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Lens Biology and Biochemistry

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Abstract

The primary function of the lens resides in its transparency and ability to focus light on the retina. These require both that the lens cells contain high concentrations of densely packed lens crystallins to maintain a refractive index constant over distances approximating the wavelength of the light to be transmitted, and a specific arrangement of anterior epithelial cells and arcuate fiber cells lacking organelles in the nucleus to avoid blocking transmission of light. Because cells in the lens nucleus have shed their organelles, lens crystallins have to last for the lifetime of the organism, and are specifically adapted to this function. The lens crystallins comprise two major families: the $\beta\gamma$ -crystallins are among the most stable proteins known and the α -crystallins, which have a chaperone-like function. Other proteins and metabolic activities of the lens are primarily organized to protect the crystallins from damage over time and to maintain homeostasis of the lens cells. Membrane protein channels maintain osmotic and ionic balance across the lens, while the lens cytoskeleton provides for the specific shape of the lens cells, especially the fiber cells of the nucleus. Perhaps most importantly, a large part of the metabolic activity in the lens is directed toward maintaining a reduced state, which shelters the lens crystallins and other cellular components from damage from UV light and oxidative stress. Finally, the energy requirements of the lens are met largely by glycolysis and the pentose phosphate pathway, perhaps in response to the avascular nature of the lens. Together, all these systems cooperate to maintain lens transparency over time.

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1. LENS BIOLOGY: OVERVIEW

This overview of lens biology is necessarily limited and will be based primarily on those functions that support transparency and focusing of light, the primary function of the lens. That function is accomplished largely by the combination of the microarchitecture of the lens, comprising anterior epithelial cells and arcuate fiber cells lacking organelles in the nucleus, and on a molecular level the densely packed lens crystallins. Other biological activities of the lens function primarily to protect these two complementary systems from disruption and damage by age and external insults, especially as related to UV light and oxidative stress. While this is a very simplistic view of the lens, it does provide a perspective for consideration of the various biological pathways required for lens function.

2. CRYSTALLINS AND LENS BIOLOGY

Crystallins are the most prevalent proteins in the lens. Comprising of two families, α - and $\beta\gamma$ -crystallins, they make up 90% of water-soluble proteins of the mammalian lens.¹ They are highly organized and provide a refractive index gradient, which allows for transparency of the lens. Once thought to be solely lens proteins, crystallins have since been identified outside of the lens, providing several important functions (see Table 1). Organelle degradation, including endoplasmic reticulum, Golgi apparatus, mitochondria, and nuclei, which occurs during late stages of lens fiber cell differentiation, is also imperative for lens transparency and any disruption of this process will result in light scattering and ultimately cataract.^{2,3} Since differentiated lens cells lack the entire apparatus to produce new proteins, crystallins are not turned over and those in the center of the lens are thus among the oldest proteins in the body. Maintenance of lens proteins is therefore crucial for ocular health and for the prevention of lens opacities.

2.1 α -Crystallins

α -Crystallin monomers include an α -crystallin domain that is highly conserved in all members of the small heat-shock protein family. In the lens, α -crystallin proteins exist as globular aggregates roughly 600–900 kDa in mass,⁴ whose quaternary structure has been suggested to behave as a protein micelle.^{5,6} α A- and α B-Crystallins have been shown to occupy equivalent and dynamic positions in the aggregate, with subunit exchange occurring easily,⁶ and by a synchrotron radiation X-ray solution scattering study of a truncation mutant^{7–9} retaining chaperone activity. This suggests that α B-crystallin is composed of flexible monomers with an extended surface area.¹⁰ Cryo-electron microscopy has shown that recombinant α -crystallin has variable monomer packing consisting of a hollow central core with a surrounding protein shell.¹¹ α -Crystallin monomers appear to dimerize and then associate into a large complex, with amino and carboxyl regions of the α B-crystallin monomers interacting with residues from the corresponding region of their partners.¹² Although α A- and α B-crystallins seem to occupy equivalent positions in the α -crystallin complex, they show different tissue-specific expression patterns, show different phenotypes when knocked out in mice,^{13,13} differ in their phosphorylation patterns,^{14,15} structural properties, and chaperone activity.¹⁶ In addition, α B-crystallin fractionates with rough

endoplasmic reticulum while α A-crystallin fractionates with smooth membranes,¹⁷ and only α B is stress inducible^{18,19} suggesting that each fulfills a unique role in the lens.

α -Crystallins are phosphorylated in both cyclic adenosine mono-phosphate (cAMP)-dependent^{20,21} and -independent^{14,15} manner. They can be dephosphorylated by a calcineurin-related enzyme found primarily in the lens epithelial cells and to a lesser extent in fiber cells.²² α -Crystallin phosphorylation is of unknown significance as it does not seem to affect chaperone activity²³ or inhibition of glial fibrillary acidic protein (GFAP) and vimentin assembly.²⁴ However, it does stabilize actin filaments and decrease cytochalasin-dependent depolymerization.²⁵ The phosphorylation of α B-crystallin is stimulated by stress, including hydrogen peroxide treatment of cultured lenses²³ and heat treatment of heart and diaphragm muscles.²⁶

Both α A- and α B-crystallin can protect β - and γ -crystallins as well as other lens proteins from thermal aggregation, although not recycling these proteins.^{27,28} α -Crystallin can improve revival of glutathione reductase activity in human cortical extracts of both cataractous and clear lens by thioredoxin and thioredoxin reductase²⁹ and regenerate sorbitol dehydrogenase activity.³⁰ This does not require adenosine triphosphate (ATP) hydrolysis, although it is enhanced in the presence of ATP, and α B-crystallin chaperone activity is increased by the products of common metabolic pathways such as glutathione and pantethine.³¹ α -Crystallin chaperone activity requires its C-terminal domain,³² whose IXI/V motif is important in inter-subunit interactions and chaperone site accessibility.³³ During this process, the target protein apparently lodges in the fenestrated outer shell of the α -crystallin complex,³⁴ and its structure transitions into a multimeric molten globular state resulting in the appropriate placement of its hydrophobic surfaces.³⁵ Binding of target proteins by α -crystallin is cooperative, and mutations of β B1-crystallin increasing binding by α A- but not α B-crystallin.³⁶ Zinc binds to α -crystallin and appears to increase its stability and chaperone activity.³⁷ α A-Crystallin peptides inhibit amyloid fibril formation by amyloid beta protein,^{38,39} and α -crystallin suppresses microtubule assembly, maintaining a pool of unassembled tubulin.⁴⁰ All of these characteristics probably protect against cataractogenesis by reducing the aggregation of partially denatured proteins that accumulate within the lens during aging, holding them in high-molecular-weight complexes that do not form a nidus for precipitation and light scattering. Thus, the chaperone function of α -crystallins serves to protect the lens from toxicity caused by damaged proteins denaturing with age and it probably acts similarly in nonlenticular tissues. In addition, peptides from α -crystallin can inhibit apoptosis of lens epithelia and lens opacification.⁴¹

α -Crystallin expression is not limited to the lens. α B-crystallin is widely expressed, including in the heart, skeletal muscle, kidney, lung, brain, and retina, although at lower levels than in the lens.^{42,43} In skeletal muscle, α B-crystallin expression increases by stretching,⁴⁴ and in the heart it forms aggregates of 400–650 kDa.⁴⁵ In contrast, α A-crystallin is expressed constitutively at low levels in many tissues and somewhat higher in the spleen and thymus.⁴⁶ α B-Crystallin has been shown to block p53-dependent apoptosis by inhibiting RAS activation,⁴⁷ and mitochondria in stressed cardiac muscle.⁴⁸ α -Crystallin enhances survival of optic nerve axons after a crush injury⁴⁹ and is upregulated in regenerating neuron axons in monkeys.⁵⁰ As mentioned above, in cultured cells α B- but not

α A-crystallin is inducible by stress, including heat¹⁹ and osmotic shock.¹⁸ Possibly related to this, α B-crystallin is found at high levels in the brains of patients with Alexander disease,⁵¹ brains of scrapie-infected hamsters,⁵² and fibroblasts from patients with Werner syndrome.⁵³

The ability of the α -crystallins to inhibit apoptosis is well documented.^{54,55} α -Crystallins have been shown to prevent apoptosis induced by a number of factors, including etoposide and staurosporine,⁵⁶ UV radiation,⁵⁴ sorbitol,⁵⁶ TNF α ,⁵⁷ hydrogen peroxide,⁵⁸ and okadaic acid.⁵⁹ Kamradt and colleagues demonstrated that overexpressing α B-crystallin slows caspase-3 maturation, thus inhibiting apoptosis.⁶⁰ Similarly, over-expression of α A- or α B-crystallin in lens epithelial cells confers resistance against several forms of stress, including thermal and photochemical.⁵⁷ Andley and colleagues demonstrated that α A-crystallin shows two- to threefold higher levels of antiapoptotic activity than equivalent amounts of α B-crystallin in cultured lens epithelial cells.⁵⁷ Mutations in α -crystallin, that were shown to reduce chaperone function, also diminished the antiapoptotic activity of α A-crystallin.⁶¹ Similarly, activation of apoptosis was seen in α B-R120G-induced desmin-related myopathy.⁶² α A-/ α B-Crystallin double-knockout mice exhibit caspase-dependent secondary lens fiber cell disintegration.⁶³

The mechanism of apoptotic inhibition by α -crystallin has been shown to be different for the two subunits. α B-crystallin is able to prevent apoptosis through repression of Raf/MEK/ERK signaling, whereas α A-crystallin activates the Akt surviving pathway to inhibit apoptosis.⁵⁴ α A-Crystallin has also been shown to prevent apoptosis by enhancing phosphoinositide 3-kinase activity.⁶⁴ Moreover, α B-crystallin was shown to prevent caspase 3 activation and therefore apoptosis in both astrocytes⁶⁵ and cardiomyocytes.⁶⁶

Preservation of mitochondrial function is essential for viability of both lens and retinal cells.^{67–69} It has been demonstrated that α B-crystallin translocates to the mitochondria under oxidative stress conditions, protecting its function and preventing oxidation of cytochrome *c*. However, the chaperone function of α -crystallin was not required for this mitochondrial protective function.⁷⁰

2.2 $\beta\gamma$ -Crystallins

The β - and γ -crystallins show sequence similarities of 30% in the aligned regions of their central globular domains, which have a similar tertiary structure, and together form a $\beta\gamma$ -crystallin superfamily.^{71–73} $\beta\gamma$ -Crystallins have a common two-domain structure comprising four repeated “Greek key” motifs because they resemble the classical pattern found on the borders of ancient Greek pottery. Each Greek key motif consists of an extremely stable, torqued β -pleated sheet.⁷⁴ The first and second Greek key motifs make up the N-terminal domain, while the third and fourth form the C-terminal domain of the protein. The two domains are connected by a linker or connecting peptide (Fig. 1). Because hydrogen bonding occurs between amino acids of different motifs in different domains, all four motifs need to be present for the high stability seen in the $\beta\gamma$ -crystallins.⁷⁵ In addition, interactions between the interface of the amino- and carboxyl-terminal domains help to stabilize the structure.⁷⁶

While each of the four Greek key motifs is similar to the others, the first motif shows higher homology to the third, and the second to the fourth, consistent with an evolution of the $\beta\gamma$ -crystallin family starting from a single-motif through a two motif intermediate that is reduplicated, creating the present $\beta\gamma$ -crystallin core structure (see Fig. 1).⁷⁷ This intermediary evolutionary step is represented by spherulin 3a, *Ciona* $\beta\gamma$ -crystallin, and nitrollin, while absent in melanoma 1 (AIM1) has expanded to six domains.^{78,79} Many evolutionary $\beta\gamma$ -crystallin homologs bind Ca^{2+} , which increases their stability,⁸⁰ as has also been suggested for mammalian β -crystallins.⁸¹ In addition to being extremely stable, the Greek key motif structure can also quench UV irradiation, helping to prevent photooxidation of the lens as it ages.⁸²

β -Crystallin monomers associate initially into homo- and heterodimers of about 50 kDa, which then further associate in a more complicated fashion into complexes of 150–200 kDa, especially in the lens.⁸³ The crystal structure of $\beta\text{B}2$ -crystallin dimers shows an extended linker connecting the domains. The widely separated amino and carboxyl domains of one β -crystallin polypeptide pair through “domain swapping” in which the amino domain of the first pairs with the carboxyl domain of the second, while the carboxyl domain of the first pairs with the amino domain of the second. In contrast, in the monomeric γ -crystallins, this connecting peptide folds back on itself so that the two domains of a single molecule pair with each other.⁸⁴ The domain swapping pairing of the β -crystallins allows higher oligomerization through further association of the dimers.^{85,86} $\beta\text{B}1$ -Crystallin has a stronger tendency than $\beta\text{B}2$ -crystallin to form higher order oligomers both *in vitro*⁸⁷ and *in vivo*.⁸⁸ Under physiological conditions, β -crystallin complexes are able to exchange monomers rapidly, indicating that they are in a reversible equilibrium rather than being static structures.⁸⁹ Formation of β -crystallin dimers is entropically driven, with the tightness of association as the temperature increases.⁹⁰ Substitution of the $\gamma2$ -crystallin connecting peptide into murine $\beta\text{A}3$ -crystallin and, conversely, part of the $\beta\text{B}2$ -crystallin connecting peptide into $\gamma2$ -crystallin by site-specific mutagenesis has no effect on association, suggesting that the connecting peptide itself does not explain dimerization of β -crystallins while the γ -crystallins remain monomers.^{91,92}

While it is unclear whether cleavage of β -crystallin arms represents development or aging, loss of the terminal extensions definitely alters their association and stability. Truncation of the amino-terminal extensions increases affinity of association of monomers of $\beta\text{A}3$ -crystallin into dimers⁹³ but decreases that of $\beta\text{B}2$ -crystallin.⁹⁰ Truncation of the terminal arms of $\beta\text{A}3$ -crystallin increases its susceptibility to photooxidation by ultraviolet B (UVB).⁹⁴ Truncation of $\beta\text{B}1$ -crystallin increases its tendency to oligomerize and causes it to undergo two phase transitions, resembling those for cold cataract in γ -crystallins.⁹⁵ However, removal of amino- and carboxyl-terminal arms of rat $\beta\text{B}2$ -crystallin⁹⁶ did not alter its association properties nor did changing the sequence or deleting part of the amino-terminal arms of chick $\beta\text{B}1$ -crystallin, or conversion of rat $\beta\text{A}3$ - from $\beta\text{A}1$ -crystallin by deletion of the 17 amino acids by which they differ.⁹⁷ As with the bonding of γ -crystallins at their interdomain interface, dimerization of β -crystallins stabilizes them and increases their solubility.⁹⁸

The highly symmetrical and correspondingly extremely stable γ -crystallins have a molecular mass of about 21 kDa.⁸⁴ The γ -crystallins are expressed specifically in the lens fibers and are thus the major crystallins in the lens nucleus, contributing to it having the highest protein concentration and being the most dehydrated and hence the hardest section of the lens. The γ -crystallins are adapted for high-density molecular packing.⁹⁹ The γ -crystallins are abundant in almost all mammals including humans, but not in birds and reptiles, which use other proteins as their major nuclear lens crystallins. Species, such as fish and rodents, with high concentrations of γ -crystallins, have hard lenses that lack the accommodative powers of the softer lenses found in birds and reptiles. γ -Crystallins, and especially γ DEF, can form “cold cataracts”, a reversible opacity which occurs on cooling of the lens.¹⁰⁰

γ S-Crystallin, which was previously termed β s-crystallin, represents an intermediate between the β - and γ -crystallins.^{101,102} γ S-Crystallin shares many physical and chemical properties with the β -crystallins. The γ S-crystallin protein is slightly larger than most γ -crystallins, having 177 residues, and includes an amino-terminal arm like the β -crystallins.¹⁰¹ Its isoelectric point is lower than that of most γ -crystallins, being closer to those of the β -crystallins. Finally, as opposed to other γ -crystallins, the amino-terminus of γ S-crystallin is blocked, similar to the β -crystallins. The human γ S-crystallin gene is located on chromosome 3, while the remaining γ -crystallin genes are located in a cluster on chromosome 2q33–35. Developmentally, γ S-crystallin is expressed later than other γ -crystallins, and continues into adulthood, when expression of other γ -crystallins is low or has ceased.¹⁰³ Unlike other γ -crystallins, γ S-crystallin is found in birds and reptiles, as are the β -crystallins.^{104,105} However, unlike β -crystallins, γ S-crystallin exists in solution as a monomeric protein as do the remaining γ -crystallins. One important criterion for assigning γ S-crystallin to the γ -crystallins is that its gene structure consists of three exons, as do the other γ -crystallins but not the β -crystallins, which consist of six exons.^{6,106}

In contrast to the ubiquitous α - and β γ -crystallins, taxon-specific crystallins are expressed at high concentrations in the lens (usually at least 10% of the soluble protein), but tend only to be present in one or a few species.¹⁰⁷ Many taxon-specific crystallins are likely to have arisen by “gene-sharing,” in which a single gene product usually retaining its original function in nonlens tissues, acquires an additional function in the lens.^{13,108,109} Use of a single gene product for multiple separate functions subjects it to additional evolutionary selection. In addition, a mutation in a regulatory sequence altering gene expression may result in an additional function for the encoded protein before or without gene duplication or loss of its original function. Gene duplication and divergent evolution and specialization of function for one of the two resulting proteins may occur later, as has been suggested to have happened for the α - and δ -crystallins.¹¹⁰ While it is expected that taxon-specific crystallins might have enzymatic activity in addition to their role in the lens, similar evolutionary and functional principles appear to apply to the ubiquitous crystallins as well, as the extralenticular roles of the crystallins have been examined.

Novel roles of crystallins in autophagy and tissue remodeling have recently come to light. For example, loss of Cryba1 was shown to lead to lysosomal dysregulation and the impairment of both autophagy and phagocytosis in retinal pigment epithelium (RPE) cells.¹¹¹ Also, mutation of the rat β A3/A1-crystallin gene (*Nuc1* allele) impairs phagosome

degradation in rat RPE.¹¹² In addition, autophagy was inhibited in the α B-crystallin R120G mutant lenses leading to larger autophagosomes compared to wild-type lenses, possibly due to a defect in protein degradation after autophagosome formation.¹¹³ α -Crystallin has also been localized to the leading edges of migrating lens epithelial cells and possibly plays a role in actin dynamics during cell migration,¹¹⁴ suggesting a possible function for α -crystallin in tissue remodeling. A potential role for β - and γ -crystallins in the vascular remodeling of the eye has also been proposed.¹¹⁵ A possible role has also been suggested for γ S-crystallin in fiber cell maturation due to its functional role in the stabilization of actin and “shepherding” of filaments.¹¹⁶ Finally, it has been recently suggested that α B-crystallin acts as a molecular guard in mouse decidualization, playing a role during early pregnancy.¹¹⁷

A number of mouse models have been generated to help analyze the functions of crystallins *in vivo* further. Knockout models for α A- and α B-crystallin and double α A/ α B knockouts have been made. The lenses of α B-knockout mice appeared normal compared to wild-type mice, although skeletal muscle degeneration was observed.¹¹⁸ However, the lenses of α A-crystallin-deficient mice were smaller and exhibited progressive opacification several weeks after birth.¹¹⁹ The double-knockout mice were born with small opaque lenses, much more severe than the individual subunit knockouts, suggesting a compensatory activity by the two α -crystallin subunits.⁶³ Knocking out the mouse γ S-crystallin gene appears to result in disorganized actin filaments and interferes with fiber cell maturation,¹¹⁶ and knockout of the β B2-crystallin gene results in age-related cataract at several months of age.¹²⁰ The “noncrystallin” functions of crystallins include a range of activities, including inhibition of apoptosis, chaperone-like functions, cytoskeletal remodeling, kinase activity, regulation of autophagy, cytochrome *c* redox state, and denucleation, although which of these specific functions are responsible for the phenotypes in knockout mice is at present unclear.

3. MEMBRANE PROTEINS

Membrane-associated proteins account for approximately 2% of lens proteins. They have molecular masses ranging from about 10 to over 250 kDa. Some, such as N-cadherin, a 135-kDa intrinsic membrane protein which may be involved in cell–cell adhesion, are components of the cyto-skeletal structure.¹²¹ Similarly, the calpactins, extrinsic membrane proteins attached to the membrane through calcium, are probably involved in membrane–cytoskeleton interactions and fiber cell elongation.^{122,123} Neural-cell adhesion molecule 2 (NCAM 2) appears to play a role in cell adhesion, contributing to the proper arrangement of gap junctions in the developing lens fiber cells.¹²⁴ Other membrane proteins include enzymes such as glyceraldehyde 3-phosphate dehydrogenase, other glycolytic enzymes and channels on the endoplasmic reticulum,¹²⁵ and a variety of other enzymes such as adenosine triphosphatases (ATPases).

In addition, there are two additional intrinsic membrane proteins that are also highly expressed in lens fiber cells. Major intrinsic protein (MIP), a hydrophobic 28 kDa, 263-amino-acid protein is the most abundant. It is part of the aquaporin family of water channels, and is also known as aquaporin 0 (AQP0). AQP0 is highly homologous to AQP1, and like it has six transmembrane domains, three extracellular loops, two intracellular loops around a

central channel, and cytoplasmic amino- and carboxyl-terminal domains.¹²⁶ The MIP monomer sequence also shows a structural symmetry of two tandem repeats, each containing a “hemipore” composed of three transmembrane helices with a hydrophobic loop containing a conserved Asn-Pro-Ala (NPA) motif. These fold into the membrane to form a functional water pore. The presence of two evolutionarily conserved NPA-core motifs, directly involved in water transport, is consistent with a gene duplication event in the aquaporin gene family.¹²⁷

X-ray and electron crystallography has largely confirmed the originally predicted hourglass structure of MIP monomers, and further confirmed that MIP forms homotetramers, consistent with the square arrays observed by ultra-structural analyses of native plasma membranes from the lens core. Not only can MIP purified from the lens core form single-layered 2D crystals of tetramers, but it also can form head-to-head tetramers (double-layered 2D crystals of junctional octamers) of similar size and thickness to the 11–13 nm thin junctions seen in ultrastructural analysis of lens plasma membranes.^{126,128,129} Conversion of nonjunctional MIP tetramers to junctional MIP octamers is associated with posttranslational proteolytic truncation of the cytoplasmic N- and C-termini, and stabilized by proline-mediated hydrophobic interactions between the extracellular loops of apposed tetramers.^{126,130,131} In addition, atomic force microscopy shows that AQP0 arrays are surrounded by connexins to form junctional microdomains important for both adhesion and channels for intercellular communication and transport.¹³²

Physiological studies have shown that MIP functions as a relatively slow water channel, being less than 10-fold as active as AQP1 in both the classical heterologous expression system in *Xenopus* oocytes as well as membrane vesicles isolated from lens fiber cells.¹³³ However, in spite of its low conductivity, experiments in lens membrane vesicles isolated from MIP-deficient mice show that because of its abundance in the lens, aquaporin provides about 80% of water transport in wild-type mouse lenses.^{133,134} In addition, the water channel activity of MIP in lens vesicles increases as the pH is lowered to 6.5 and is raised by increasing Ca²⁺ ion concentration in a calmodulin-dependent manner.^{135,136}

Recently, molecular dynamic simulations have indicated that both junctional and nonjunctional forms of MIP can transport water at similar rates, with both showing an average channel occupancy of about five water molecules.¹³⁷ In addition, extracellular water appears to access the pores of junctional MIP,¹³⁸ suggesting that junctional MIP could carry out both the water transport and cell-to-cell adhesion functions needed for lens homeostasis. In addition to the self-association into tetramers shown in water transport and junction formation, MIP is suggested to interact with several other proteins in the lens, including connexins,¹³⁹ filensin and phakinin (beaded filament proteins),¹⁴⁰ and crystallins¹⁴¹ through its C-terminus, perhaps providing a means of regulation or for additional functions.

4. GAP JUNCTION PROTEINS

In the mature lens, which is avascular, a network of gap junction channels facilitates intercellular communication and metabolic cooperation.^{142,143} This is accomplished by exchange of ions such as Na⁺, K⁺, Ca²⁺, and Cl[−], second messengers such as cAMP, cGMP,

and inositol trisphosphate (IP3), and small metabolites including glucose and amino acids. There are at least three gap junction proteins, called connexins, in the lens, having overlapping but distinct expression patterns. Gap junction protein A1 (GJA1, connexin-43, Cx43), with a molecular mass of about 43 kDa, is expressed predominantly in the anterior epithelia.¹⁴⁴ Gap junction protein A8 (GJA8, connexin 50, Cx50), with a molecular mass of about 50 kDa, is expressed in anterior epithelial cells along with GJA1, but its expression continues in the elongating fiber cells along with gap junction protein A3 (GJA3, connexin 46, Cx46), with a molecular mass of about 46 kDa, to make them the most highly expressed gap junction proteins in the lens nucleus.^{145,146} Each gene consists of a single coding exon and encodes a protein with four transmembrane domains separated by two extracellular loops and a cytoplasmic loop with cytoplasmic amino- and carboxyl-termini.³⁵ Connexin monomers associate to form hexamers, also known as hemichannels or connexons, and gap junction channels are formed when two connexons in the membranes of adjacent cells associate in the extracellular space. Gap junction plaques are formed in lens fiber cell membranes when up to hundreds of gap junction channels cluster to form, and appear as 16–18 nm thick junctions seen in electron micrographs.¹²⁸ In addition, as discussed above, connexins appear to associate with AQP0 to form junctional microdomains.¹³²

Gja3 and Gja8 function in the internal microcirculation system of the lens, which delivers glucose, amino acids, and antioxidants such as glutathione to the central fiber cells.¹⁴³ In this model, it is proposed that Na⁺ ions carry the current with metabolites into the lens primarily via the extracellular spaces at the anterior and posterior poles, and also carry the current out of the lens through cytoplasmic diffusion gradients through gap junctions to be transported out of the lens by the Na⁺–K⁺ pumps in the anterior epithelial cells. In mice with both Gja3 and Gja8 knocked out, the lenses develop swelling and degeneration of mature fiber cells in the inner lens, resulting in cataract formation. This suggests that these two connexins participate in the intercellular transport of proteins, metabolites, and ions within the central lens nucleus.¹⁴⁷ In this light, Gja3 and Gja8 have been implicated in helping to establish the uniform distribution of protein seen among cells of the lens core, and GJA3 gap junctions have been shown to provide a pathway for glutathione (GSH) diffusion from the outer cortex to the lens nucleus.^{148,149} In lens fiber cells, both connexins GJA3 and GJA8 undergo posttranslational phosphorylation. This is hypothesized to regulate proteolytic cleavage and turnover as well as downregulate cell–cell communication and increase protein stability.^{150–152}

5. CYTOSKELETAL PROTEINS

Many cytoskeletal proteins common to other tissues are found in the lens, such as actin, α -actinin, ankyrin, myosin, spectrin, and vimentin. An extended network of proteins including spectrin and actin is found immediately below the cell membrane, similar to that seen in erythrocytes,¹⁵³ and through tropomodulin 1 contributes both to early lens development to the maintenance of hexagonal cell shape of differentiating fiber cells of the lens cortex.^{154–156} Microtubules, which are rare in epithelial cells but are found arrayed lengthwise in the peripheral cytoplasm of cortical fiber cells and decrease again in nuclear fiber cells, contain α - and β -tubulins.¹⁵⁷ Along with other cytoskeletal components, microtubules may contribute to establishing and maintaining the elongated shape of fiber

cells as well as being involved in the migration of chromosomes in dividing lens epithelial cells.¹⁵⁸ Actin filaments interact closely with cell membranes of lens cells^{159–160} through adherens junctions as well as proteins such as ezrin, radixin, and protein 4.1.^{161–163} It has been suggested that they might facilitate accommodation.^{164–166} These components of the basal membrane complex undergo rearrangement as the fiber cells elongate and migrate from the capsule to the lens sutures,¹⁶⁷ forming a terminal web to stabilize the ends of lens fibers at the sutures.¹⁶⁸ Nonmuscle isoforms of β - and γ -actin also occur in lens microfilaments (also called thin filaments).¹⁶⁹ Tropomodulin and α -actinin have been shown to associate with actin in lens microfilaments, especially in elongating cortical fiber cells.^{170,171}

Vimentin is usually found in cells of mesenchymal origin, but it is part of intermediate filaments in lens cells.¹⁵⁵ These 10 nm filaments can occur as extrinsic membrane proteins, but are more commonly found in the cytoplasm.¹⁷² Vimentin-based intermediate filaments occur primarily in anterior epithelial cells, where they are required for repair of damage to the lens epithelium.¹⁷³ While some vimentin is expressed in superficial cortical cells, vimentin-containing filaments are replaced by BFSP2 filaments deeper in the cortex.¹⁵⁵ Vimentin can be phosphorylated¹⁷⁴ and defects in its phosphorylation can cause aneuploidy of lens epithelia resulting in micro-phthalia and cataract.¹⁷⁵ Vimentin expression is requisite on pigment epithelium-derived factor (PEDF) expression in epithelia cells,¹⁷⁶ and triples in lens development in embryonic chickens, decreasing after hatching.¹⁷⁷ Transfection of vimentin promoters into cultured cells has identified a complex set of positive and negative cis-regulatory 5'-flanking sequences that control vimentin expression.^{177,178} An intermediate filament protein GFAP, usually found in cells of neuroectodermal origin, shows a similar expression pattern: high in anterior epithelial cells and decreasing upon differentiation to fiber cells.^{106,179}

As vimentin-containing intermediate filaments disappear during fiber cell differentiation, they are replaced by beaded filaments,¹⁸⁰ cytoskeletal proteins that appear to be uniquely expressed in the lens.^{77,181} Beaded filaments consist of a central backbone filament 7–9 nm wide containing BFSP1 (CP-115, filensin), along which 12–15 nm globular protein particles containing BFSP1 as well as BFSP2 (CP-49, phakinin) are spaced.¹⁵⁵ Both BFSP1 and BFSP2 belong to the intermediate filament family, although they are highly divergent.¹⁸² They appear to be critical in maintaining the shape, size, and stiffness of the lens,¹⁸³ probably through regulating fiber cell geometry in coordination with the spectrin-actin membrane skeleton mediated through Tmod1.¹⁸⁴ This is critical for lens transparency and coincides with the rearranging and remodeling of fiber cells as they transit from the cortical lens to the nucleus.¹⁸⁵

The α -crystallins appear to be critical for the assembly, maintenance, and remodeling of the lens cytoskeleton. BFSP1 and BFSP2 copolymerize *in vitro*, forming 10 nm fibers similar in appearance to intermediate filaments seen in nonlens tissues.^{186,187} These proteins fail to assemble with vimentin, and substitution of the vimentin rod or tail domain for that of BFSP1 will inhibit fiber assembly.¹⁸⁸ However, addition of α -crystallin during the assembly process results in the formation of a structure similar to a beaded chain.¹⁸⁶ In addition, not only will addition of α -crystallins result in the correct beaded filament-type

structure, but it also inhibits assembly of both GFAP and vimentin into filaments in an ATP-dependent manner,²⁴ resulting in their transition from formed filaments to a soluble state.

6 LENS METABOLISM

6.1 Energy Metabolism

The intermediary metabolism of the lens is generally similar to those of other tissues. The major differences arise from the lens being avascular, so that it has to obtain most of its nutrients through the aqueous humor.¹⁸⁹ In addition, the loss of intracellular organelles, including mitochondria, during fiber cell differentiation places additional requirements on lens metabolism. Thus, utilization of various metabolic pathways for energy production in the whole lens is quite different from most tissues, with most glucose in the lens metabolized through anaerobic glycolysis. Enzymes composing the citric acid cycle are only found in the anterior epithelia, which retain their mitochondria. Because of its greater efficiency, the citric acid cycle provides 20–30% of the total lens while consuming only roughly 3% of the glucose.^{190,191} Although the lens can control its ion balance, maintain high-energy phosphate levels, and carry out protein synthesis in the absence of oxygen, blocking the Embden–Meyerhof pathway by exposing the lens to iodoacetate, which inhibits 3-phosphoglyceraldehyde dehydrogenase, causes ionic changes with accompanying swelling and cataracts.^{192,193} Conversely, exposure of cultured lenses to oxidative stress induced by exposure to hydrogen peroxide¹⁹⁴ or hyperbaric oxygen¹⁹⁵ increases hexokinase activity and thus stimulates the pentose phosphate pathway.

The osmotic hypothesis suggests that aldose reductase activity might increase sorbitol and thus protect the lens from daily diet and disease-related changes in osmolality of the aqueous humor,¹⁹⁶ similar to the action of sorbitol in the renal medulla.¹⁹⁷ This proposes that cataracts secondary to diabetes mellitus and galactosemia share a common pathogenic mechanism in which aldose reductase reduces glucose to sorbitol and galactose (more readily) to galactitol.¹⁹⁸ As opposed to sorbitol, which is further metabolized by sorbitol dehydrogenase, galactitol remains in the lens, potentially damaging the lens cells by increasing its intracellular fluid in response to the increased osmotic pressure. This can cause swelling of the lens cells, increasing their membrane permeability with secondary electrolyte abnormalities, and finally resulting in metabolic dysfunction.¹⁹⁹ Cataracts occurring secondary to polyol accumulation occur in transgenic mice expressing the aldose reductase gene in their lenses, which normally lack this enzyme.²⁰⁰ While human lenses have reduced aldose reductase compared to the rat, human lenses from diabetic patients cultured *in vitro* accumulate more polyols compared to lenses from nondiabetic controls, and aldose reductase inhibitors inhibit this polyol accumulation.²⁰¹ Osmotic stress can also induce a variety of FGF and TGF- β signals and increased signaling through the mitogen-activated protein kinase (MAPK) pathway.²⁰² However, the mechanism of sugar cataract pathogenesis remains controversial, and the possibility that inhibition of aldose reductase might reduce accumulation of polyols and the accompanying osmotic damage and cataract in diabetics is still being investigated.²⁰³

6.2 Maintenance of a Reduced State

As discussed in Section 4 of the chapter Overview of the Lens, oxidative damage to lens constituents, especially that from photooxidation, is a major threat to lens transparency. This accumulates over the lifetime of an individual and can include not only proteins but also lipids and even nucleic acids. It is particularly problematic when it affects those very systems that provide for lens homeostasis. Loss of the reducing environment in lens cells can result in a variety of oxidative modifications to lens proteins, including ascorbylation. When this impacts the lens crystallins, it can alter their short range interactions, causing aggregation and cataract.²⁰⁴ Hyperbaric oxygen treatment of guinea pigs results in disulfide bond formation and cross-linking of lens crystallins with formation of aggregates and resultant light scattering.²⁰⁵ When β B3-crystallin is oxidized, it interacts with γ -crystallins to increase their sensitivity to thermal aggregation and cause light scattering.²⁰⁶ One major source of oxidative stress in the lens is photooxidation from ultraviolet light, and it is possible that other types of insults such as osmotic stress might also tax the reducing environment of the lens.^{207,208} In the normal human eye, peroxides are generated in both lens fiber and anterior epithelial cells, maintaining the concentration of H_2O_2 at around 30 mM, although it can be significantly elevated with cataracts.²⁰⁹ In addition, it has been suggested that vitreal degeneration can increase oxidative stress on the posterior lens.²¹⁰

Under optimal conditions, the lens can respond to chronic oxidative stress by increasing production of reducing agents, and has developed a comprehensive and versatile system to defend against oxidative stress, and repair oxidative damage when the reducing environment is over-whelmed.²¹¹ The most notable of these defenses is high levels of glutathione, the most abundant low-molecular-weight thiol in the human lens, which serves to maintain a strong reducing environment.^{212,213} The high levels of reduced glutathione are maintained by a variety of intracellular enzymes including glutathione reductase as well as reducing power generated through the pentose phosphate pathway. A gradient of reduced glutathione exists in the lens, from the peak concentration in the metabolically active anterior epithelium followed by the cortical regions and finally lowest in the nucleus.²¹⁴ While glutathione levels in the anterior epithelium and cortex are not age dependent in the normal lens, they are decreased markedly in most types of cataractous lenses.²¹⁵ Lens glutathione serves as a sulfhydryl buffer that maintains protein thiol groups in a reduced state as well as protecting other residues against oxidative damage. However, under oxidative stress, glutathione is found in mixed disulfides with various lens proteins, including crystallins such as γ S-crystallin, possibly altering its association behavior.²¹⁶ However, thioredoxin and thioredoxin reductase can rescue enzymatic function of oxidized glyceraldehyde 3-phosphate dehydrogenase, and this activity is enhanced by α -crystallin.²⁹ Similarly, methionine sulfoxide reductase can reverse oxidation of methionine, conferring additional resistance to oxidative stress.⁶⁷

Detoxification of hydrogen peroxide in the lens can be carried out by both the glutathione redox cycle and catalase, although glutathione reductase activity appears to be more critical for this function.²¹⁷ Catalase is found in peroxisomes and acts on higher levels of H_2O_2 .²¹⁸ In contrast, enzymes such as glutathione reductase and peroxidase, that are part of the glutathione redox cycle, occur throughout the cytoplasm. Although the mercapturic acid

pathway, including glutathione *S*-transferase, also helps to protect lens proteins from oxidative damage,²¹⁹ glutathione reductase has a primary role in maintaining lens glutathione in a reduced state.²¹³ That glutathione *S*-transferases have been important in lens biology for a long period during evolution is suggested by their close similarity to *S*-crystallins, the major crystallins in cephalopods.²²⁰ Because of the effectiveness of these combined reducing pathways, only about 2–5% of glutathione in the lens is normally in its oxidized state. The oxidative defense systems including catalase, glutathione reductase, and glutathione peroxidase cooperate and show considerable functional overlap, so that defects in one pathway can be partially compensated by others.²²¹ Efforts are underway to utilize peroxidases as anticataract agents by protecting the lens for oxidative stress.⁵⁰⁴ Additional protection against oxidative stress is provided by heat-shock proteins, especially α -crystallins, which can also act through protection of mitochondria in a manner independent of their chaperone activity.⁷⁰

6.3 Osmoregulation

Osmoregulation in the lens occurs through active transport, in which Na^+/K^+ -dependent ATPase exchanges sodium for potassium into the lens, and is associated with the lens microcirculation mentioned earlier.²²² Most of the lens Na^+/K^+ -dependent ATPase is found in the apicolateral membranes of the anterior epithelial cells,^{223,224} but there is also some activity in the anterior cortex and the fiber membranes abutting the sutures.^{225,226} The actively exchanged cations are followed passively by diffusion of chloride and water, and transport might be purinergic control.²²⁷ Osmotic and ionic homeostasis of the lens is a complex process. There is ion transport through the intercellular spaces of epithelia and fiber cells, followed by water and solutes. These are then actively transported into the fiber cells, and access the central fiber cells through coupling by gap junctions. Finally, metabolic end products exit the fiber cell mass following through the lens equator, probably coupled to Na^+/K^+ transport.²²⁸ This microcirculation occurs largely in the superficial cortex. There is also transport of macromolecules and cholesterol by caveolae and coated vesicles.²²² Similarly, calcium homeostasis is also complex, involving not only Ca^{2+} -ATPase but also calcium channels and a variety of means to sequester calcium intracellularly including binding proteins and active transport across the endoplasmic reticulum. Ca^{2+} -ATPase shows the highest specific activity in the anterior epithelium, so that the lens has a lower Ca^{2+} concentration than the aqueous or vitreous humor.^{229–231} Among other problems, disruption of the Ca^{2+} circulation can lead to opacity through increased calcium levels resulting in the activation of proteinases and then cleavage and aggregation of crystallins.²³²

The lens controls movement of various macromolecules in different fashions. It has been proposed that metabolites might pass from the aqueous chamber into the lens, and this has been demonstrated for albumin.²³³ The first barrier a molecule must pass to reach the avascular lens is the capsule. It provides a limit for diffusion: horseradish peroxidase, with a molecular weight of 40,000 kDa, can diffuse across the lens capsule, but ferritin, with a molecular weight of 500,000, cannot,²³⁴ and experiments with dextran show an exclusion limit of about 150 kDa.²³⁵ Thus, low-molecular-weight crystallins can penetrate the lens capsule but not the higher molecular weight α -crystallin.²³⁶ A second barrier is the layer of anterior epithelial cells, but these can also be penetrated by low-molecular-weight proteins

such as horseradish peroxidase and various dyes.²³⁷ However, passage of metabolites between the epithelial cells and fiber cells is probably through transcytotic processes mediated by caveolae and clathrin-coated vesicles rather than, or perhaps in addition to, the relatively small number of gap junctions connecting them.^{238,239} Lens fiber cells are connected by an extensive network of communicating channels.¹⁴³ Sugar transport in the lens occurs with glucose transporters 1 and 3 (GLUT1 and GLUT3), similarly to that seen in many other tissues, including myocytes and blood cells.^{240–242}

This brief overview cannot provide a detailed examination of all the protein families, metabolic pathways, and homeostatic systems that are present in the lens. A number of additional systems have not been covered at all, including RNA granules during lens, and especially fiber cell development,²⁴³ autophagy in lens development and maintenance of transparency,²⁴⁴ and the underappreciated role of epithelial cell mitochondria in preserving lens fiber cell function.²⁴⁵

ABBREVIATIONS

AIM	absent in melanoma
AQP0	aquaporin 0, or MIP, major intrinsic protein
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BFSP1	beaded filament specific protein 1, or CP-115, or filensin
BFSP2	beaded filament specific protein 2, or CP-49, or phakinin
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
ERK	extracellular signal-regulated kinase, or MAPK, mitogen-activated protein kinase
FGF	fibroblast growth factor
GFAP	glial fibrillary acidic protein
GJA1	gap junction protein A1, or connexin-43, Cx43
GJA3	gap junction protein A3, or connexin-46, Cx46
GJA8	gap junction protein A8, or connexin-50, Cx50
GLUT1	glucose transporter 1
GLUT3	glucose transporter 3
GSH	glutathione
IP₃	inositol trisphosphate

MAPK	mitogen-activated protein kinase, or ERK, extracellular signal-regulated kinase
MEK	mitogen-activated protein kinase kinase
MIP	major intrinsic protein, or AQP0, aquaporin 0
NCAM	neural-cell adhesion molecule
PEDF	pigment epithelium-derived factor
Raf	Raf-1 protooncogene
RPE	retinal pigment epithelium
TGF-β	transforming growth factor beta
UVB	ultraviolet B

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Figure 1. Evolution of the genomic and protein structures of the ubiquitous α -, β -, and γ -crystallins. The α -crystallins are chaperones related to heat-shock proteins, while the $\beta\gamma$ -crystallins belong to a superfamily related to stress proteins. During evolution, multiple events have increased divergence of the crystallin families, including gene duplication, fusion of exons, and the use of multiple transcription and protein synthesis initiation sites.

Table 1

Summary of Lens Crystallin Gene Families

Distribution	Crystallin	(Related) or Identical
Represented in all vertebrates	α	(Small heat-shock proteins) (<i>Schistosoma mansoni</i> antigen p40)
	β	(<i>Myxococcus xanthus</i> protein S) (<i>Physarum polycephalum</i> spherulin 3a)
	γ	(EDSP) (A1M1, AIMIL, CRYBG3)
Birds and reptiles	$\delta 1$	(Argininocuccinate lyase)
	$\delta 2$	Argininosuccinate lyase
	ϵ	Lactate dehydrogenase B
Some mammals	ζ	(NADPH:quinone reductase), CR YZ and CR YZL1 in humans
	π	Glyceraldehyde 3-phosphate dehydrogenase
	ι	(Cellular retinol-binding protein)
	η	Cytoplasmic aldehyde dehydrogenase
	λ	(Hydroxyacyl CoA dehydrogenase)
	μ	(Ornithine cyclodeaminase) CRYM in humans
	BHMT	Betaine–homocysteine methyltransferase (BHMT2)
Many species	τ	α -Enolase
Frogs	ρ	(NADPH-dependent reductases)
Cephalopods	SL11/LOPS4	Glutathione <i>S</i> -transferase
	S	(Glutathione <i>S</i> -transferase)
	Ω/L	(Aldehyde dehydrogenase)
Jellyfish	J	(Novel proteins)