

# Enigma of the Abundant Water-Soluble Cytoplasmic Proteins of the Cornea

## The “Refracton” Hypothesis

Joram Piatigorsky, Ph.D.

**Abstract:** It is accepted that the taxon-specific, multifunctional crystallins (small heat-shock proteins and enzymes) serve structural roles contributing to the transparent and refractive properties of the lens. The transparent cornea also accumulates unexpectedly high proportions of taxon-specific, multifunctional proteins particularly, but not only, in the epithelium. For example, aldehyde dehydrogenase 3 (ALDH3) is the main water-soluble protein in corneal epithelial cells of most mammals (but ALDH1 predominates in the rabbit), whereas gelsolin predominates in the zebrafish corneal epithelium. Moreover, some invertebrates (e.g., squid and scallop) accumulate proteins in their corneas that are similar to their lens crystallins. Pax-6, among other transcription factors, is implicated in development and tissue-specific gene expression of the lens and cornea. Environmental factors appear to influence gene expression in the cornea, but not the lens. Although no direct proof exists, the diverse, abundant corneal proteins may have evolved a crystallinlike role, in addition to their enzymatic or cytoskeletal functions, by a gene sharing mechanism similar to the lens crystallins. Consequently, it is proposed that the cornea and lens be considered as a single refractive unit, called here the “refracton,” to emphasize their similarities and common function.

**Key Words:** Corneal proteins—Crystallins—Gene expression—Refracton.

Until recently, the major water-soluble lens proteins, the  $\alpha$ - and  $\beta/\gamma$ -crystallins, were thought to be specialized for their ability to make the lens transparent and generate a gradient of refractive index.<sup>1–3</sup> It has been also generally accepted that diverse proteins can fulfill the optical needs of the lens ever since comparative studies showed the existence of taxon-specific crystallins.<sup>4,5</sup> A greater stir occurred when it was first discovered that the ubiquitous  $\alpha$ -crystallins are homologous to small heat-shock proteins of *Drosophila* species.<sup>6</sup> This was followed by the surprise finding that the taxon-specific crystallins are similar or identical to metabolic enzymes (i.e., lactate dehydrogenase B<sub>4</sub>/ $\epsilon$ -crystallin in ducks,  $\alpha$ -enolase/ $\tau$ -crystallin in turtles and argininosuccinate lyase in birds and reptiles).<sup>7–9</sup> In many cases, these enzyme–crystallins are

metabolically active. An essential finding was that the active enzyme–crystallin and the enzyme expressed in nonlens tissues are encoded by the same gene, a situation called “gene sharing.”<sup>10–12</sup> In some cases, such as  $\delta$ 1-crystallin in birds<sup>11</sup> or glutathione S-transferase/S-crystallins in cephalopods,<sup>13</sup> gene duplications have led to lens specialization. Thus, the identical gene encodes a protein that functions as a structural crystallin at high concentration in the lens and as a metabolic enzyme, or stress protein, at low concentration in other tissues.

Although no direct proof exists, it is accepted that these metabolic enzymes act as structural lens crystallins in view of the refractive function of the clear lens and the abundance of a given enzyme–crystallin in the lens (10%–90% of the water-soluble protein, depending on enzyme–crystallin and species). A number of arguments favor this idea. One is that it does not make sense for an enzyme to exist in the lens at such a high concentration if its only role is to catalyze metabolic reactions. Another argument for a structural role of an enzyme–crystallin is that it may be active in the lens of one species and relatively inactive in another species. For example, argininosuccinate lyase/ $\delta$ -crystallin is active in the duck lens, whereas  $\delta$ 2-crystallin is highly expressed and inactive in the chicken lens, where  $\delta$ 1-crystallin predominates.<sup>11–14</sup>

## ABUNDANT CORNEAL PROTEINS

It has been known for a long time that similar to the lens, the cornea accumulates unusually high proportions of a few intracellular, water-soluble proteins.<sup>15</sup> The first reported abundant intracellular protein of the cornea, BCP 54 for bovine corneal protein of 54 kilodaltons accounted for 20% to 40% of the water-soluble protein.<sup>16–18</sup> BCP 54 was subsequently shown to be active aldehyde dehydrogenase 3 (ALDH3).<sup>19–22</sup> ALDH3 accumulates in the corneas of most mammals but not in other classes, indicating that it is taxon-specific like the enzyme–crystallins of the lens.<sup>17,23,24</sup> The unexpectedly high proportion of BCP 54/ALDH3 in the corneal cells of mammals led to the suggestion that it may have a structural role for transparency.<sup>18,25</sup> Thus, the idea that corneal cells use abundant proteins as crystallins is not new.

The taxon specificity of the abundant corneal proteins is as striking as that of the lens crystallins (Table 1). In some cases, there are gross species-specific differences in the major enzymes of the cornea. Examples include the abundance of isocitrate dehydrogenase in the bovine cornea<sup>26</sup> and ALDH3<sup>18</sup> and transketolase (TKT) in corneas of mammals<sup>27</sup> but not of birds, reptiles or fish.<sup>28</sup>

Submitted March 28, 2001. Accepted June 14, 2001.

From the Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

Address correspondence and reprint requests to Dr. J. Piatigorsky, Laboratory of Molecular and Developmental Biology, National Institutes of Health, 6 Center Drive, Building 6, Room 201, Bethesda, MD 20892-2730, U.S.A.

**TABLE 1.** Abundant cytoplasmic water-soluble corneal proteins

Enzyme	Species	Reference
Aldehyde dehydrogenase class 3	Most mammals	17, 18, 20, 22, 29
Aldehyde dehydrogenase class 1	Rabbit	23
Transketolase	Mammals	27
Isocitrate dehydrogenase	Cattle	26
Peptidyl-prolyl cis-trans isomerase	Chicken	29
Gelsolin	Zebrafish	24
Glutathione S-transferase/ S-crystallins	Squid	29

However, there are also intriguing subtle differences in the choice of enzymes that accumulate in the corneas of different species. For example, whereas ALDH3 predominates in the corneas of most mammals,<sup>28</sup> it is replaced by ALDH1 in rabbit corneas.<sup>23</sup> There are numerous structural and substrate differences between these two ALDH isoforms, again consistent with the idea that there are more uses for the abundant corneal enzymes than metabolism per se.

Another feature of the abundant water-soluble proteins of the cornea that resembles the lens crystallins is that these multifunctional proteins are not always enzymes. In the lens, the  $\alpha$ -crystallins were recruited from ubiquitous, nonenzymatic small heat-shock proteins,<sup>6</sup> and  $\iota$ -crystallin was recruited from cellular retinol-binding protein in geckos.<sup>30,31</sup> In the cornea, gelsolin is the predominant water-soluble, intracellular protein in the corneas of some fish.<sup>24</sup>

### ABUNDANCE OF GELSOLIN IN THE ZEBRAFISH CORNEA

We recently have shown that gelsolin, which binds and dissociates actin fibrils, represents approximately 40% of the water-soluble protein of the zebrafish cornea.<sup>24</sup> ALDH3 is negligible, if present at all, in these corneas. Actin is also abundant (approximately 15%) in the cornea, but less so than gelsolin, as judged by the staining patterns on the sodium dodecyl sulfate–polyacrylamide gels. Together, gelsolin and actin appear to account for at least 50% of the water-soluble proteins of zebrafish corneas. In situ hybridization and immunocytochemistry showed that the zebrafish gelsolin is located in the epithelium. Rhodamine–phalloidin staining of the zebrafish cornea indicated that most of the abundant

actin is not polymerized as F-actin, as it is in the mouse cornea, presumably because of its dissociation by gelsolin. This is consistent with the deduced primary structure of zebrafish gelsolin, which has the conserved binding sequences for actin, calcium, and phosphatidylinositol biphosphate.

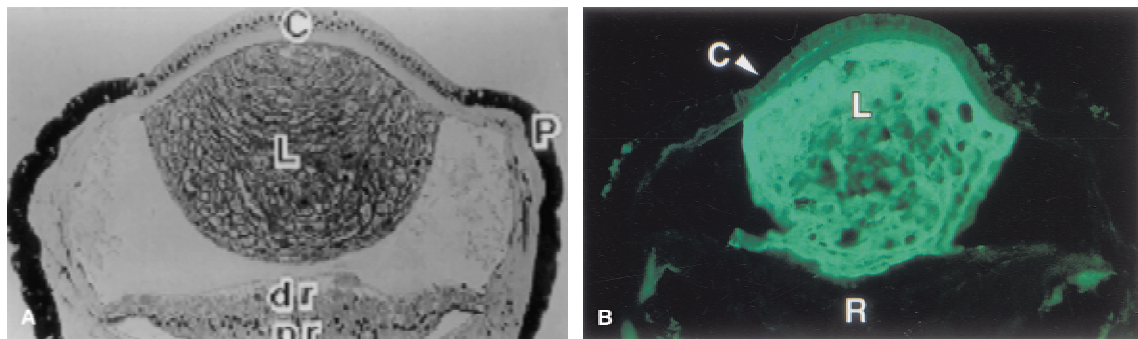
Thus, gelsolin and actin–gelsolin complexes might fill the cytoplasm of the zebrafish corneal epithelial cells in much the same way as the crystallins fill the cytoplasm of the lens cells.

It is not known how prevalent gelsolin overexpression is in the cornea of different species of fish. Gelsolin is the main water-soluble corneal protein of two other freshwater fish (rosey barb and tricolor shark).<sup>24</sup> Trout and sculpin corneas were also tested and appear to have considerably less gelsolin, although rhodamine–phalloidin staining of their corneas was weak, as in the zebrafish cornea, suggesting that the corneas of these fish also contain proteins that can dissociate F-actin.

### CORNEAL PROTEINS OF INVERTEBRATES

Many invertebrates have cellular lenses and corneas, although they have structural differences from those of vertebrates.<sup>13</sup> Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis indicated that the main water-soluble proteins of the squid cornea are similar to the S-crystallins of the squid lens.<sup>28</sup> S-crystallins are a complex family of proteins with multiple genes derived from that of encoding glutathione S-transferase,<sup>13</sup> and it is not known whether the same S-crystallin genes are expressed in the squid lens and cornea. Nonetheless, there is a striking similarity between the protein profile in the lens and cornea of the squid,<sup>29</sup> more so than of any other species investigated to date.

Scallops also have well-developed eyes with cellular lenses, with their corneas being a single layer of epithelial cells separated from the lens by extracellular material.<sup>32,33</sup>  $\Omega$ -crystallin, the sole crystallin of the scallop lens, is an inactive, dimeric protein belonging to the ALDH 1/2 class.<sup>33</sup> The only other significant expression of  $\Omega$ -crystallin in the scallop eye is in the cornea, according to an immunocytochemical test. The photomicrographs in Figure 1 show the scallop eye with its corneal monolayer of epithelial cells (Fig. 1, left) and the  $\Omega$ -crystallin/ALDH immunofluorescence pattern in the lens and cornea (Fig. 1, right). Note how the scallop



**FIG. 1.** **A:** Light micrograph of a transected eye from a mature sea scallop, *Placopecten magellanicus*. The eye was fixed in 4% paraformaldehyde, embedded in methacrylate, sectioned longitudinally along the central axis, and stained with toluidine blue (courtesy of Dr. W. Gerald Robison, Jr.). **B:** Immunofluorescence of a sea scallop eye using a primary polyclonal antibody made against squid L-crystallin/ALDH of the light organ lens and a biotinylated secondary antibody. The eye was fixed in paraformaldehyde, embedded in Tissue-Tek OCT compound, frozen (Sakura Finetechnical Company, Tokyo, Japan), and cut with a cryostat (Leica SM 3050, Heidelberg, Germany) (courtesy of Dr. Ernst Tamm; magnification,  $\sim 100\times$ ). Further details can be found elsewhere.<sup>33</sup> C, cornea; dr, distal retina; L, lens; P, pigmented epithelium; pr, proximal retina; R, retina.

cornea and lens appear as an integrated structure. Indeed, the thickened extracellular material between the corneal epithelium and lens can be imagined as analogous to the corneal stroma of a vertebrate eye lying against the lens instead of the endothelium. It is still possible that the cross-reactive corneal protein is not  $\Omega$ -crystallin or that the relative proportion of  $\Omega$ -crystallin is not particularly high in the scallop cornea as it is in the lens. However, the data raise the possibility that  $\Omega$ -crystallin is an abundant water-soluble protein in the cornea and lens of the scallop.

Cubomedusan jellyfish are another invertebrate with complex eyes, called ocelli.<sup>34</sup> The ocellus has a cellular lens and a monolayer of epithelial cells as a cornea, as does the scallop, although it lacks the thickened extracellular layer between the cornea and lens that is present in the scallop (Fig. 2). In the jellyfish eye, it is striking how the proximity of the lens and cornea resemble the epithelium and fibers of the vertebrate eye. The jellyfish lens crystallins consist of three monomeric bands of 35-, 20-, and 19-kilodalton proteins called J1-, J2-, and J3-crystallin, respectively. J1-crystallin is composed of three extremely similar, novel proteins, J1a, b, and c, encoded in distinct, intronless genes.<sup>35</sup> We are investigating whether one or more of the jellyfish crystallins are present in the cornea.



**FIG. 2.** Paraffin section transecting the ocellus of the cubomedusan jellyfish, *Tripedalia cystophora*, stained with hematoxylin and eosin (Magnification,  $\sim 500\times$ ). Further descriptions of the cubomedusan ocellus can be found elsewhere.<sup>34</sup>

Thus, the available evidence, albeit scanty, suggests that at least some invertebrates express their lens crystallins or a member of the same protein family in their cornea. This contrasts with vertebrates in which the abundant water-soluble corneal proteins differ from the lens crystallins. One possible exception that needs investigation in the vertebrate eye is the elephant shrew that contains ALDH1/ $\eta$ -crystallin in the lens<sup>36</sup> and presumably accumulates an ALDH in the cornea, as do other mammals.

### CORNEA- AND LENS-PREFERRED GENE EXPRESSION: A ROLE FOR PAX-6

Transgenic mouse and transfection experiments have shown that lens specificity is achieved largely, if not entirely, at the transcriptional level and may be independent of species.<sup>37</sup> Although no universal *cis*-control element has been found as the basis of lens specificity, a group of transcription factors have emerged as being critical for expression of crystallin genes in the developing lens.<sup>38</sup> Pax-6, retinoic acid receptors, maf family members, and Sox family members are among those that appear to be used for activity of a number of different crystallin promoters in the lens.

Pax-6 appears to be important for preferential gene expression in the cornea and lens. First, Pax-6 is expressed in the surface head ectoderm that forms the lens and cornea.<sup>39–41</sup> Moreover, lens and cornea express Pax-6 and have defects resulting from its mutations or misexpression.<sup>42–45</sup> It is well established that Pax-6 can regulate crystallin promoter activity in transfected cells.<sup>38,46</sup> Gene expression has been studied less intensively in the cornea than in the lens. However, *in vivo* tests in rabbits using a gene gun<sup>47</sup> and transfection experiments using human corneal epithelial cells<sup>48</sup> have provided evidence that the cornea-specific mouse keratin K12 promoter is activated by Pax-6. In addition, gelatinase-B promoter activity has been linked with Pax-6 during wound healing of the corneal epithelium.<sup>49</sup> Our current cotransfection experiments indicate that Pax-6 activates the mouse ALDH3 promoter (Davis and Piatigorsky, unpublished data). The existence of an upstream enhancer in the Pax-6 gene that directs promoter activity specifically to the lens and cornea of transgenic mice<sup>50,51</sup> further links tissue-preferred gene expression in the cornea and lens.

Despite the common use of Pax-6 and possibly other transcription factors for lens- and cornea-preferred gene expression, lens specificity is achieved without corneal expression in transgenic mouse experiments using various lens crystallin promoters fused to reporter transgenes.<sup>37</sup> Conversely, cornea-specific promoter activity, excluding the lens, has also been shown in transgenic mice. A 4-kilobase mouse ALDH3 promoter: reporter transgene functioned specifically in corneal epithelial cells<sup>52</sup> and a 3.2-kilobase keratocan promoter-reporter transgene was active selectively in corneal keratocytes<sup>53</sup> of transgenic mice. Although the 4-kilobase ALDH3 promoter fragment contained intron 1, a 12-kilobase ALDH3 promoter fragment lacking intron 1 was active in a number of tissues and in the cornea, suggesting that corneal specificity may require an interaction between elements in intron 1 and the 5' flanking sequence. It is also noteworthy that the keratocyte-specific keratocan promoter fragment contained a portion of the first intron.

Finally, control elements regulating corneal and lens specificity of the mouse  $\alpha$ B-crystallin promoter are in proximity if not intimately related. Activity of the  $-164/+44$   $\alpha$ B-crystallin promoter

fragment lacking the upstream  $\alpha$ B-crystallin enhancer required for expression in nonocular tissues was shown originally to be lens-specific in transgenic mice<sup>54</sup> and activated by Pax-6<sup>55</sup> and retinoic acid–retinoid receptors<sup>56</sup> in transfected cells. Recent tests, however, showed that the –164/+44 promoter fragment is also active, albeit weakly, in the mature corneal epithelium of transgenic mice.<sup>57</sup> The transgene was active in the lens but not the cornea of the 16-day-old embryonic transgenic mice. Promoter activity was detected in the cornea and the lens in the 6-week-old transgenic mice. The delayed appearance of  $\alpha$ B-crystallin promoter activity in the cornea may simply reflect a difference in the sensitivity of our assay. However, it is also possible that there is an important regulatory difference in cornea-preferred promoter activity in the lens and cornea. One possibility is that environmental effects may contribute to tissue-preferred gene expression in the cornea, but not in the lens.

### DEVELOPMENTAL VERSUS ENVIRONMENTAL CONTROL OF CORNEAL GENE EXPRESSION

ALDH3 and alcohol dehydrogenase activities of the mouse cornea are upregulated between 7 and 16 days after birth<sup>58</sup> (Kays and Piatigorsky, unpublished data). Similarly, mRNA and protein levels of ALDH3 (Kays and Piatigorsky, unpublished data) and TKT<sup>28,59</sup> are increased in mouse corneal epithelial cells between 10 and 25 days after birth. These increases in corneal gene expression coincide with eyelid opening, which occurs 12 or 13 days after birth in the mouse.

Developmental factors and light and other stresses affect the increases in these enzymes in the cornea. Comparisons between mice that were raised in the dark and those raised in 12-hour light–dark cycles showed slightly increased ocular ALDH3 and alcohol dehydrogenase activities in the latter.<sup>58</sup> It has also been shown that TKT mRNA levels were twice as high in the corneas of 25-day-old mice raised in cycling light–dark conditions than in the corneas of mice raised in near total darkness.<sup>28</sup> Exclusion of light did not affect TKT mRNA levels in adult mice. Light exposure also prevented a decrease in ALDH3 gene expression in cultured rat corneal epithelial cells<sup>60</sup> and increased TKT mRNA levels in excised eyes of newborn mice.<sup>59</sup>

Environmental influences on corneal gene expression may not be confined to light. For example, TKT mRNA was increased by oxidative stress of cultured lens cells.<sup>60</sup> In addition, acceleration of eyelid opening by epidermal growth factor increased TKT mRNA levels in corneas of mice raised in the dark for 28 days.<sup>28</sup> Although the reasons for these increases are not known, the data are consistent with the possibility that developmental and environmental factors influence gene expression in the cornea. The range of environmental factors (e.g., oxygen-to-carbon dioxide ratios, pH, and oxidative stress) and mechanisms of their action require further investigation. Whatever the environmental parameters, these control mechanisms apparently do not extend to the lens, where crystallin gene expression is under developmental control<sup>38</sup> and unaffected by light.<sup>61</sup>

### FUNCTION OF THE ABUNDANT WATER-SOLUBLE INTRACELLULAR CORNEAL PROTEINS

The functions of the abundant cytoplasmic proteins of the cornea remain an enigma. By analogy with the taxon-specific multi-

functional lens crystallins, it seems reasonable to explore the idea that they serve crystallinlike roles in addition to their known enzymatic or other cellular roles.<sup>15</sup> Their precise noncrystallin roles would depend on the protein and species, as suggested for the taxon-specific lens crystallins. For example, the lenses of diurnal geckos that are exposed to high light intensities accumulate  $\iota$ -crystallin, a cellular retinol-binding protein type 1 adapted to bind vitamin A<sub>2</sub>.<sup>30,31</sup> In addition to presumably affecting the refractive index of the lens, the  $\iota$ -crystallin–vitamin A<sub>2</sub> complex is thought to protect the retina by absorbing shortwave radiation. By analogy with the dual roles of lens crystallins, ALDH3 may protect against oxidative damage of light-induced lipid peroxidations and absorption of ultraviolet light in the mammalian cornea.<sup>19,20,62,63</sup> Conversely, the abundant gelsolin in zebrafish cornea may affect wound healing or provide structural stability rather than protect against ultraviolet light that is probably a minor problem in the fish lacking eyelids.<sup>24</sup> The unifying idea is that the abundant corneal proteins may be serving multiple functions, as is thought to be the case for lens crystallins, by a gene sharing strategy.<sup>15</sup>

Indeed, at our current state of knowledge, it is possible to consider that in some cases, the primary role of an abundant corneal enzyme is structural rather than catalytic. Again, it is instructive to use the lens as a model.  $\delta$ -Crystallin has high argininosuccinate activity in the duck lens and low activity in the chicken lens,<sup>11</sup> raising the possibility that its enzymatic activity may not be significant for the lens, at least in some species. If enzyme activity were the primary function for corneal ALDH3, it is peculiar that this enzyme is most abundant in mice, which are nocturnal and exposed to relatively little ultraviolet light, and negligible in birds, which fly in the sky and are bombarded with shortwave radiation.

We have obtained ALDH3 null<sup>64</sup> and TKT heterozygote<sup>65</sup> mice by homologous recombination to investigate the role of these enzymes in the mouse cornea. No viable TKT null mice were obtained. Surprisingly, ALDH3 null and TKT heterozygote mice have clear corneas. It is noteworthy in this connection that  $\alpha$ B-crystallin null mice have clear lenses,<sup>66</sup> yet presumably this small heat-shock protein–crystallin has a refractive function in the lens. We are currently searching for the biologic role(s) of ALDH3 in the mouse corneal epithelium. Thus, the abundant, taxon-specific proteins in the cornea remain an intriguing puzzle.

### CONSIDERATION OF THE LENS AND CORNEA AS ONE FUNCTIONAL UNIT: THE ‘REFRACTON’ HYPOTHESIS

In view of the analogies between the lens crystallins and the abundant corneal proteins, it seems worthwhile to explore the idea that the lens and cornea have more in common than is generally appreciated. Both tissues are derived from the same ectodermal tissue during embryogenesis and use similar transcription factors for development and specific gene expression, with Pax-6 a currently notable example.

The transparent cornea and lens function together to eliminate spherical aberrations and cast a focused image onto the retina. The relative importance of the cornea or lens for focusing in the eye depends on niche. Approximately two thirds of the refraction is performed by the cornea in terrestrial vertebrates, in which the air–liquid interface and tissue curvature play dominant roles,

whereas the refraction is performed principally by the lens in aquatic species. Moreover, the cornea and lens have the difficulty of dealing with oxidative stresses, especially ultraviolet radiation, and must develop protective mechanisms.

Although the extracellular stroma has an important role in corneal transparency, the problem of cellular transparency in the cornea remains.<sup>64</sup> The refractive index of corneal cells must be optimized for transparency and refraction just as in the lens. In this regard, rabbit keratocytes significantly reduce their ALDH1 and TKT contents and become reflective after freeze-injury of their corneas, suggesting that these abundant enzymes are necessary for keratocyte transparency in the rabbit cornea.<sup>23</sup>

Although it may be fortuitous, it is striking how the simplified cornea (i.e., the transparent epithelial sheet anterior to the lens) in some invertebrates appears anatomically similar to the lens epithelium of vertebrates. Moreover, at least in some invertebrates (e.g., squid and scallop), the cornea expresses proteins that are similar if not identical to the crystallins in their lenses.

Consequently, it is proposed to consider the cornea and lens as the anterior and posterior element, respectively, of a single functional unit, called herein the "refracton" to signify its biologic role. The cornerstone of the refracton hypothesis is that the lens and cornea have accumulated diverse, taxon-specific multifunctional proteins by a gene sharing strategy, which contribute to its optical properties. Detailed comparative studies between the lens and cornea would be enlightening. Identification of their common features, such as gene sharing, may deepen our understanding of fundamental processes in both tissues. The mechanistic differences that are found between common processes in the lens and cornea may be equally revealing. For example, the putative role of environmental factors for gene expression in the cornea, but not the lens, may reflect evolutionary mechanisms that give new insights into gene sharing and the recruitment of novel functions for proteins by changes in the expression of their genes. There are presumably other common biochemical, biophysical, and physiologic parameters of the lens and cornea unifying these two transparent tissues, which will strengthen the refracton concept. Exciting times are ahead.

## CONCLUSIONS

By analogy with the taxon-specific multifunctional lens crystallins, it is proposed that the abundant, water-soluble, cytoplasmic proteins of the cornea have crystallin-like functions, and that the lens and cornea together form a functional unit called the refracton.

**Acknowledgment:** The author thanks Drs. Janine Davis, Frederick Bettelheim, and Zbynek Kozmik for critically reading the manuscript.

## REFERENCES

- Benedek G. Theory of the transparency of the eye. *Appl Optics* 1971; 10:459-73.
- Bettelheim FA, Siew EL. Effect of change in concentration upon lens turbidity as predicted by the random fluctuation theory. *Biophys J* 1983;41:29-33.
- Delaye M, Tardieu A. Short-range order of crystallin proteins accounts for eye lens transparency. *Nature* 1983;10:415-8.
- Rabaey M. Electrophoretic and immunoelectrophoretic studies on the soluble proteins in the developing lens of birds. *Exp Eye Res* 1962;1: 310-6.
- Wistow G, Piatigorsky J. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Ann Rev Biochem* 1988;57:479-504.
- Ingolia TD, Craig EA. Four small *Drosophila* heat shock proteins are related to each other and to mammalian  $\alpha$ -crystallin. *Proc Natl Acad Sci USA* 1982;79:2360-4.
- Wistow G, Mulders JWM, de Jong WW. The enzyme lactate dehydrogenase as a structural protein in avian and crocodilian lenses. *Nature* 1987;326:622-4.
- Wistow G, Piatigorsky J. Recruitment of enzymes as lens structural proteins. *Science* 1987;236:1554-6.
- Wistow G, Lietman T, Williams LA, et al.  $\tau$ -crystallin/ $\alpha$ -enolase: one gene encodes both an enzyme and a lens structural protein. *J Cell Biol* 1989;107:2729-36.
- Hendriks W, Mulders JWM, Bibby M, et al. Duck lens  $\epsilon$ -crystallin and lactate dehydrogenase B4 are identical: a single-copy gene product with two distinct functions. *Proc Natl Acad Sci USA* 1988;85:7114-8.
- Piatigorsky J, O'Brien WE, Norman BL, et al. Gene sharing by  $\delta$ -crystallin and argininosuccinate lyase. *Proc Natl Acad Sci USA* 1988;85: 3479-83.
- Piatigorsky J, Wistow G. Enzyme/crystallins: gene sharing as an evolutionary strategy. *Cell* 1989;57:197-9.
- Tomarev SI, Piatigorsky J. Lens crystallins of invertebrates. Diversity and recruitment from detoxification enzymes and novel proteins. *Eur J Biochem* 1996;235:449-65.
- Piatigorsky J, Horwitz J. Characterization and enzyme activity of argininosuccinate lyase/ $\delta$ -crystallin of the embryonic duck lens. *Biochem Biophys Acta* 1996;1295:158-64.
- Piatigorsky J. Gene sharing in lens and cornea: facts and implications. *Prog Ret Eye Res* 1998;17:145-74.
- Holt WS, Kinoshita JH. The soluble proteins of the bovine cornea. *Invest Ophthalmol Vis Sci* 1973;12:114-26.
- Alexander RJ, Silverman B, Henley WL. Isolation and characterization of BCP 54, the major soluble protein of bovine cornea. *Exp Eye Res* 1981;32:205-16.
- Silverman B, Alexander RJ, Henley WL. Tissue and species specificity of BCP 54, the major soluble protein of bovine cornea. *Exp Eye Res* 1981;33:19-29.
- Evces S, Lindahl R. Characterization of rat cornea aldehyde dehydrogenase. *Arch Biochem Biophys* 1989;274:518-24.
- Abedinia M, Pain T, Algar EM, et al. Bovine corneal aldehyde dehydrogenase: the major soluble corneal protein with a possible dual protective role for the eye. *Exp Eye Res* 1990;51:419-26.
- Verhagen C, Hockzema R, Verjans GMGM, et al. Identification of bovine corneal protein 54 (BCP 54) as an aldehyde dehydrogenase. *Exp Eye Res* 1991;53:283-4.
- Cooper DL, Baptist EW, Enghild JJ, et al. Bovine corneal protein 54K (BCP 54) is a homologue of the tumor-associated (class 3) rat aldehyde dehydrogenase (RATALD). *Gene* 1991;98:201-7.
- Jester JV, Moller-Pedersen T, Huang J, et al. The cellular basis of corneal transparency: evidence for 'corneal crystallins.' *J Cell Sci* 1999;112:613-22.
- Xu Y-S, Kantorow M, Davis J, et al. Evidence for gelsolin as a corneal crystallin in zebrafish. *J Biol Chem* 2000;275:24645-52.
- Rabaey M, Segers J. Changes in the polypeptide composition of the bovine corneal epithelium during development. In: Trevor-Roper PD, ed. *Congress of the European Society of Ophthalmology*. London: Academic Press, 1981:41-4.
- Sun L, Sun TT, Lavker RM. Identification of a cytosolic NADP<sup>+</sup>-dependent isocitrate dehydrogenase that is preferentially expressed in bovine corneal epithelia. A corneal epithelial crystallin. *J Biol Chem* 1999;274:17334-41.
- Sax CM, Salamon C, Kays WT, et al. Transketolase is a major protein in the mouse cornea. *J Biol Chem* 1996;271:33568-74.
- Sax CM, Kays WT, Salamon C, et al. Transketolase gene expression in the cornea is influenced by environmental factors and developmentally controlled events. *Cornea* 2000;19:833-41.
- Cuthbertson RA, Tomarev SI, Piatigorsky J. Taxon-specific recruitment of enzymes as major soluble proteins in the corneal epithelium of three mammals, chicken, and squid. *Proc Natl Acad Sci USA* 1992; 89:4004-8.
- Roll B, Amon R, de Jong WW. Vitamin A<sub>2</sub> bound to cellular retinol-

- binding protein as ultraviolet filter in the eye lens of the gecko *Lygodactylus picturatus*. *J Biol Chem* 1996;271:10437–40.
31. Werten PJJ, Roll B, van Aalten DMF, et al. Gecko  $\nu$ -crystallin: how cellular retinol-binding protein became an eye lens ultraviolet filter. *Proc Natl Acad Sci USA* 2000;97:3282–7.
  32. Barber VC, Evans EM, Land MF. The fine structure of the eye of the mollusc, *Pecten maximus*. *Z Zellforsch* 1967;76:295–312.
  33. Piatigorsky J, Kozmik Z, Horwitz J, et al.  $\Omega$ -crystallin in scallop. A dimeric aldehyde dehydrogenase class 1/2 enzyme-crystallin. *J Biol Chem* 2000;275:41064–73.
  34. Piatigorsky J, Horwitz J, Kuwabara T, et al. The cellular lens and crystallins of cubomedusan jellyfish. *J Comp Physiol A* 1989;164:577–87.
  35. Piatigorsky J, Horwitz J, Norman BL. J1-crystallins of the cubomedusan jellyfish lens constitute a novel family encoded in at least three intronless genes. *J Biol Chem* 1993;268:11894–901.
  36. Graham C, Hodin J, Wistow G. A retinaldehyde dehydrogenase as a structural protein in a mammalian eye lens. Gene recruitment of  $\eta$ -crystallin. *J Biol Chem* 1996;271:15623–8.
  37. Piatigorsky J, Zelenka PS. Transcriptional regulation of crystallin genes: *cis*-elements, *trans*-factors, and signal transduction systems in the lens. *Adv Dev Biochem* 1992;1:211–56.
  38. Cvekl A, Piatigorsky J. Lens development and crystallin gene expression: many roles for Pax-6. *BioEssays* 1996;18:621–30.
  39. Li H-S, Yang J-M, Jacobson RD, et al. Pax-6 is first expressed in a region of ectoderm anterior to the early neural plate: implications for stepwise determination of the lens. *Dev Biol* 1994;162:181–94.
  40. Grindley JC, Davidson DR, Hill RE. The role of Pax-6 in eye and nasal development. *Development* 1995;121:1433–42.
  41. Collinson JM, Hill RE, West JD. Different roles for Pax-6 in the optic vesicle and facial epithelium mediate early morphogenesis of the murine eye. *Development* 2000;127:945–56.
  42. Schedl A, Ross A, Lee M, et al. Influence of PAX6 gene dosage on development: overexpression causes severe eye abnormalities. *Cell* 1996;86:71–82.
  43. Koroma BM, Yang J-M, Sundin OH. The Pax-6 homeobox gene is expressed throughout the corneal and conjunctival epithelia. *Invest Ophthalmol Vis Sci* 1997;38:108–20.
  44. Lyon MF, Bogani D, Boyd Y, et al. Further genetic analysis of two autosomal dominant mouse eye defects, Ccw and Pax6. *Mol Vis* 2000;6:199–203.
  45. Duncan MK, Cvekl A, Li X, et al. Truncated forms of Pax-6 disrupt lens morphology in transgenic mice. *Invest Ophthalmol Vis Sci* 2000;41:464–73.
  46. Duncan MK, Haynes JI II, Cvekl A, et al. Dual roles for Pax-6: a transcriptional repressor of lens fiber cell-specific  $\beta$ -crystallin genes. *Mol Cell Biol* 1998;18:5579–86.
  47. Shiraiishi A, Converse RL, Liu C-Y, et al. Identification of the cornea-specific keratin 12 promoter by in vivo particle-mediated gene transfer. *Invest Ophthalmol Vis Sci* 1998;39:2554–61.
  48. Liu JJ, Kao WW-Y, Wilson SE. Corneal epithelium-specific mouse keratin K12 promoter. *Exp Eye Res* 1999;68:295–301.
  49. Sivak JM, Mohan R, Rinehart WB, et al. Pax-6 expression and activity are induced in the reepithelializing cornea and control activity of the transcriptional promoter for matrix metalloproteinase gelatinase B. *Dev Biol* 2000;222:41–54.
  50. Williams SC, Altmann CR, Chow RL, et al. A highly conserved lens transcriptional control element from the Pax-6 gene. *Mech Dev* 1998;73:225–9.
  51. Kammandel B, Chowdhury K, Stoykova A, et al. Distinct *cis*-essential modules direct the time-space pattern of the Pax6 gene activity. *Dev Biol* 1999;205:79–97.
  52. Kays WT, Piatigorsky J. Aldehyde dehydrogenase class 3 expression: identification of a cornea-preferred gene promoter in transgenic mice. *Proc Natl Acad Sci USA* 1997;94:13594–9.
  53. Liu C, Arar H, Kao C, et al. Identification of a 3.2 kb 5'-flanking region of the murine keratocan gene that directs beta-galactosidase expression in the adult corneal stroma of transgenic mice. *Gene* 2000;250:85–96.
  54. Gopal-Srivastava R, Piatigorsky J. Identification of a lens-specific regulatory region (LSR) of the murine  $\alpha$ B-crystallin gene. *Nucl Acids Res* 1994;22:1281–6.
  55. Gopal-Srivastava R, Cvekl A, Piatigorsky J. Pax-6 and  $\alpha$ B-crystallin/small heat shock protein gene regulation in the murine lens. *J Biol Chem* 1996;271:23029–36.
  56. Gopal-Srivastava R, Cvekl A, Piatigorsky J. Involvement of retinoic acid/retinoid receptors in the regulation of murine  $\alpha$ B-crystallin/small heat shock protein gene expression in the lens. *J Biol Chem* 1998;273:17954–61.
  57. Gopal-Srivastava R, Kays WT, Piatigorsky J. Enhancer-independent promoter activity of the mouse  $\alpha$ B-crystallin/small heat shock protein gene in the lens and cornea of transgenic mice. *Mech Dev* 2000;92:125–34.
  58. Downes J, Holmes R. Development of aldehyde dehydrogenase and alcohol dehydrogenase in mouse eye: evidence for light-induced changes. *Biol Neonate* 1992;61:118–23.
  59. Salamon C, Chervenak M, Piatigorsky J, et al. The mouse transketolase (TKT) gene: cloning, characterization, and functional promoter analysis. *Genomics* 1998;48:209–20.
  60. Boesch JS, Lee C, Lindahl RG. Constitutive expression of class 3 aldehyde dehydrogenase in cultured rat corneal epithelium. *J Biol Chem* 1996;271:5150–7.
  61. Augusteyn RC. The effect of light deprivation on the mouse lens. *Exp Eye Res* 1998;66:669–74.
  62. King G, Holmes RS. Human corneal aldehyde dehydrogenase: purification, kinetic characterization and phenotypic variation. *Biochem Mol Biol Internatl* 1993;31:49–63.
  63. Downes JE, Swann PG, Holmes RS. Differential corneal sensitivity to ultraviolet light among inbred strains of mice. Correlation of ultraviolet B sensitivity with aldehyde dehydrogenase deficiency. *Cornea* 1994;13:67–72.
  64. Nees D, Wawrousek EF, Piatigorsky J. A homozygous knockout of aldehyde dehydrogenase 3 in mice. *Invest Ophthalmol Vis Sci* 2000;41:S752.
  65. Xu Z-P, Wawrousek EF, Piatigorsky J, et al. Transketolase in the cornea: species-specificity, production in darkness, and creation of hemizygous mice. *Invest Ophthalmol Vis Sci* 2000;41:S261.
  66. Brady JP, Wawrousek EF. Targeted disruption of the mouse  $\alpha$ B-crystallin gene. *Invest Ophthalmol Vis Sci* 1997;38:S935.