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The transparent lens and cornea in the mouse and zebra fish eye

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Abstract

The lens and cornea combine to form a single optical element in which transparency and refraction are the fundamental biophysical characteristics required for a functional visual system. Although lens and cornea have different cellular and extracellular specializations that contribute to transparency and refraction, their development is closely related. In the embryonic mouse, the developing cornea and lens separate early. In contrast, zebra fish lens and cornea remain connected during early development and the optical properties of the cornea and lens observed by slit lamp and quasielastic laser light scattering spectroscopy (QLS) are more similar in the zebra fish eye than in the mouse eye. Optical similarities between cornea and lens of zebrafish may be the result of similarities in the cellular development of the cornea and lens.

Keywords

refracton; lens; cornea; zebra fish; transparency; optical development

In the vertebrate visual system, image formation requires the development of symmetric, transparent lenticular and corneal optics that refract and transmit light to the retina. In contrast to glass or plastic compound lenses which are composed of uniform synthetic materials, the cornea and lens consist of layers of cells and extracellular matrix [1,2]. The cornea consists of three different cell types and transparent layers of extracellular refractile type I collagen fibrils produced by keratocytes. The lens consists of concentric layers of transparent, elongated epithelial cells known as lens fibers which express and concentrate soluble cytoplasmic proteins known as crystallins to increase the refractive index. Together, the lens and cornea form an optical system in which highly concentrated intracellular or extracellular proteins increase the index of refraction, and short range order of the constituent proteins creates glass-like transparent structures [3,4].

In the developing lens and cornea multifunctional crystallin and crystallin-like proteins are expressed but their precise contribution to the development of optical function is not known. That the lens and cornea act as a single optical unit in the eye and have common characteristics of transparency and refraction is considered in the 'refraction hypothesis' which states that similar genetic programs may be responsible for the normal development of the lens and cornea [5].

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The molecular basis for the coordinated developmental process that results in the fundamental optical properties of the lens and cornea remains to be understood. The lens and cornea are derived embryologically from ectoderm which normally produces epithelial and neural cells that scatter light and are not refractile or transparent. During development, the cellular organization and protein composition of the lens and cornea become specialized to provide refractive transparent structures. In the cornea, the specialized organization of the extracellular stroma which constitutes 90% of the corneal thickness in mammals results in a transparent refractile tissue. The stroma consists of ordered collagen fibers of uniform diameter, approximately 30 nm, in both mouse and human, which are produced by interspersed keratocytes [6–8]. A cellular endothelium and epithelium help maintain corneal transparency by regulating fluid transport and hydration. While transparent in the anterior – posterior direction, light scattering is easily observed from corneal cells and stroma at an angle of approximately 40 degrees in slit lamp photos of the mammalian eye, unlike the normal lens which is transparent at all angles (Fig 1A). The lens is the only tissue in the mammal that consists of optically transparent cells that do not scatter incident light. During lens fiber cell differentiation all structures large enough to scatter light including nuclei, mitochondria, golgi apparatus and endoplasmic reticulum are broken down and removed from the cells. The concentration of lens crystallins increases dramatically which increases the index of refraction. Establishment of short range, glass-like order of the cytoplasmic proteins minimizes light scattering of lens cells. The synchronized differentiation of the cells results in symmetric, close-packed hexagonal organization of lens fibers in the transparent adult structure.

In the mammal, the development of the transparent cornea is separate from the development of the transparent lens fibers even though the optical functions of lens and cornea are inseparable. Both the lens and cornea are derived from cranial ectoderm in separate stages of early embryonic development (Fig 2A'–E'). Soon after the neuroectoderm invaginates to form the neural crest, a projection which will become the optic cup extends toward the interior of a region of the surface ectoderm that will become the lens and cornea [9]. As the optic cup approaches, the overlying cells of the surface ectoderm thicken to form the lens placode. Next the lens placode invaginates to form a pit which continues to invaginate until a hollow lens vesicle separates from the surface ectoderm. In the mammal, further development of the lens accompanies the separate development of the cornea [10]. For the purposes of this manuscript, it is important to emphasize that the embryonic primordium of the mouse lens and cornea are separated into two distinct tissues early in development, well before the differentiation of characteristic refractive and transparent extracellular and intracellular specializations.

From jellyfish to humans, the biological optics of the lens and cornea have much in common [11,12]. That coupled with the common use of developmental transcription factors, especially PAX6, has led to the concept that the biological optics descend from common primordial forms [13]. Although invertebrate eyes arise from a variety of developmental processes, basic eye organogenesis is generally assumed to be similar among vertebrates [14,15]. In this context, it is noteworthy that zebra fish and mouse optical elements develop differently. In contrast to the mouse and human in which the lens and cornea develop separately, the optical elements of the zebra fish eye remain connected during a significant period of their development (Fig 2A–E). Cornea and lens cells differentiate contiguously from cells of the surface ectoderm in the embryonic zebra fish [16]. Rather than invagination of a fluid-filled lens vesicle, the lens placode in the zebra fish thickens and proliferates to form a cellular mass that remains attached to the posterior surface of the site of the developing cornea. A vesicle is not formed. Instead, the proliferating lens cells delaminate from the deep surface of the corneal ectoderm to establish an intact, solid lens. Continued growth of the lens results from the synchronized proliferation, migration and elongation of the embryonic lens epithelium surrounding the core mass of delaminated cells to produce a symmetric layered tissue. The result is an optical system in which lens and cornea are closely apposed and separated by a very narrow aqueous chamber.

Lens delamination is probably not unique to zebra fish, as there is evidence that *Xenopus* does not form a lens vesicle stage either [16]. In the eyes of invertebrates including jellyfish and scallops, the lens and cornea are fused throughout life [17,18]. Development of zebra fish optics may be the result of an intermediate process between the fused optics of invertebrates and separated optics of the mouse. The common differentiation of the cellular elements in the zebra fish optics suggests that the biophysical properties of the lens and cornea in zebra fish may be more similar than in the lens and cornea in mice.

Slit lamp views comparing a living mouse eye with a living fish eye provide the first indication that this is the case. In the mouse, the cornea and the lens epithelium appeared as separate bright arcs that scatter light in an optical section at ~40 degrees from the anterior – posterior axis because they contain structural elements (scatterers) that are large with respect to the wavelength of visible light (Fig 1A). The dark band separating the lens epithelium from the corneal endothelium is the aqueous chamber. Scattering from the differentiated lens fiber cells deep to the lens epithelium is minimal because the organization and size of the constituent lens proteins is much different than that of the cornea. Lens crystallins are soluble proteins, approximately 10 to 20 nm in diameter, and are organized in the cytoplasm in glass-like short range order. In contrast, corneal collagen fibrils are organized in layers with the long axis of the fibers perpendicular to the incident light. Although the cornea is transparent in the anterior-posterior axis, light scattering increases with the angle of incidence of the slit lamp light source. In the slit lamp view of the mouse eye, distinct differences in the scattering properties of the lens and cornea are obvious. The slit lamp view of the zebra fish eye is very different from the mouse eye; scattering is minimal in both the lens and cornea (Fig 1B). The similar transparency of the two tissues makes it difficult to distinguish that the lens and cornea are separated by a very narrow aqueous chamber. On the basis of slit lamp observations, it can be hypothesized that the similarities between cornea and lens proteins are greater in the zebra fish than in the mouse.

An important technology used to compare molecular light scatter in lens and cornea is QLS (quasielastic laser light scattering spectroscopy), which analyzes the time-dependent fluctuations in the intensity of scattered light to evaluate the size and mobility of the molecular scatterers [19,20]. When protein scatterers are small and mobile, their motion in solution is rapid and the corresponding QLS autocorrelation function decay is rapid. When protein scatterers are large and/or immobile their motion is slow and the decay of the autocorrelation function is slow. There are relatively few large immobile scatterers in a transparent mouse lens, and like other mammalian lenses, the predominant scatterers are alpha and beta/gamma crystallins [21–23]. Because lens crystallins are small scatterers, the autocorrelation function of the time dependent intensity fluctuations decays rapidly (Fig 3A). In mouse cornea large, immobile collagen fibrils are the predominant scatterers and in contrast to lens, the time dependent decay in the autocorrelation function is slow (Fig 3B). In zebra fish the decay of the autocorrelation function for the time dependent fluctuations is similar for the lens and cornea indicating greater similarities in the constituent protein scatterers of lens and cornea in the zebra fish than in the mouse (Fig 3C, D). The similarities in the slit lamp and QLS measurements of light scattering are consistent with the hypothesis that the similarities in the biophysical properties of the cornea and lens in the zebra fish are the result of a close connection during development.

The optical environment of aquatic vertebrates is quite different from that of terrestrial mammals [24–27]. In a mouse eye most of the refraction occurs at the air-cornea interface where the refractive index of air is approximately 1.0 and cornea is approximately 1.3. In a fish eye, refraction of light is minimal at the water – cornea interface where the refractive index of water is approximately 1.3 and nearly identical to the refractive index of the cornea. As a result, much more refraction occurs in a fish lens where the index of refraction (1.41–1.55

[28]) is greater than in a rodent lens (1.38–1.48 [29]) and it is anticipated that the refractive index of the zebra fish lens may be even higher. For the cornea and lens to function as a single optical element in any refractive environment, developmental adaptations may be coordinated through common overlapping pathways, and similar transcription factors in common pathways of gene expression result in development of the lens and cornea from embryonic surface ectoderm [5]. The phenotype of the optical systems of many invertebrates in which the cornea and lens are fused (like commercial compound camera lenses) is consistent with the refracton hypothesis. A corollary to the refracton hypothesis is that diverse, taxon-specific, multifunctional crystallin proteins contribute to transparent cellular structure of both lens and cornea as a result of gene sharing [30]. This does not necessarily mean that eyes of all species develop from single common primordium but that lens and corneal development results in biological tissues that refract light, are transparent, and share common optical properties necessary for visual function.

While it is preliminary, our data suggest a closer relationship between the biophysical properties of the lens and cornea in the zebra fish eye than in the mouse eye. In the mouse eye, the abundant lens crystallins interact to provide short range order, high refractive index, and the long term stability necessary for the normal optical function of the transparent lens. By analogy, corneal crystallins may make similar functional contributions to the optical properties of the transparent cornea, although the function of the corneal crystallins remains to be established [30,31]. All vertebrate species express alpha and beta/gamma crystallins in lens in addition to other taxon specific crystallins in some species [32]. In contrast, corneal crystallins are more divergent. Some amphibian species have lens alpha, beta, and gamma crystallins in the cornea [33] but this is exceptional and not the case in all vertebrates. In mice, 50% of the soluble protein in cornea is ALDH3a1 and 10% is transketolase [34,35]. In zebra fish cornea, 40% of the soluble protein is scinla (C/L-gelsolin) and 14% is G-actin [5,36,37]. There is no homolog of scinla that accumulates as a crystallin in the cornea of mammals, and zebra fish cornea has negligible amounts ALDH3a1 and transketolase [36–38], emphasizing the differences in protein composition between zebrafish and mouse cornea (Table 1). On the basis of our preliminary observations, we propose that the crystallins are important for the normal development of optical elements in the eye which could account for the greater similarities between the zebra fish cornea and lens than the mouse cornea and lens. It is our expectation that the observed differences in light scattering from mouse lens and cornea are greater than the observed differences in light scattering in zebra fish lens and cornea because of the differences in the developmental function of corneal crystallins on formation of optical elements. To be more specific, the corneal crystallins influence the development and maintenance of corneal transparency and refraction in both zebra fish and mouse even though the gelsolin-like protein, scinla, is not homologous to ALDH3a1. The slit lamp and QLS observations in the mouse and zebra fish eye suggest that the physical functions of the crystallins in zebra fish cornea resemble the crystallins in the zebra fish lens more than the crystallins in mouse cornea resemble the crystallins in the mouse lens. Should this hypothesis be correct, it would be of great interest to determine precisely the common functional properties of the non-homologous crystallins of the zebra fish lens (alpha and beta/gamma crystallins) and zebra fish cornea (the gelsolin protein scinla). Clearly the importance of the functional properties of corneal and lens crystallins in development of optical function in the eye need to be understood in greater detail.

In vertebrates, the presence of crystallin proteins in both the transparent cornea and lens may be more fortuitous than functional ... or is it? The lens and cornea of zebra fish remain connected during development and also share similar optical characteristics including minimal light scattering, mobility of scattering elements, and refractive power. In contrast, the cells of the future cornea and lens in mice that separate early in development have greater differences in their optical characteristic while sharing a common function. While this observation does

not rule out the possibility of similar genetic programs influencing the development of optical function of the lens and cornea, the correlation between developmental connection and biophysical properties is striking.

IN SUMMARY

Refraction, transparency and symmetry are fundamental biophysical properties that distinguish the optical elements of the eye from all other vertebrate tissues. Lens fibers are elongated, transparent cells containing highly concentrated crystallin proteins that increase the index of refraction and establish short-range, transparent order. In contrast, the cornea is largely an extracellular matrix of collagen fibrils produced by cells containing abundant water-soluble proteins which, by analogy with lens crystallins, may contribute to the fundamental optical properties of the cornea. Lens and cornea may share properties of refraction and transparency as a result of similar influences of lens and corneal crystallins on their optical development.

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Figure 1.

Slit lamp views of the mouse eye and zebrafish eye. (A) The slit lamp view of a 6 week old normal C57Bl/6 mouse eye resembles an optical section of the anterior segment of the eye. There are distinct bands of scattering from the cornea and lens epithelium/capsule. The aqueous, vitreous and lens fibers show minimal scattering. The slit beam is traveling from right to left in the image. The first bright arc on the right side is the cornea. Directly behind the cornea is a dark band, the aqueous chamber, which contains fluid with no structures large enough to scatter light. Behind the aqueous chamber, the thin bright arc is the anterior epithelium and capsule of the lens. Deep to the epithelium is the mass of the transparent lens fibers which scatter very little light. The vitreous in the mammal is transparent because the concentration of scattering molecules is so small that scattering is minimized. The difference between the cornea and the lens is the result of the organization of the structural components. The cornea consists of type I collagen fibers that are approximately 30nm in diameter. In contrast, the lens fibers consist of concentrated crystallins ordered by cytoskeletal filaments that are less than 25nm in diameter. (B) In the slit lamp view of an 8 month old normal zebrafish eye the differences in light scattering from the cornea and lens are minimal. The cornea is difficult to observe because, in contrast to the mouse eye, there is no bright band of corneal scattering. There is modest scattering at the anterior edge of the zebrafish lens but an aqueous chamber is not apparent. The slit lamp view demonstrates that the scattering from the zebra fish cornea is minimal and resembles the normal transparent zebra fish lens. White bars are 250 μ m.

Figure 2.

Difference in development of cornea and lens in zebrafish and mouse (reprinted with permission [16]). Both species form a lens placode (A, A'). During much of the development of the zebra fish anterior segment of the eye, the lens and cornea remain connected. Cell proliferation on the deep surface of the ectoderm results in the lenticular cell mass without obvious organization (B). With continued development, elongating cells begin to surround the cells in the lens core (D) to form a spherical lens in the mature eye (E). There is no vesicle stage during lens development in the zebra fish. The mouse lens follows the developmental stages that are well known in mammals and in the chick. The invagination of the surface ectoderm (B') leads to separation of the fluid filled lens vesicle (C'). At this early stage of

embryogenesis, the development of the lens and cornea are separated. In lens, elongation of the posterior epithelial cells obliterates the vesicle stage (D') and establishes an embryonic core of cells that is present in the adult as the embryonic nucleus of the mammalian lens. Secondary fibers surrounding the embryonic nucleus are formed by the proliferation, elongation and posterior migration of the differentiating lens fibers (E'). The differences in development between mouse and zebrafish suggest that the cells in the lens and cornea may have much more in common in the zebra fish than in the mouse.

Figure 3.

Autocorrelation functions of QLS measurements in normal lens and cornea of the mouse and zebra fish. A thin infrared laser beam was directed into the eye of a mouse (top) or zebra fish (bottom). The insets are photos of the laser beam passing through the cornea and aqueous and into the lens. A small circle indicates the sampling volume for each measurement. The autocorrelation function for light scattered from the mouse lens decays rapidly which is a measure of the small, mobile crystallin proteins in the mouse lens (A) and the autocorrelation function for the mouse cornea decays slowly which is a measure of the large, immobile collagen fibers in the mouse cornea (B). The zebra fish is much different. The decay of the

autocorrelation functions for the lens (C) and cornea (D) are similar, suggesting that the proportion of small and large protein scatterers is similar in zebra fish lens and cornea.

TABLE 1

Characteristics of normal cornea and lens in zebra fish and mouse. Rows 1 and 2 compare corneal thickness. The zebra fish epithelial and stromal layers are much thinner in the zebra fish with the stroma comprising only ~30% of the total thickness as compared to 90% in mouse and humans. Row 3 compares corneal crystallins which are different between the two species. Row 4 compares lens crystallins. Both species contain alpha, beta, and gamma crystallin but the relative percentages (besides that of alpha crystallin) are unknown in zebra fish. Although the percentage of soluble alpha crystallin in zebra fish lens is much lower than in rat, the percentage of total alpha crystallin protein (soluble + insoluble fractions) in rat and zebra fish lens remains to be determined. Row 5 compares the lens index of refraction in mouse and fish. While the refractive index for the lens of the zebra fish has not been measured, mouse lens refractive index is expected to be much lower than zebra fish lens refractive index.

		MOUSE	ZEBRAFISH	References
CORNEA	Epithelial thickness	50µm at P30	12.5µm at 2mon/mature	[39,40]
	Stromal thickness	82µm at P30, ~90% of total	6µm at 2mon/mature, ~30% of total	[39,40]
	Corneal crystallins (percent of Soluble protein)	50% ALDH3a1	<i>ALDH3 negligible</i>	[34,36]
		10% transketolase	<i>Very little transketolase activity</i>	[35,38]
		<i>No scinla ortholog in terrestrial animal eyes</i>	40% scinla (C/L-gelsolin)	[5,37]
		<i>F-actin most common</i>	14% dissociated G-actin	[36]
LENS	Lens crystallins (percent of soluble protein)	α 28% (rat)	α 2.3%	[29,41]
		β 41% (rat)	β unknown	
		γ 31% (rat)	γ unknown	
	Index of refraction	1.38 – 1.41 (rat)	Up to ~1.5 in most fish studied	[28,29,42–44]