evolution)? Monophyletic evolution would make diverse eyes homologous (inherited similarities from a common ancestor); polyphyletic evolution would make eyes homoplasious (independently acquired similarities). Historically, anatomical and developmental differences among eyes of different species favored homoplasy; however, recent molecular data indicating that all eyes employ a similar cascade of transcription factors (proteins regulating gene expression) for development have suggested homology. Comparative studies on invertebrates and vertebrates suggest that the use of common networks of developmental transcription factors may be due to parallel evolution, a form of homoplasy by independent recruitments of similar genes and transcriptional networks. Remarkably, the photoreceptors of lens-containing jellyfish eyes have ciliary photoreceptors, like vertebrate photoreceptors, and apparently employ a vertebrate phototransduction system (linked biochemical processes converting light into nervous electrical impulse), consistent with parallel evolution between jellyfish and vertebrate eyes. Finally, the major proteins conferring the lens optical properties—the crystallins—were recruited by a gene-sharing process (the addition of a new gene function without loss of the original function) from various stress proteins and common metabolic enzymes in different species by convergent mutations (derived independently, not related by common ancestry) in their promoters (gene regulatory sequences) leading to high lens expression. Thus, the data indicate that homology or homoplasy of diverse eyes depends upon the level of analysis.

Keywords Homology · Convergent evolution · Parallel evolution · Gene sharing · Crystallins · Lens · Retina · Opsin · Phototransduction · Jellyfish

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Introduction

The complex and specialized nature of eyes has challenged evolutionists ever since Darwin wrote famously in his treatise on the origin of species [(Darwin 1859) p. 186] that "to suppose that the eye, with all its inimitable contrivances for adjusting the focus for different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree." Nonetheless, Darwin went on to express confidence that eyes did indeed evolve, and the accessibility and widespread distribution of eyes throughout the animal kingdom have made them a favorable subject for evolutionary studies (Arendt 2003; Arendt and Wittbrodt 2001; Fernald 2006; Land and Fernald 1992; Land and Nilsson 2002). The question as to whether the eye evolved once very early in the history of animals and diversified thereafter (monophyletic evolution) or whether an eye evolved multiple times independently in various forms as evolution progressed (polyphyletic evolution) has led to much discussion.

Agreeing on a proper definition for an eye can be itself a confounding issue, especially when considering eye evolution. Clearly there are different considerations if an eye is defined as simply a photoreceptor that responds to light in some fashion or if an eye is defined as a complex organ

used in vision. For the present discussion an eye will be defined as an organ that can distinguish the directionality of light and that therefore has a dark shielding pigment along with two or more photoreceptors. Indeed, Land and Nilsson (1992) require that eyes must have the ability to detect an image, no matter how crude, by simultaneously comparing light from different directions; that is, eyes must have spatial vision. Although it is not possible to determine at present, if ever, how many times a light-responsive cell (photoreceptor) combined independently with a dark-shielding cell or other structure to create a primitive eye spot, I review here some salient features that favor the idea that diverse eyes have evolved with a considerable degree of independence and have different levels of homology (Land and Nilsson 2002; Fernald 2004).

Are Eyes Monophyletic or Polyphyletic?

Historically, the major anatomical and developmental differences between complex lens-containing eyes of vertebrates and some invertebrates (i.e., squid or octopus) or between lens-containing and compound eyes consisting of hundreds of ommatidia (separate facets with photoreceptors) were taken to mean that eyes arose multiple times or polyphyletically. Polyphyletic eye evolution is consistent with the facts that the morphology of the photoreceptors (retinal cells containing the protein, opsin, which coverts light to an electrical signal allowing vision) and the phototransduction signaling cascades (the linked biochemical processes in photoreceptors converting light into a nerve impulse) of invertebrates and vertebrates are generally different (except see last section of this review): invertebrates have rhabdomeric (microvillar) photoreceptors and employ a phospholipase C-based phototransduction cascade, while vertebrates have ciliated photoreceptors and employ a phosphodiesterase-based phototransduction cascade (Fernald 2004, 2006; Land and Nilsson 2002; see Gregory, this issue for further discussion). Moreover, early studies suggested that photoreceptors evolved 40–60 times (Salvini-Plawen and Mayr 1977), although this idea was challenged by Eakin, who believed that all ciliated photoreceptors had a common ancestor (Eakin 1979). Nevertheless, Eakin supported two origins for photoreceptors, ciliary and rhabdomeric. Computer-assisted estimations that complex eyes could evolve relatively quickly (within half a million years) starting from a light-sensitive skin patch gave credibility to the notion that different eyes evolved independently numerous times (Nilsson and Pelger 1994). That eyes exist only in about one-third of the animal phyla also supports polyphyletic eye evolution since there is no obvious reason why so many species would lose eyes and dispense with the selective advantage of vision.

In contrast to multiple origins of eyes, the common function of vision and the conserved use of opsin family members for phototransduction, despite phototransduction signaling differences in invertebrates and vertebrates, seemed consistent with the possibility that a primitive eyespot originated once and diversified thereafter during evolution (monophyletic evolution; see Gregory in this issue for further discussion). The recent influx of molecular data showing that a similar (although not necessarily identical) developmental regulatory network is employed for development of diverse eyes supported the notion that eyes are linked by common ancestry (Gehring 2005; see below). Indeed, reexamination of eye evolution in terms of developmental mechanisms (i.e., an evolutionary developmental biology, or evo–devo approach) greatly impacted the ideas of how diverse eyes originated.

Homology (Divergence) and Homoplasy (Parallelism and Convergence)

Understanding the basis for similarities and differences in tissues and organs is a major challenge for evolutionary biologists. Delving into the evolutionary questions of eye diversity requires defining certain terms that are used copiously in the scientific literature. The clarifications of evolutionary terms connected with homology are especially necessary because the concept of homology has differed with investigators, conflating structure and function and now, development (Fitch 2000). A simplified diagrammatic representation of homologous structures that evolved divergently and homoplasious structures that evolved by parallel or convergent evolution is presented in Fig. 1.

Gould has written an extensive book on evolution which reviews the historical basis and present understanding of homology, homoplasy, parallelism, and convergence (Gould 2002; see especially Chapter 10). Although Gould's

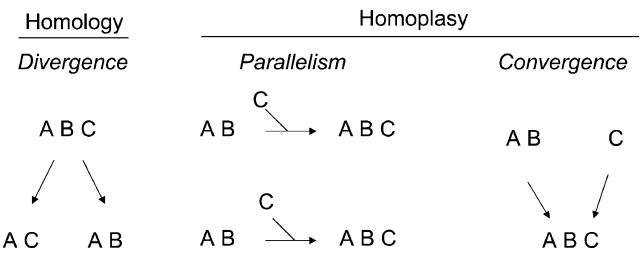

Fig. 1 Diagrammatic representation of the differences between homology and homoplasy. *Arrows* denote evolution. The *left panel* indicates that homologous structures (*AC* and *AB*) arise by divergence from a single ancestor (*ABC*) during evolution. The *two right panels* indicate that homoplasious structures may appear similar (*ABC*) either by independently recruiting the same element (*C*) by parallel evolution (*middle panel*) or by utilizing different elements (*AB* and *C*) and processes by convergent evolution (*right panel*). See text for further discussion

discussion is beyond the scope of the present article, it is worthwhile highlighting a few points to help understand the complexity of eye evolution. In brief, homology is similarity by inheritance from a common ancestor, and homoplasy is similarity by independent evolution. However, Gould points out that these concepts can become muddled and overlap by the fact that homoplasy can be divided into two processes: parallelism and convergence. Parallelism involves independent recruitment of similar, conserved regulatory processes, while convergence involves independent evolution of analogous functions using different regulatory processes. Gould states the following (p. 1074): "If we decide that the crucial distinction between homology and homoplasy should rest upon common ancestry vs. independent origin, then one important phenomenon, necessarily included within homoplasy by the defining criterion of independent origin for similar structures, shares too much conceptual overlap with homology to permit a clear and comfortable *theoretical* separation (however firm the *descriptive* division): independent origin channeled by common internal constraints of homologous genes or developmental pathways—in other words, the phenomenon known as *parallelism*." Even though parallelism involves independent origin and homology-inherited evolution, these distinct evolutionary concepts can become intertwined, as expressed by Gould (p. 1079): "At the level of an overt phenotypic structure under explicit consideration, parallelism denies homology and asserts independent origin. But, at the level of the generators for the overt feature—the genes regulating its architecture and the developmental pathways defining its construction—parallelism affirms homology as the concept's fundamental meaning and *raison d'etre*, and the basis for its dichotomous contrast with convergence as alternatives within the more inclusive category of homoplasy. Thus, parallelism does require independent regimes of similar selection, but the resulting phenotypic likenesses must also be channeled from within by homologous generators." Homology as a descriptor of inherited relatedness, then, can be applied to different levels of biological or developmental organization. Gould distinguishes parallelism from convergence as follows (p. 1075): "...parallelism marks the formal influence of internal constraint, while convergence reflects the functional operation upon two substrates different enough to exclude internal factors as influences upon the resulting similarity." In other words, the developmental mechanics of the evolution of a structure by parallelism or by convergence are different.

Gould's descriptions help us appreciate the complexities of resolving the extent to which diverse eyes are monophyletic or polyphyletic, or stated otherwise, the extent to which diverse eyes evolved by the ancient eye scenario (monophyletic evolution) and by the parallel recruitment and convergent scenarios (polyphyletic evolution; Abouheif et al. 1997; Hodin 2000). For eyes to be monophyletic would require that each species inherited its eye developmental network (ancient eye scenario) rather than having independently recruited a similar developmental network (parallel recruitment scenario) or an entirely different developmental network (convergent scenario). The first alternative would make diverse eyes homologous (common descent from an ancestral form), the second alternative would make diverse eyes homoplasious by being independently derived in their lineages despite the utilization of similar developmental networks, and the third alternative would make diverse eyes homoplasious by being independently derived in their lineages using different developmental networks. The distinctions between these different aspects of evolution are not always obvious and can be a matter of degree, especially if one considers developmental potential that is not always utilized. Indeed, it has been suggested (Abouheif 2008) that parallelism may be considered a special and distinct phase of evolution characterized by "the flickering on or off of characters between closely related species through time." This special phase encompassing patterns of parallel evolution using distinct mechanisms has been called "mesoevolution" (Abouheif 2008).

As discussed below, the presence of diverse eyes cannot be understood in terms of any one of these evolutionary processes by itself. Eye evolution involves homology, parallelism and convergence, depending on the level being considered.

Similar Transcriptional Networks Have Been Used for Eye Development During Evolution

A turning point away from a polyphyletic view of eye evolution based on the marked differences in eye anatomy towards a monophyletic view came with the demonstration that the gene encoding the developmental transcription factor Pax6 (called Eyeless in *Drosophila*) is essential for eye development in flies (Quiring et al. 1994) and humans (Ton et al. 1991; Glaser et al. 1992; Hanson 2003) and can even induce eyes in anatomical regions that do not normally have eyes (such as legs, antennae, or wings) in flies (Halder et al. 1995; Tomarev et al. 1997) and vertebrates (Chow et al. 1999). Recent studies show that these extra supernumerary eyes (often called ectopic eyes) in strange places respond to light and may be functional in the sense that they extend nerve projections to the central nervous system (Clements et al. 2008). Comparative studies throughout the animal kingdom suggested that *Pax6* is a "master gene" for eye development (Gehring 2004; Gehring and Ikeo 1999). In addition, a plethora of investigations showed that the Pax6 protein consistently works within a similar group of proteins (called a developmental cascade

or network of transcription factors) that directs eye development by regulating the expression of many different genes. The conserved developmental cascade of transcription factors regulating gene expression is known as the *Pax*/*Six*/*Eya*/*Dach* regulatory gene network for eye development; it is also called the retinal determination gene network (Silver and Rebay 2005; Kozmik 2008). The repeated employment of a similar developmental cascade of transcription factors for eye development during evolution reinforced the notion that an ancestral eyespot was constructed once and was subsequently modified during evolution in different species, producing the diversity of eyes seen today, and could be interpreted as the ancient eye scenario, namely, monophyletic eye evolution by inheritance from a common ancestor

The idea that all eyes are homologous because that they use Pax6 within a similar developmental cascade, or network, of transcription factors is compelling; however, it is not without difficulties (Simpson and Price 2002; Van Heyningen and Williamson 2002). First, there are species that have members of the eye developmental cascade but do not have eyes. The nematode *Cenorhabitis elegans* is among the best-studied eyeless species, where Pax6 is used for head and sensory neuron development (Chisholm and Horvitz 1995; Zhang and Emmons 1995). There are also cases in which eyes develop without Pax6. These include the adult eyes of the polychaete annelid worm (*Platynereis*; Arendt et al. 2002), the Hesse eyecup of the cephalochordate amphioxus (*Branchiostoma*; Glardon et al. 1998), and eye regeneration in the planaria flatworms (Pineda et al. 2002). In addition, the hierarchy of the transcription factors used in the cascade may differ within the network; moreover, the "master gene" concept is complicated by the fact that other proteins within the cascade can induce ectopic eyes (Silver and Rebay 2005; Kumar and Moses 2001a; Pichaud et al. 2001) or lenses (Oliver and Gruss 1997). The different transcription factors comprising the developmental cascade function with different degrees of importance during eye development in different species, making some members of the cascade critical for eye development in one species but not in another species. An example is the *eyes absent* (*eya*) gene which is essential for eye development in flies, but its role in eye development in mice is much less clear [see (Donner and Maas 2004) for review].

Other questions arise when considering homology on the basis of the conserved use of a similar cascade of transcription factors for the development of diverse eyes. A general issue is that gene regulatory networks have been referred to as "tool kits" because of their promiscuous use in multiple tissues (Conway Morris 2003). For example, the critical transcription factor Pax6, for eye development, is also used for the development of the brain, nose, and pancreas in mice (Dahl et al. 1997), and the *Pax*/*Six*/*Eya*/*Dach* regulatory gene network, or at least members of the gene families used in this network, direct not only eye development but also that of many other tissues depending upon the species (Silver and Rebay 2005; Kozmik 2008; Donner and Maas 2004; Relaix and Buckingham 1999; Heanue et al. 1999; Kawakami et al. 2000; Kardon et al. 2004; Rebay et al. 2005; Kozmik et al. 2007). However, biology is complex, and more information is required in order to establish homology by the use of similar developmental cascades of transcription factors. Different combinations of the members of a developmental cascade may lead to significant differences in its function, and gene regulatory networks respond to various signaling cues and may contain different members of duplicated genes that are closely related but not identical (see Silver and Rebay 2005; Kumar and Moses 2001b; Zuber et al. 2003; Schlosser 2006). In addition, changes in the genetic makeup of the regulatory networks used for eye development in different species can occur during evolution leading to diversification between eyes sharing a common ancestor. In this connection, it has been proposed that developmental pathways are modified by a process called intercalary evolution (Gehring and Ikeo 1999). Intercalary evolution can occur by gene duplication and divergence, by recruitment of novel genes into the pathway via changes in gene regulation, or by various forms of evolutionary tinkering.

Thus, a type of monophyletic eye evolution based on the conservation of a gene regulatory cascade for eye development cannot be refuted at the present time. Additional data in the future should provide new insights into the evolutionary significance of the common use of similar genetic networks for eye development in different species.

Lens and Crystallins: The Concept of Gene Sharing

Complex eyes contain transparent cellular lenses just behind (posterior to) the cornea. Lenses differ in shape, relative size, and hardness in different species of invertebrates and vertebrates in accordance with the different mechanisms used for focusing (accommodation) and improve vision by increasing refractive power while allowing more light into the eye (Land and Nilsson 2002; Jonasova and Kozmik 2008). Lenses accumulate globular, water-soluble cytoplasmic proteins called crystallins. Crystallins are named as such because they are present in the crystal-clear lens. Crystallins form a refractive index gradient in the lens, highest in the center and lowest at the periphery which shortens the focal length of the transmitted light and eliminates spherical and chromatic aberrations. Hard spherical lenses dominate the refractive power in eyes of aquatic species because there is a

negligible difference in the refractive index of the surface cornea and the surrounding water. In the environment of nonaquatic species, where the refractive index between the cornea and air is large, the cornea located at the surface of the eye provides about two-thirds and the internal lens about one-third of the refractive power of the eye.

In addition to their structural importance in the lens, crystallins are interesting from the viewpoint of evolution. Lenses are specialized for transparency and refraction. By analogy with other tissues performing highly specialized functions, for example erythrocytes filled with globin for oxygen transport, one would expect the crystallins to be uniquely specialized for their optical functions in the lens. However, this is not the case. Crystallins are surprisingly diverse, water-soluble proteins that may differ between taxonomic groups of animals (i.e., some crystallins are taxon-specific; Wistow and Piatigorsky 1988; Piatigorsky 1989; de Jong et al. 1989). Figure 2 diagrams the distribution of different taxon-specific crystallins in vertebrates (left panel) and invertebrates (right panel), respectively. Unexpectedly, then, entirely different proteins may serve as crystallins to allow a lens to form a focused image lacking spherical aberration on the retinal photoreceptor cells (Fig. 3).

Taxon-specific crystallins are often (not always) common metabolic enzymes (known as enzyme crystallins) that are expressed at lower levels in many tissues where they have nonrefractive roles. Even the crystallins that are present in all vertebrate eyes (i.e., the α-crystallins and the β/γ-crystallins) and would seem to be highly specialized for their structural lens role in vision are also expressed in other tissues where they have non-optical roles. For example, the α-crystallins are stress-inducible small heat shock proteins (Ingolia and Craig 1982; Klemenz et al. 1991; Horwitz 1992), and if not for their accumulation in the lens where they have a refractive function, they would be classified strictly as members of the small heat shock protein family. We have called this dual role for an identical protein of refraction in the lens and catalysis or stress protection within or outside of the lens, "gene sharing" (Piatigorsky et al. 1988; Piatigorsky and Wistow 1989; Piatigorsky 1992). In other words, a crystallin gene encodes a protein with more than one biochemical or molecular function. Thus, gene sharing expands the functional significance of a gene, which clearly has evolutionary implications.

As a point of interest, although a digression from the focus of this review, gene sharing is not restricted to lens crystallins and occurs extensively throughout the animal kingdom (Piatigorsky 2007). One implication is that the function of a protein can be directly related to the expression of its gene (Fig. 4). In the case of crystallins, an enzyme expressed at low levels in a non-lens tissue has a strictly catalytic role, while the identical enzyme expressed at high levels in the lens has a refractive function. The enzyme-crystallin may or may not use its catalytic potential in the lens, depending upon circumstances. A change in the molecular function of a protein by a change in the regulation of its gene means that gene duplication is not a prerequisite for functional innovation (Piatigorsky 1992; Piatigorsky and Wistow 1991; Hughes 1994, 1999, 2005), as was thought for many years (Ohno 1970; Kimura and Ota 1974; Taylor and Raes 2004).

Gene sharing among crystallins provides a striking example of how tissue homology can be hierarchical. Lenses

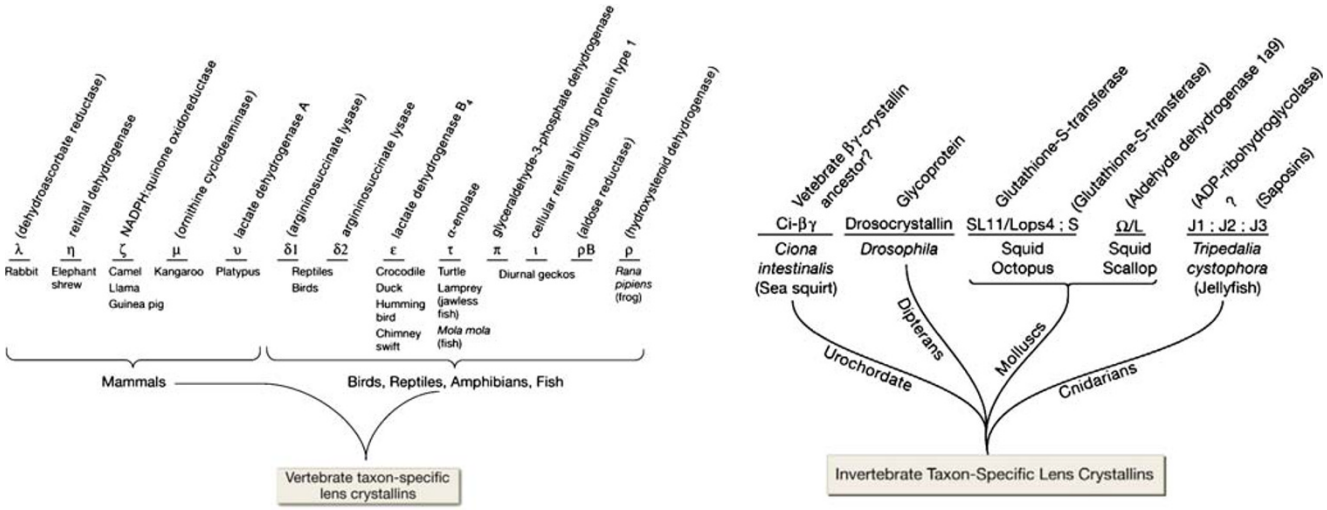

Fig. 2 Diagrammatic representation of vertebrate (*left panel*) and invertebrate (*right panel*) taxon-specific lens crystallins. Note the use of common metabolic enzymes that function as lens crystallin. Ci-βγ-crystallin in the urochordate, *Ciona intestinalis*, is thought to be ancestral to the βγ-crystallins of vertebrates although *C. intestinalis* does not have a lens in its larval eye. *Enzymes in parenthesis* have undergone mutations and lack activity. Reprinted by permission of the publisher from GENE SHARING AND EVOLUTION: THE DIVERSITY OF PROTEIN FUNCTIONS by Joram Piatigorsky, pp. 62 and 64; Cambridge, Mass.: Harvard University Press, Copyright © 2007 by the President and Fellows of Harvard College

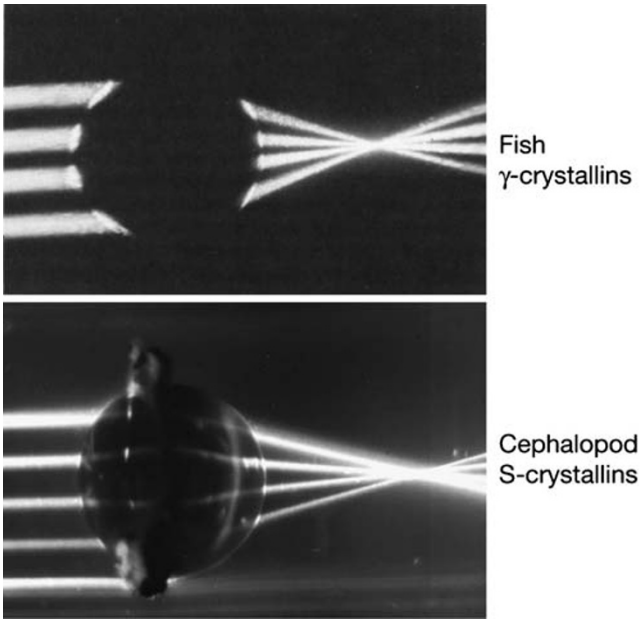

Fig. 3 Refraction by the lens of the African cichlid fish, *Haplochromis burtoni* (*upper*; supplied by Dr Robert Fernald, Stanford University, Stanford, CA, USA), and of the squid, *Sepiotheuthis lessoniana* (*lower*; supplied by Dr. Jacob Sivak, University of Waterloo, Ontario, Canada). The fish lens contains α-, β-, and mostly γ-crystallins, while the squid lens contains almost exclusively S-crystallins, which are homologous to the enzyme glutathione S-transferase although they lack enzyme activity. Note that despite that the fish and squid lens have entirely different crystallins, they both have the ability to focus an image without spherical aberration. From (Piatigorsky 2007)

of different species may have elements of homology as judged by using similar inherited genetic networks to direct their development; yet, they acquired independently a variety of different crystallins for their optical roles. In short, there is no one answer to the question of whether eyes or lenses are homologous or not: it depends on the level of analysis.

Recruiting Unrelated Crystallin Genes by Convergent Evolution

Both the multiple functions and the taxon-specificity of crystallins were totally unexpected in view of the specialized nature and overall similarity in structure and function of lenses in different species. Apart from all crystallins being water-soluble, their defining characteristic is their abundance in the lens, which is a requirement to affect the refractive index of the transparent tissue. What, then, might account for the diversity of lens crystallins?

One method of accumulating a protein in a tissue is to have its gene very active in that tissue. Gene activity is controlled by specific, short DNA sequences (called *cis*-control elements) in the promoter and enhancer regions associated with the gene. Promoters are situated just in front of the protein-coding region of the gene, while enhancers can be in front of, within, or even behind the gene. In addition to controlling the intensity of gene activity (i.e., the amount of messenger RNA made by the gene), promoters and enhancers also determine the tissues in which the gene is expressed by binding different combinations of transcription factors. Indeed, this is the molecular basis of how the specific genetic cascades discussed above function, namely the transcription factor members of the cascade sequentially bind the promoters and enhancers of different genes that must be activated to direct eye development. Expression of a gene in a particular tissue, then, requires that certain transcription factors present in that tissue bind to and activate the promoter and enhancer of that gene.

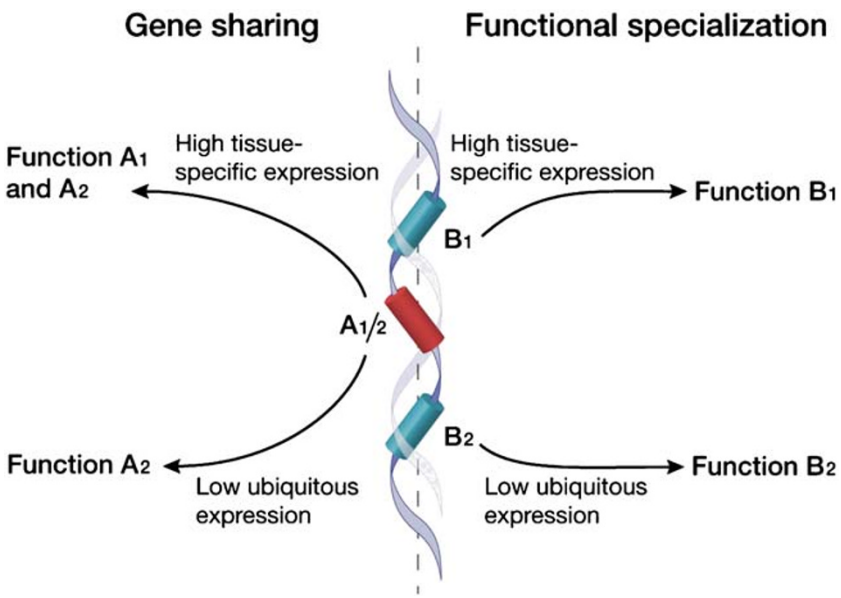

Fig. 4 Diagrammatic representation of two distinct molecular functions (*A1* and *A2*) performed by differential regulation of a single gene (Gene *A1/2*; *red*) in contrast to the functional specialization of sibling genes (*B1* and *B2*; *blue*) after gene duplication. The use of the single gene for two or more molecular functions is called gene sharing. Reprinted by permission from GENE SHARING AND EVOLUTION: THE DIVERSITY OF PROTEIN FUNCTIONS by Joram Piatigorsky, p. 4; Cambridge, Mass.: Harvard University Press, Copyright © 2007 by the President and Fellows of Harvard College

Numerous experiments involving genetic engineering have now established that the promoters and enhancers of different, unrelated (nonhomologous) crystallin genes are similar enough to bind similar transcription factors that cause high expression of the gene in the lens (Chepelinsky et al. 1985; Kondoh et al. 1987; Klement et al. 1989; Cvekl and Piatigorsky 1996; Carosa et al. 2002) What happened during evolution is that short stretches of DNA sequence, namely the *cis*-control elements, in the promoters and enhancers of certain genes that were not originally crystallin genes underwent independent sequence modifications making them able to bind transcription factors used for lens development. These convergent promoter and enhancer mutations were the basis for the recruitment of crystallin genes by virtue of making them expressed highly in the lens. Pax6 is one of the critical transcription factors causing high lens expression of nonhomologous genes recruited to become lens crystallin genes (Cvekl et al. 1994, 1995, 2004). It is of interest that Pax6 is also responsible for expressing the rhodopsin gene in the photoreceptor cells, linking rhodopsin and crystallin gene expression during eye evolution (Sheng et al. 1997). Convergent mutations in promoters and enhancers have been the basis for many phenotypic changes in evolution (Wray 2007). Figure 5 portrays diagrammatically the binding of some of the same transcription factors (except for jellyfish; see below) to different crystallin genes to activate high expression in the lens.

The diverse crystallins are thus unified less by the nature of their proteins than by the similarities in the regulation of their genes, which was achieved by independent evolution of gene regulatory sequences in promoters and enhancers. The sequence changes recruiting or modifying regulatory elements did not necessarily extinguish the original expression patterns of their associated genes in other tissues. Many of the recruited crystallin genes continued their original expression patterns and functions in addition to serving as lens crystallin genes, fulfilling the criterion for gene sharing of serving more than one molecular function.

In conclusion of this section, it is important to underline that the recruitment of nonhomologous crystallin genes occurred by independent changes in their promoters and enhancers, resulting in their gaining affinity for transcription factors that are members of the conserved developmental network used for eye and lens development in all species that have been studied. Crystallin evolution, thus, involved a combination of homologous and convergent processes.

Evidence for Parallel Eye Evolution in Cnidarians and Vertebrates

Astonishingly, cubozoan jellyfish, which are ancient cnidarians, have complex lens-containing eyes (Laska and

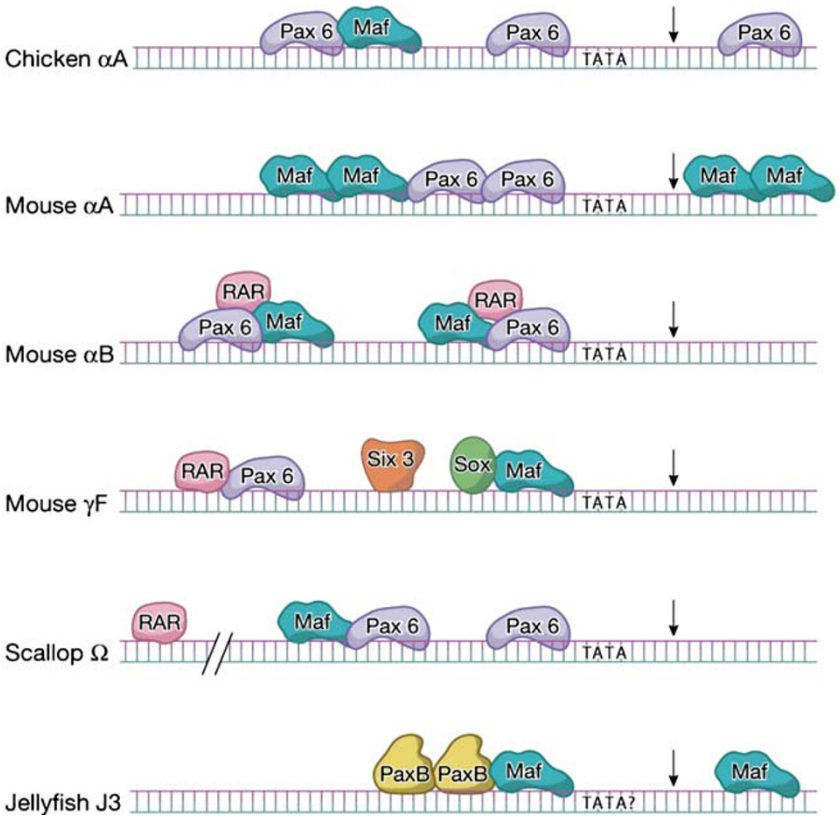

Fig. 5 Transcription factors that are used for lens development and activation of crystallin promoters in different species. Many more transcription factors are used, of course, but these are critically important and serve as examples of the convergent evolution of the recruitment and expression of nonhomologous crystallin genes in different species. Reprinted by permission from GENE SHARING AND EVOLUTION: THE DIVERSITY OF PROTEIN FUNCTIONS by Joram Piatigorsky, p. 85; Cambridge, Mass.: Harvard University Press, Copyright © 2007 by the President and Fellows of Harvard College

Hundgen 1982; Piatigorsky and Kozmik 2004). Cnidarians, the likely sister group to the Bilateria, are the earliest branching phylum with a well-developed visual system, making them of special interest with respect to evolution. The cubozoan jellyfish, *Tripedalia cystophora*, has four sensory structures called rhopalia, each containing two complex lens-containing eyes plus two pit-shaped and two slit-shaped pigment cup eyes (also called ocelli; Fig. 6). The rhopalia are integrated parts of the central nervous system of the jellyfish (Garm et al. 2006). The smaller, upper lens-containing eyes gaze directly towards the surface of the water, and the larger, lower lens-containing eyes are directed downward (Fig. 6B,C). The jellyfish lenses are cellular (Fig. 6D), as are all vertebrate and many invertebrate lenses. The photoreceptors of both lens-containing eyes have similar spectral sensitivities apparently due to a single opsin (Coates et al. 2006; Kozmik et al. 2008a), while the pit-shaped and slit-shaped non-lens eyes in the rhopalia appear to have a different opsin (Ekstrom et al. 2008). The lens of each eye has sophisticated optics due to a gradient of refractive index that eliminates spherical aberrations (Nilsson et al. 2005). Jellyfish eyes govern behavioral phototaxis responses (Coates 2003; Garm et al. 2007a, b) and control the swim pulse frequency by regulating light-sensitive swim pacemaker activity (Garm and Bielecki 2008).

The lenses of *T. cystophora* have three distinct crystallins (J1, J2, and J3), and each crystallin polypeptide is encoded in a separate gene. The J1 crystallins comprise three extremely similar polypeptides, while J2 and J3 crystallins are each a single polypeptide (Piatigorsky et al. 1989, 1993, 2001; Kozmik et al. 2008b). The jellyfish crystallins are unrelated to crystallins of other species consistent with their being recruited as crystallins independently in jellyfish. The crystallin genes are expressed in a number of jellyfish tissues, suggesting that they have non-lens functions and have evolved by a gene-sharing process as have crystallins of other species. The putative non-lens functions of the jellyfish crystallins are not known. J1 crystallins belong to a subfamily of proteins that show similarity to ADP-ribosylation enzymes (Castellano et al. 2005) and J3 crystallin shows similarity to saposins, which are multifunctional proteins involved in membrane turnover (Piatigorsky et al. 2001). J2 crystallin is a novel protein of unknown function that has not been found in other species (Kozmik et al. 2008b). The transparent lenses of jellyfish eyes, then, have high refractive power associated with a gradient of unique, independently recruited crystallins.

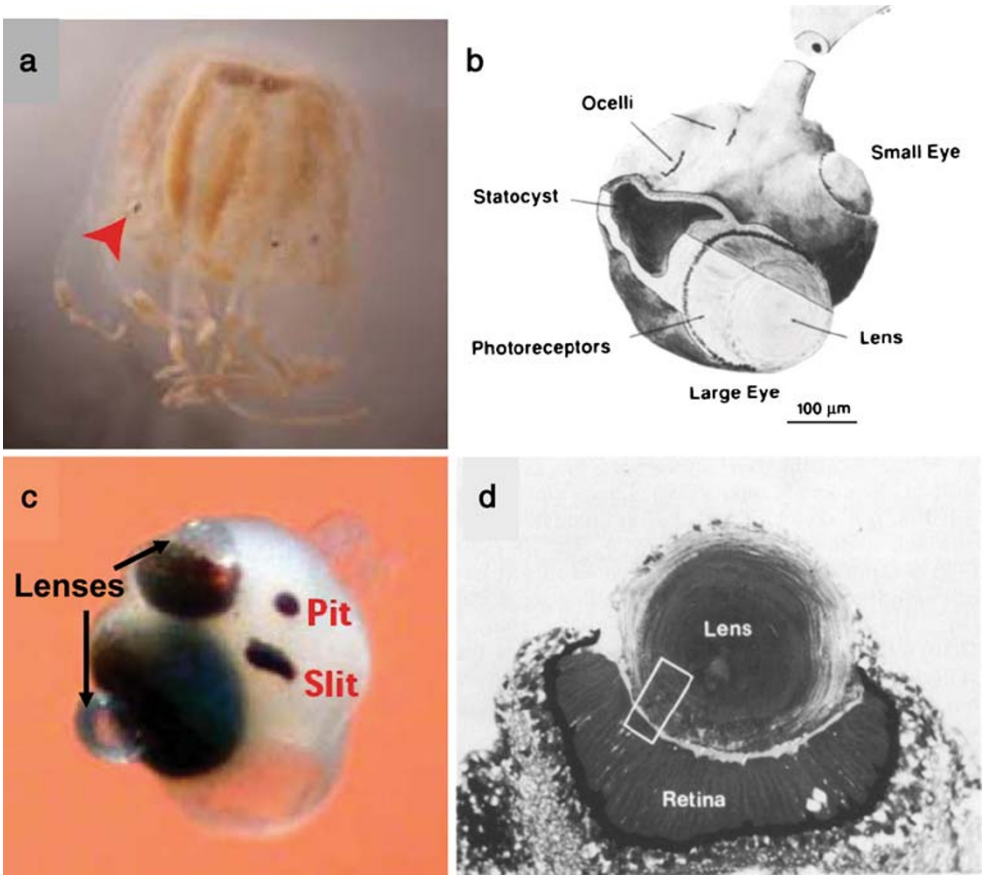

Fig. 6 The jellyfish, *T. cystophora* (**A**), a diagrammatic illustration of one of its rhopalia (**B**), an actual rhopalium showing two lens-containing camera-type eyes, a slit, and a pit eye (**C**), and a section through the large lower camera-type eye (**D**). Panels (**A**) and (**B**) are from (Kozmik et al. 2008a), and (**C**) and (**D**) are from (Piatigorsky et al. 1989). The *red arrowhead* in (**A**) points to a rhopalium

Unlike other species which activate their crystallin promoters with Pax6 (see Fig. 5), jellyfish lack Pax6 and have, instead, PaxB (Kozmik 2005). Pax6 and Pax2 (also involved in aspects of eye development) are believed to have been derived by gene duplication of a common ancestor to PaxB. PaxB, not Pax6, binds and activates the promoters of the jellyfish crystallin genes (Kozmik et al. 2003, 2008b). Jellyfish PaxB does not activate expression of crystallin genes of other species. It follows that jellyfish have independently recruited unique lens crystallins using a Pax transcription factor that does not exist in other species with lens-containing eyes.

Comparison of jellyfish and vertebrate retinas suggest that they evolved in parallel by independently recruiting similar genes in their retinas. First, the photoreceptors of the invertebrate jellyfish more closely resemble the vertebrate ciliary type than the invertebrate rhabdomeric type (Wray 2007; Kozmik et al. 2008a; Eakin and Westfall 1962). Moreover, multiple opsins from different species of cnidarians (the phylum in which jellyfish are situated) more closely resemble those of vertebrates than those of invertebrates (Suga et al. 2008). In addition, there is molecular biological evidence that the jellyfish photoreceptors employ a vertebrate-like phototransduction system (Kozmik et al. 2008a). Further studies, however, are required to establish the precise similarities and differences between jellyfish and vertebrate phototransduction processes.

Another similarity between jellyfish and vertebrate eyes involves the dark shielding pigment providing visual orientation [see (Kozmik et al. 2008a) for data, further discussion and references]. Invertebrates use a pigment comprising pterins and/or ommochromes (a polychaete annelid, *Platynereis dumerilii* and *Drosophila*) or rarely melanin (the planarian, *Dugesia*). Vertebrates use exclusively melanin as the dark-shielding pigment of the retina. It appears that jellyfish use melanin as do vertebrates for its dark-shielding eye pigment. Interestingly, the jellyfish melanin resides within the photoreceptors rather than in separate pigmented cells, as is the case in vertebrates. It is not known whether the incorporation of the pigment granules into the jellyfish photoreceptors reflects an ancestral or derived state.

Invertebrates and Vertebrates Both Have Ciliary and Rhabdomeric Photoreceptors and Opsins

Although beyond the scope of the present article, but as a point of interest in the fascinating story of eye evolution, it turns out that invertebrate and vertebrate eyes are not as markedly split into two categories defined by having either ciliary or rhabdomeric photoreceptors or opsins as implied above. The polychaete annelid worm *P. dumerilii* has rhabdomeric photoreceptors with r-opsin in the eye and ciliary photoreceptors with c-opsin in the brain (Arendt et al. 2004). These findings were interpreted as follows: a species developed an ancestral eyespot with undefined ancestral photoreceptors and opsin which underwent cell and gene duplications to give rise to rhabdomeric and ciliated photoreceptors with r-opsin and c-opsin, respectively. The rhabdomeric photoreceptors were used for vision in the retina, and the ciliated photoreceptors were used for brain activity during evolution in invertebrates. Vertebrate evolution involved a transition: the invertebrate brain ciliary photoreceptors were incorporated into the retina for vision and the invertebrate retinal rhabdomeric photoreceptors transformed into the vertebrate retinal ganglion cells, which contain the r-opsin related melanopsin and were used for photoperiodicity (Foster and Hankins 2007).

Concluding Remarks

Taken together, it appears that both parallel and convergent evolutionary processes played a role in eye evolution. The diverse lens crystallins comprise a variety of unrelated multifunctional proteins serving refractive functions in the lens and nonoptical functions (often enzymatic or stress protective functions) in other tissues utilizing a process called gene sharing. The recruitment and high lens expression of crystallins were accomplished during evolution by the independent acquisition of mutations in the *cis*-control elements of the promoters and enhancers of their genes, making the crystallin genes responsive to a similar set of transcription factors used for eye development. Thus, the diverse lens crystallins were recruited by convergent changes in the regulation of unrelated genes.

In contrast to the striking diversity of lens crystallins, the photoreceptors and the phototransduction cascades in jellyfish and vertebrates appear unexpectedly similar. Although these similarities do not prove that jellyfish and vertebrates evolved eyes in parallel by independently recruiting related genes, it is difficult to explain the similarities of these disparate species by strict inheritance, as would be required for homology in view of the differences in phototransduction between vertebrates and invertebrates.

How such a remarkable common independent gene recruitment process characteristic of parallel evolution may have taken place remains conjectural. A promising hypothesis is that parallel evolution involving similar pathways was forced by constraints that limit the options that could have been implemented (Hodin 2000; Abouheif 2008). In that connection, Kozmik (2005) has put forth a bipartite model for eye evolution based on the DNA-binding paired domain and homeodomain of Pax proteins. The case is made that the crucial role of the paired domain for eye morphogenesis, pigment cell development, and

crystallin gene expression, and the importance of the homeodomain for opsin synthesis are coupled in one molecule, the Pax transcription factor. In Kozmik's words, "…the morphological unity found in the eye, a photoreceptor linked to the shading pigment, is mirrored on the molecular level, by uniting two independent DNA-binding domains in one regulatory protein." Such a constraint would make it difficult to dissociate Pax proteins and their associated transcription factors from eye development during evolution.

Finally, a personal comment: Science often has a narrative character that rivals any great piece of literature. The scientific narratives are based on real characters (molecules) that have been studied and represented to the best of the scientist's ability. The plots are created by the experimental paths seeking answers to the questions posed by the scientist. Clearly different routes (sets of experiments) can be followed, and the data (language, imagery) can be expressed in numerous ways. No two scientists will develop identical narratives just as no two authors will write identical novels, although both of the authors or both of the scientists could address similar issues and have similar outcomes. The multiple aspects of eye evolution—gene sharing, convergence, homology, parallelism—all occurring simultaneously reflect the enormous richness of choices that Nature provides at each instant over hundreds of millions of years under changing conditions. The ambiguities in evolutionary history that we spectators-at-a-distance are left with resemble the literature qualities of science where the great conflicts are seldom resolved to finality. We are writing narratives about characters we never met that were born and in some cases died during events before our time, and that may have had similar or quite different functions during their lifetime than their progeny have today. These ambiguities are not like those of *The Trial* by Kafka, where one never learns what crime was committed but more like historical narratives where one knows the major actors, the plot, and the results yet still argues about the precise nature of the characters or the details of the environment or reported events, all of which may significantly impact the final conclusions. The ambiguities of evolutionary history in no way deny that there was such a history, but they provide space for new perspectives that can modify the narrative. We know the characters of the diverse extant eyes, we reconstruct their histories the best we can, but we are never certain to what extent and when similar properties were inherited or derived independently. It may have some value, then, to keep in mind that science, like literature, is a human endeavor holding great truths and many possibilities.

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