Eye evolution: Lens and cornea as an upgrade of animal visual system

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Abstract

Lens-containing eyes are a feature of surprisingly broad spectrum of organisms across the animal kingdom that represent a significant improvement of simple eye composed of just photoreceptor cells and pigment cells. It is apparent that such an upgrade of animal visual system has originated numerous times during evolution since many distinct strategies to enhance light refraction through the use of lens and cornea have been utilized. In addition to having an ancient role in prototypical eye formation Pax transcription factors were convergently recruited for regulation of structurally diverse crystallins and genes affecting morphogenesis of various lens-containing eyes.

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Keywords: Eye; Lens; Cornea; Evolution; Crystallin

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1. Lens and cornea: introduction

Vision is one of the most important animal senses. Eyes of some sort occur in many animal phyla (Fig. 1), but their anatomy, ontogenetic origin and degree of sophistication vary enormously [1]. The minimal setting for an eye involves a single photoreceptor in the vicinity of a cell expressing dark shielding pigment [2] situation found, for example, in Hesse eye cups of amphioxus [3]. Primitive eyes can only provide information about light intensity and direction. Adding an optical system such as lens and cornea that could increase light collection and produce an image would dramatically improve optical performance. Lens-containing (camera-type) eyes can simply be defined as those with an additional refractive element in front of the photoreceptive layer. The cornea is a transparent layer on the eye periphery (usually of a convex shape). The cornea can be represented by a single cell layer (e.g., box jellyfish) but it can also form a complex multilayer organ (e.g., vertebrates). Sometimes it is difficult to distinguish between lens and cornea which function in similar ways. Eyes where cornea and lens are united as a single unit (e.g., corneal lens in Drosophila ommatidium) are known as well as eyes where the cornea has all (e.g., terrestrial vertebrates) or almost no (e.g., underwater animals) refractive power. Apart from its refractive function, a cornea can serve as protection for the eye, a nutritive device or a light filter [4–6]. The complex of the cornea and lens is known as a refracton [7].

This review aims to discuss evolution of lens-containing eyes using a combination of morphological, ontogenetic, optical and molecular data. We have chosen six model organisms representing distantly related animals (box jellyfish, fruit fly, scallop,
squid, zebrafish and mouse) to illustrate different visual strategies. Finally we propose diverse roles of Pax transcription factors in assembly of prototypical eye and in convergent evolution of lens-containing eyes.

2. Environmental constraints affecting lens and cornea properties

Whereas primitive eyes can serve as simple dark/light sensors, sophisticated lens-containing eyes are often able to produce a sharp image in the desired plane of focus due to light refraction. Refraction occurs when light rays travel from a medium with a given refractive index to a medium with another. Therefore, the optical characteristics of the eye are primarily determined by whether it is used in air or water (or both) [8]. Terrestrial animals use the refractive power of both cornea and lens because two interfaces air/cornea and ocular fluid/lens have refractive indexes sufficiently different to cause relevant refraction. The aquatic animals mostly use the refractive power of the lens. Almost no refraction takes place on the water/cornea boundary because they have usually very similar refractive indexes [1].

The secret to increasing lens efficiency and thereby compensating for the missing function of the cornea in aquatic species is the small radius of the lens curvature. The spherical shape of the lens makes it possible to shorten the focal length and maintain an appropriate size of the eye. Unfortunately the spherical shape (as the almost universal solution for underwater animals) introduces image aberrations because the properties of the lens vary sharply between the lens center and periphery and the light rays behave differently. The rays coming through the peripheral parts focus onto a different plane than those coming through the entire lens (spherical aberration) and the rays of different wavelengths will be focused differently as well (chromatic aberration). The preferred solution to spherical aberration is a graded refractive index with the maximum in the lens center and minimum on the lens periphery. The light rays break continuously through the entire lens and a one-step refraction change does

Fig. 1. Re-occurrence of lens-containing eyes throughout the animal kingdom. A diagram of main animal phyla indicating the presence of lens-containing eyes or eyes without a lens (in the adult or in the larval stage) within each phylum. An eye is defined here as an organ of spatial vision with a minimum requirement of a single pigmented cell and at least one photoreceptor cell [2]. The representatives with lens-containing eyes in each phylum are shown in Table 1.
not occur [9]. Graded refractive index is achieved by the concentration gradient of proteins within a lens. Some aquatic lens centers contain nearly 100% protein (refractive index 1.55) in contrast to terrestrial animals where the maximum usually is 40% protein. The refractive index decreases towards the lens periphery to a value of 1.35 close to the 1.34, refractive index of sea water [10]. A gradient of protein concentration in lenses creates a problem of protein aggregation which can cause light scattering. A recent study of a large family of proteins encoding variants of glutathione S-transferase present in squid lens has provided an interesting example of crystallin evolution to achieve concentration gradient without an apparent aggregation [10].

Terrestrial species must integrate the output of two refractive parts of the eye and for this reason many eye designs emerged during evolution [1]. Although the eyes of terrestrial species often profit from the high efficiency of the cornea, some terrestrial animals have fish-like spherical lenses with a gradient of a refractive index, a strategy which maximizes light gathering and is thus particularly advantageous and common in nocturnal species such as the mouse [11,12]. An unusual example of cornea and lens diversity is found among the fish Anableps anableps [13–15]. This fish, living on the air/water interface has specially optimized eyes where one half (dorsal) is modified for air environment and second (ventral) for submerged movement. The aerial and aquatic sections are divided by a pigmented strip on the cornea and have different attributes. The lens is pyriform with two separate visual axes. Indeed two distinct images emerge on the retina. The corneas of the dorsal and ventral eye are very distinct. The dorsal (aerial) cornea is thicker and flatter and contains more proteins (gelsolin and G-actin). This part has also been shown to be glycogen rich and with thicker collagen fibers. The heavily modified upper part (dorsal) of the cornea has been discussed in the context of desiccation protection (glyco-gen content) and the increased refractive role (thickness) on air. It has been suggested that the ventral part is ineffective as underwater corneas used to be and that the longer visual axis of the lens together with strong accommodation are crucial for aquatic vision of the Anableps.

3. Molecular basis of lens and cornea mediated vision: recruitment of crystallins by a gene sharing strategy

The main optical properties of lens and cornea are transparency and refractive power. Proteins that contribute to these optical properties are collectively called crystallins. Crystallins were initially thought to be highly characteristic for their optical functions in the lens but instead they turned out to be neither specialized nor tissue restricted [16]. Crystallins are best defined as highly abundant, cytoplasmic water-soluble proteins responsible for the optical properties of the lens in both vertebrates and invertebrates. The abundance parameter is arbitrary: the protein must simply be present at a sufficient concentration to affect the refractive properties of the lens in a biologically meaningful way in order to be called a crystallin. Known crystallins are generally globular proteins present in the lens as monomers (such as γ-crystallins), dimers (some β-crystallins) or tetramers (such as δ-crystallins). Vertebrate α-crystallins form even higher order aggregates of variable size with multiple (up to about 50) interacting polypeptides. The crystallins account for up to 90 percent of the dry weight of the lens although their absolute concentrations vary between species and also with spatial location in the lens. Crystallin concentrations are generally higher in aquatic than terrestrial animals and in the center of lens than periphery. In order to avoid spherical aberrations crystallin concentration has to decline smoothly from the center to the periphery of spherical lens to create a gradient in refractive index. Lens transparency is maintained when the lens lacks discontinuities of refractive index greater than half the wavelength of the transmitted light. Large fluctuations in the concentration of closely packed crystallins could cause light scattering and affect the quality of the resulting image.

In striking contrast to the universal conservation of opsins as the visual pigments in the photoreceptors, the lens crystallins are diverse proteins that are often taxon-specific, i.e. different proteins function as crystallins in different species [17–19]. The taxon-specificity of crystallins might be a consequence, at least in some cases, of multiple independent origins of eyes and/or lenses during evolution. Thus, while there is phylogenetic inheritance of crystallins, such as for example the δ-crystallins in birds and reptiles [20], the crystallin composition within the lens generally does not reflect evolutionary relationships.

The diversity and taxon-specificity of lens crystallins throughout the animal kingdom is indicative of convergent evolution of crystallin roles. Certain proteins are preferentially recruited as lens crystallins. Proteins used as lens crystallins are often related or identical to ubiquitously expressed metabolic enzymes or physiological stress proteins. All vertebrate lenses contain the α-crystallins belonging to the family of small heat shock proteins [21,22] and the β/γ-crystallins that are related to microbial stress proteins [23,24]. Vertebrate α-crystallins are effective chaperones that protect partially denatured proteins from aggregating in the lens [25], thus helping to maintain the transparency. The co-option of a protein with chaperone-like activity as a lens crystallin makes sense in light of the lack of protein turnover in vertebrate lenses. Vertebrate lens continues to increase in diameter throughout life. As the lens grows the lens fiber cells differentiate, lose their nuclei and other organelles and are, therefore, incapable of replacing the aging crystallins. As a result, the core of the lens contains proteins produced well before birth that have to last a lifetime. The selective advantage of recruiting enzyme-crystallins is less clear [16]. First, they do not fall within a common metabolic category. The catalytic activities include but are not limited to lactate dehydrogenase (e-crystallin), argininosuccinate lyase (d-crystallin), α-enolase (τ-crystallin) or glyceraldehyde 3-phosphate dehydrogenase (π-crystallin). Furthermore, some enzyme-crystallins have lost their catalytic activity (e.g. aldehyde dehydrogenase/scallop Ω-crystallin) suggesting that, unless they have gained a new substrate specificity, their function in lens is limited to the structural (refractive) role. An interesting group of enzyme-crystallins with additional putative non-refractive function represent proteins that bind nicotinamide adenine dinucleotide cofactors (NAD-binding enzyme-crystallins). It was proposed that NAD-binding
enzyme-crystallins present in high amount in lenses could serve as UV filters to reduce glare and/or to protect against oxidative stress [26]. It is possible that NAD-binding contributed to frequent recruitment of this group of enzymes as lens crystallins.

There is an intriguing parallel between lens crystallins and abundant, taxon-specific corneal proteins (e.g. aldehyde dehydrogenase). Although their optical (refractive) function has not yet been established the close proximity and co-evolution of lens and cornea, currently viewed as a single unit, the “refracton”, suggest that these abundant proteins might be considered corneal crystallins [7].

An important general concept, ‘gene sharing’, has emerged from crystallin studies [16,19,27]. Gene sharing proposes multiple uses of a single protein encoded in one gene. Thus, the protein encoded by a single gene may perform two entirely different functions: a refractive function in the lens as a lens crystallin and a catalytic or stress function elsewhere (as well as in the lens). An important implication of gene sharing nicely illustrated by the example of lens crystallins is that a protein can evolve a new role, without losing its original function, simply by a change in gene expression [28]. Furthermore the new function is established in the absence of gene duplication [29]. It has become apparent over time that gene sharing and repeated use of proteins for new tasks in general represents a common evolutionary strategy [16].

4. Transcriptional control of lens crystallin genes: convergent evolution of Pax regulatory sequences

Crystallin protein levels in the lens can be affected by multiple parameters such as transcriptional rate, translational efficiency, mRNA or protein stability. However, it appears that the tissue-specific transcriptional regulation plays a key role in recruiting a gene to become crystallin gene. Remarkably, the high lens-preferred expression of non-homologous crystallin genes is regulated by a similar set of transcription factors in vertebrates and invertebrates [30–33]. Many of these transcription factors are either known regulators of lens (or eye) development (e.g. Pax, Sox, Maf, Prox1) or transcription factors sensing oxidative stress (e.g. AP1, CREB) [33].

Perhaps the most remarkable is the widespread conservation of Pax transcription factors as regulators of eye development and crystallin gene expression (reviewed in [34–38]). Molecular dissection of crystallin promoters and enhancers identified a number of functional Pax regulatory elements. First of all, Pax6 can activate or repress many vertebrate crystallin reporter genes in transfection assays. This appears to be a direct effect since many Pax6 binding sites were identified in crystallin regulatory regions by either in vitro or in vivo (chromatin immunoprecipitation) approaches. In vertebrates, Pax6 has been implicated in regulation of chicken and mouse \( \alpha \)-crystallin [39–41] mouse \( \beta \)-crystallin [42], \( \beta \)B1-crystallin [43], chicken \( \delta \)-crystallin [44,45], mouse \( \gamma \)/\( \gamma \)F-crystallins [46,47] and taxon-specific guinea pig \( \zeta \)-crystallin [48]. Much less is known about Pax6-mediated crystallin gene regulation in invertebrates. The promoter of the scallop \( \Omega \)-crystallin gene has an arrangement of putative cis-control elements, including functional Pax6 binding sites, surprisingly similar to that used for lens promoter activity of the unrelated \( \alpha \)-crystallin genes [31]. Cnidarians who lack a true Pax6 gene [32] have recruited another member of Pax gene family, designated PaxB, for crystallin activation. PaxB corresponds to an ancestral Pax gene encoding a paired domain, homeodomain and an octapeptide [49]. The presence of two independent DNA-binding domains is shared by Pax6 and PaxB. However, specific amino acid changes within paired domain are responsible for their distinct DNA-binding specificities. In box jellyfish Tripedalia cystophora possessing well-developed lens-containing eyes PaxB binding sites were identified in J-crystallin promoters. Furthermore, PaxB binding sites appeared functional as judged by site-specific mutagenesis and transfection tests ([32]; Kozmik et al., submitted). The data indicate that modern Pax2 and Pax6 genes evolved from PaxB ancestor by duplication and diversification in higher metazoans. Thus the structurally similar yet functionally distinct members of Pax gene family were recruited to achieve lens-specific crystallin expression in diverse phyla. It is interesting to note that in all known cases of Pax-mediated crystallin regulation it is the paired domain and not the homeodomain, which mediates the effect. This situation is reminiscent of the role of individual domains in general morphogenesis of animal eyes. At least on the basis of genetic data in vertebrates and flies, the Pax6 paired domain seems to be more important than is the homeodomain for eye morphogenesis [50,51].

The presence of similar cis-acting elements for a common set of transcription factors, especially Pax, in non-homologous crystallin genes in various animal species is due to convergent evolution. It reflects the situation that the diverse crystallin genes have been recruited independently during evolution and hence their regulatory elements to achieve lens-preferred expression must have been acquired by independent events as well.

5. Building up the lens: a tale of endless diversity and multiple origins

The level of sophistication of animal eyes is generally not correlated with the complexity of animal body plan. Lens-containing eyes representing a significant improvement of visual system are sprinkled throughout the animal kingdom (Fig. 1, Table 1). The first metazoan eyes with lens are found in the basal phylum Cnidaria. Two out of seven main ecdysozoan’s phyla developed an eye with a lens. Onychophorans (velvet worms) possess paired eyes with secreted cuticular cornea and secreted acellular lens from granular material, with the whole eye being of epidermal origin [52]. The huge phylum of arthropods includes mainly species with compound eyes (either sessile or stalked) or single-chambered eyes with corneal lens. The lophotrochozoan group includes large phyla such as mollusks, annelids and platyhelminthes. Mollusks are characterized by unusual diversity of their visual system including sophisticated camera-type (squid) or mirror-containing eyes (scallop). Within the annelid phylum there are representatives with lens eyes among polychaetes. A simple cup eye,
a lensed cup eye (nereid, platyneris) as well as complex eyes with spherical lens (Vanadis, Odontosyllis) occur in this diverse class [53–55]. Really peculiar lenses with a likely mitochondrial origin have been identified within the phylum Plathelminthes [56,57]. Proteins are not the only refractive material used for a lens as exemplified by the echinoderm phylum. Some brittlestars use a unique system of vision based on calcitic lenses [58]. Similar calcitic lenses have been discovered also within trilobite fossils [59]. The origin of vertebrate lens is probably the most interesting question in relation to our own vision. When we follow the proposed vertebrate line – cephalochordates (amphioxus)–tunicates (ciona)–craniates (cephalochordates) split [3]. Based on the fossil record of early chordates, such as pikaya, it appears that those animals did not have prominent paired eyes. A frontal eye of the amphioxus with ciliated photoreceptor cells therefore most likely represents an ancestral condition from which paired bilateral eyes of vertebrates formed rather than a derived (regressed) state. Already in tunicates which were shown to be closer to vertebrates than cephalochordates [60], there is an ocellus with three-cell lens in the larval stage of some species. Study of Ciona βγ-crystallin suggests that chordate βγ-crystallin ancestor was already expressed in a cell-specific manner in derivatives of a primitive visual system prior to the evolution of the lens in vertebrate lineage [61]. Hagfish have a poorly developed visual system (probably as a result of regression) in contrast to lampreys that previously had relatively small but fish-like eyes with almost spherical multicellular lens covered closely with a thin cornea [62]. And finally, the spherical lens with appropriate gradient of refractive index is the best solution for aquatic animal; consequently this design is usually maintained among fishes, the true vertebrates [9].

Diversity of lens-containing eyes is observed at every level of comparison–structural design, ontogenetic origin or the crystallin content. Yet the final morphological appearance of lens-containing eyes is often remarkably similar suggesting independent origins by convergent evolution. In the following paragraphs we compare and contrast eyes of six organisms representing distantly related animals (box jellyfish, fruit fly, scallop, squid, zebrafish and mouse) to illustrate different visual strategies (Fig. 2).

In box jellyfish (Tripedalia cystophora) very complex structures characteristic of more advanced animals appear such as the camera-type eye situated within four sensory organs called rhopalia. Each rhopalia carries six eyes: two pit-shaped, two slit-shaped and two complex with a cellular lens. The eyes with lens are covered with a thin cornea, but differ in size, lens shape, retinal geometry, as well as in gradient of a refractive index [63]. The cornea is de facto a part of a single layer covering the entire rhopalia and has no refractive capacity. The large biconvex lens has a homogenous center and a peripheral gradient but the drop-shaped lens of the small eye has an almost perfect gradient through the lens correcting its aberrations. The resulting image produced by the lenses is severely out of focus and, i.e., blurred. It can be functional as a low-pass filter for this animal which would not be able to use a more complex image [63]. The rhopalia develops during metamorphosis of the polyp into the medusa stage from a dedifferentiated cell derived from fused polyp tentacles and thus the lenses are originally derived from myoepithelial cells [64]. None of the Tripedalia crystallins have a sequence relationship to other crystallins in any other species [65–68]. Two crystallins, J1 and J2, provide a majority of crystallins within the lens of both the small and large eye.

### Table 1
A table comprising those phyla where lens-containing eye occurs

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Representative</th>
<th>Lens type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnidaria</td>
<td>Cladonema radiatum</td>
<td>Lens bodies</td>
<td>[101]</td>
</tr>
<tr>
<td>Hydrozoa</td>
<td>Tripedalia cystophora</td>
<td>Cellular lens</td>
<td>[65]</td>
</tr>
<tr>
<td>Cubozoa</td>
<td>Peripatoida novaezealandiae</td>
<td>Non-cellular lens</td>
<td>[52]</td>
</tr>
<tr>
<td>Arthropods</td>
<td>Porcello scaber</td>
<td>Corneal lens and crystalline cone</td>
<td>[102]</td>
</tr>
<tr>
<td>Cheliceriformes</td>
<td>Portia fimбриa</td>
<td>Corneal lens and secondary retinal lens</td>
<td>[103]</td>
</tr>
<tr>
<td>Myriapoda</td>
<td>Craterostigmus tasmanianus</td>
<td>Corneal lens</td>
<td>[104]</td>
</tr>
<tr>
<td>Hexapoda</td>
<td>Drosophila melanogaster</td>
<td>Corneal lens and crystalline cone</td>
<td>[105]</td>
</tr>
<tr>
<td>Plathelminthes</td>
<td>Entobdella soleae</td>
<td>Mitochondrial lens</td>
<td>[56]</td>
</tr>
<tr>
<td>Nemertea</td>
<td>Odontosyllis enopla</td>
<td>Cellular lens</td>
<td>[53]</td>
</tr>
<tr>
<td>Mollusks</td>
<td>Hermisenda crassicornis</td>
<td>Non-cellular lens (secreted)</td>
<td>[107]</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>Pecten maximus</td>
<td>Cellular lens</td>
<td>[69]</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>Loligo opalescens</td>
<td>Non-cellular lens (fused procceses)</td>
<td>[73]</td>
</tr>
<tr>
<td>Echinoderm</td>
<td>Ophiocoma wendii</td>
<td>Calcitic lens</td>
<td>[58]</td>
</tr>
<tr>
<td>Cephalopoda</td>
<td>Ciona intestinalis</td>
<td>Three-cell lens</td>
<td>[106]</td>
</tr>
<tr>
<td>Tunicates</td>
<td>Anableps anableps</td>
<td>Cellular lens</td>
<td>[13]</td>
</tr>
<tr>
<td>Craniates</td>
<td>Tripedalia cystophora</td>
<td>Cellular lens</td>
<td>[101]</td>
</tr>
<tr>
<td>Phylum</td>
<td>Representative</td>
<td>Lens type</td>
<td>Reference</td>
</tr>
</tbody>
</table>
| Cnidaria      | Cladonema radiatum | Lens bodies | [101]  
| Hydrozoa      | Tripedalia cystophora | Cellular lens    | [65]  
| Cubozoa       | Peripatoida novaezealandiae | Non-cellular lens | [52]  
| Arthropods    | Porcello scaber | Corneal lens and crystalline cone | [102]  
| Cheliceriformes | Portia fimбриa | Corneal lens and secondary retinal lens | [103]  
| Myriapoda     | Craterostigmus tasmanianus | Corneal lens | [104]  
| Hexapoda      | Drosophila melanogaster | Corneal lens and crystalline cone | [105]  
| Plathelminthes | Entobdella soleae | Mitochondrial lens | [56]  
| Nemertea      | Odontosyllis enopla | Cellular lens | [53]  
| Mollusks      | Hermisenda crassicornis | Non-cellular lens (secreted) | [107]  
| Gastropoda    | Pecten maximus  | Cellular lens | [69]  
| Bivalvia      | Loligo opalescens | Non-cellular lens (fused procceses) | [73]  
| Echinoderm    | Ophiocoma wendii | Calcitic lens | [58]  
| Cephalopoda   | Ciona intestinalis | Three-cell lens | [106]  
| Tunicates     | Anableps anableps | Cellular lens | [13]  

For each phylum at least one representative with lens type specification and reference is given.
Fig. 2. Lens-containing eyes of selected model organisms. (A) Phylogenetic distances of selected model organisms and their distribution across the animal kingdom. Splits between clades are indicated in million years (mya). (B) Hematoxylin-eosin stained sections of animal eyes showing differences in lens and cornea shape and structure. Note the crystalline core of the fish lens (medaka) and an unusual shape of scallop’s lens. L, lens; c, cornea. (C) A schematic diagram illustrating the eye morphology, crystallin variability and refractive index (n) in the center of the lens of selected model animals [84,63,69,10,100].
The lens in the scallop (*Argopecten irradians*) eye, unlike other underwater animals, has a homogeneous refractive index and a hemispheric shape [69]. This is because the image is produced by the mirror on the posterior wall of the eye but not by the lens. The mirror formed by the layer called argentea or tape-tum consists of layers of guanine crystals and has a refractive index of 1.8 [69]. Light rays coming through the lens reflect on the distal retina neighboring the mirror. The lens probably has a role in correcting the spherical aberration of the mirror. Lens of the bay scallop was described as soft (in contrast to fish) and filled with one major crystallin (\(\Omega\)-crystallin) identified as an aldehyde dehydrogenase and noted ALDH1A9 [31]. The co-option of an ALDH enzyme as a crystallin has occurred several times in vertebrates or in invertebrates. Scallop \(\Omega\)-crystallin has homology to squid and an octopus minor lens \(\Omega\)-crystallin [70]. \(\Omega\)-crystallins are enzymatically inactive. It is also expressed in the cornea and together with other records of corneal crystallin expression supports the idea of its role as a corneal crystallin [71].

A squid (*Loligo opalescens*) possesses an eye with a spherical lens that has a graded refractive index compensating for spherical and other aberrations [72]. A cephalopod lens differs markedly in development compared to vertebrates (although the resulting lens design of fish and squid is very similar). Vertebrate lenses develop by invagination or delamination of the surface ectoderm. The squid lens originates also from the ectoderm but from two distinguishable parts. Each part is a primordium of one of the two future halves of the lens. Lentigenic cells of the anterior and posterior primordium give rise to a bipartite lens which is derived from ectodermal cellular processes and thus does not have a completely cellular state. The posterior lens primordium arises as the first and later the anterior forms a smaller plate-like element of the lens which is thickened during development. The two parts of the lens are separated by septum, a thin layer of living cells [73,74]. In addition, the cornea is derived from epithelial tissue. This tissue from the posterior part of the eye is probably moved by neighboring muscle cell contractions and migrates over the eyeball [75]. Modified glutathione S-transferase is a major crystallin (S-crystallin) of the squid ocular lens [76] but aldehyde dehydrogenase has also been identified as a minor crystallin (\(\Omega\)-crystallin). This enzyme has been further recruited for the lens of the light organ where it was denoted as L-crystallin [77]. Of special interest is the fact that there are more than 25 isoforms of the S-crystallin in the ocular lens of the squid that are differentially expressed to maintain optical performance and clarity of lens [10]. It has been suggested that gene duplications followed by mutations to accumulate positive charge in S-crystallin were essential to achieve concentration gradient without an apparent aggregation [10].

The compound eye of the fruit fly (*Drosophila melanogaster*) consists of hundreds of ommatidia which are composed of two main refractive parts: corneal lens secreted by underlying corneagen cells and a four-part crystalline cone (pseudocone) produced by cone (Semper) cells. The receptive part under the corneal lens and cones consists of photoreceptors (rhododemes) with rhabdoms. In addition to the refractive and receptive parts are pigment cells which restrain the receptive field and separate ommatidia. The compound eye originates during metamorphosis from a simple epithelium of the eye disc involving highly complex pattern-creating events. The corneal lens as well as cones are epithelial derivatives (secreted by cone cells, corneagen cells and primary pigment cells) and serve as independent surfaces for multiple refraction [1,55]. A 52 kDa large glycoprotein (known as drosocrystallin) has been described as a major protein of the corneal lens [78]. It was shown that drosocrystallin is a homologue of a cuticle protein indicating that a gene-sharing strategy has been used in arthropods.

The eyes of zebrafish (*Danio rerio*) are laterally placed and the protuberant lenses allow for panoramic vision. A zebrafish lens is spherical having a refractive index gradient with the maximum refractive index in the center of the lens. The whole eye is developed within first 3 days after fertilization but growth continues for 1 month [79,80]. The development is similar to that of other vertebrates but important differences have been found. The lens does not originate from the surface ectoderm by the process of invagination as known for other vertebrates but a thickened lens placode undergoes delamination. Thus the lens vesicle rises as a compact formation and primary fiber cells are formed from the central cell cluster (and not from posterior epithelial cells) forming the nucleus of the lens. Three classes of crystallins (\(\alpha\)-, \(\beta\)-, \(\gamma\)-) typical for vertebrates are expressed in zebrafish lens. Among those more than sixteen \(\gamma\)-crystallins have been found including nine \(\gamma\)M-crystallins characteristic for aquatic animals and two \(\gamma\)N-crystallins which were described as evolutionary hybrids of \(\beta\)- and \(\gamma\)-crystallins [81]. Corneal crystallins of the gelsolin protein family have been described for zebras [82]. It is a diverged parologue of the vertebrate scinderin gene with calcium- and actin-binding and actin-severing capabilities. Six genes have been cloned and sequenced, three of them with preferential expression in the cornea [83]. Gelsolin-like proteins were found in zebrafish as well as in *Anableps* where the expression varies between ventral and dorsal cornea [14,83].

The nocturnal mouse (*Mus musculus*) has a large spherical lens and curved cornea that are ideal for very short focal length and maximal light gathering capacity [1,84]. Both cornea and lens function in light focusing in mouse. A critical region of cornea for refraction is collagen rich stroma where collagen fiber bundles form a highly ordered three-dimensional network [85,86]. The lens has an almost spherical shape that suffers from spherical aberrations and has a smooth gradient of refractive index [87]. Taken together, mouse is able to gather much more light than the human eye can, but the quality of the image is decidedly worse [87]. Mouse lens arises by invagination of surface ectoderm for which the interaction with optic vesicle (neural ectoderm) is a key inductive step (for review see [88]). The corneal epithelium develops at first from anterior surface ectoderm. Migrating periciliar mesenchymal cells (neural crest and paraxial mesoderm cells) give rise to presumptive corneal stroma. The posterior part of the cornea differentiates later into corneal endothelium. As in zebrafish, all three major vertebrate crystallin classes (\(\alpha\)-, \(\beta\)-, \(\gamma\)-) are present in the mouse lens. \(\alpha\)A-crystallin is expressed mainly in the lens while \(\alpha\)B-crystallin is an ubiquitous protein. The elimination of \(\alpha\)A-crystallin in mice leads smaller and opaque lenses [89] while \(\alpha\)B-crystallin-
deficient mice exhibit more pleiotropic phenotypes associated primarily with muscles and heart [90,91]. A cluster of six γ-crystallins (A–F) typical for placental mammals is expressed within the mouse lens [92]. The main corneal crystallin in mouse is ALDH3a1 [93,94]. Interestingly, although ALDH3a1 comprises nearly one-half of the water-soluble protein fraction in the corneal epithelium, the ALDH3a1 knockout mouse contains no detectable defects in corneal morphology or transparency [95].

6. Summary: implication of lens and cornea upgrade for eye evolutionary history

The eye has challenged generations of evolutionary biologists. Whether an eye originated once (monophyletic origin) or multiple times (polyphyletic origin) still remains a controversial topic. Despite obvious anatomical, developmental and organizational differences between mouse and *Drosophila* eyes, genetic studies have indicated that a homologous gene, *Pax6*, directs development of eyes in both species [96,97]. Since then more evidence has emerged for an almost universal re-deployment of *Pax* genes for animal eye development [32,34]. Such common underlying genetic mechanism provides a powerful argument for monophyletic eye origin [34] and might reflect an ancient role of *Pax* genes in functioning of a “primitive eye” (eye prototype) composed of a photoreceptor cell and a shielding pigment cell. A bipartite model proposes that the two independent DNA binding domains within a single *Pax* transcription factor have been co-opted for two essential features of the prototypical eye [35] (Fig. 3, top). Production of a dark shielding pigment ('pigmentation' program) has been controlled by paired domain and production of a photopigment ('opsin' program) by homeodomain. Once such transcriptional regulatory network was established the two genetic programs being driven by two independent DNA binding domains within a single transcription factor became virtually inseparable.

In contrast, the enormous morphological diversity found among animal eyes seems incompatible with monophyletic
origin but is rather consistent with convergent evolution and multiple independent origins [98]. Theoretical modeling suggested that a camera-type eye can evolve from a flat layer of photosensitive epithelium in less than 400,000 generations [99]. We can view camera-type (lens-containing) eyes as a significant improvement of primitive eyes that has allowed animals to gather more light onto their photoreceptors and to obtain a focused image. Such an upgrade of eyes by addition of a lens (and cornea) was a milestone during eye evolution. As described above amazingly broad and distinct strategies of how to design lens and cornea have been utilized by different animals. It follows that the lens/cornea upgrade of animal eyes has a polyphyletic origin. It is almost impossible to find anything uniting corneal lens of jumping spiders and sperical lens of fish. There is no doubt that even in the case of cellular lenses with their crucial refractive ability provided by an accumulation of crystallins it is safe to argue for multiple independent origins because the genes encoding lens (and corneal) crystallins are almost always taxon-specific [16]. It is intriguing that the same transcription factors, especially Pax, were used once again as key regulators in lens/cornea upgrade. Pax regulatory elements were essential for convergent recruitment of lens and corneal crystallins as well as for driving the divergent morphogenesis of various types of lens-containing eyes (Fig. 3, bottom). In summary, animal eyes have evolved by convergent mechanisms a variety of lenses and corneas containing diverse crystallins and displaying multiple differences for achieving their visual functions.

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References


