

Protein misfolding and aggregation in cataract disease and prospects for prevention

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The transparency of the eye lens depends on maintaining the native tertiary structures and solubility of the lens crystallin proteins over a lifetime. Cataract, the leading cause of blindness worldwide, is caused by protein aggregation within the protected lens environment. With age, covalent protein damage accumulates through pathways thought to include UV radiation, oxidation, deamidation, and truncations. Experiments suggest that the resulting protein destabilization leads to partially unfolded, aggregation-prone intermediates and the formation of insoluble, light-scattering protein aggregates. These aggregates either include or overwhelm the protein chaperone content of the lens. Here, we review the causes of cataract and nonsurgical methods being investigated to inhibit or delay cataract development, including natural product-based therapies, modulators of oxidation, and protein aggregation inhibitors.

The eye lens and cataract disease

The ocular lens is responsible for the fine focusing of light onto the retina, and its transparency is vital for visual acuity (Figure 1a). The lens anterior is lined with a single layer of epithelial cells, overlaying the lens cortex and nucleus, both of which are composed of elongated fiber cells. The epithelial cells maintain metabolic activity and undergo mitosis to produce daughter cells that migrate to the lens equator where they begin differentiating into fiber cells [1]. The differentiating cells elongate to yield long, thin, ribbon-like structures that form the onion-like layers of the lens. During this time major intracellular changes occur, including very high expression of soluble crystallin proteins followed by organelle degradation [1].

The center of the lens, known as the nucleus, contains terminally differentiated fiber cells of which the innermost are formed *in utero*. The outer layers of fiber cells, known as the lens cortex, surround the nucleus and maintain some level of protein turnover and metabolic activity. The development and structure of the lens is such that it contains some of the oldest cells and proteins in the entire body that must maintain their molecular structure and organization over an entire lifetime.

To enable sight, the lens must remain transparent to visible light and numerous strategies have evolved to reduce or remove light-scattering structures from the tissue. The

lens is avascular with no arterial or venous circulation. Fiber cells are organized to compact membranes and reduce intercellular space [2]. Coordinated organelle degradation is initiated during fiber cell maturation to remove nuclei, mitochondria, endoplasmic reticulum (ER), ribosomes, and other organelles, which reduces light scattering [3]. Crystallin protein expression is highly upregulated during differentiation, resulting in the crystallins comprising 90% of protein in the mature lens. Short-range ordered packing of the crystallins at concentrations of 250–400 mg/mL contributes to the transparency of the concentrated solution and a polydisperse mixture of crystallins avoids crystallization [4,5]. Because the lens is formed *in utero* and mature fiber cells lack protein synthesis and degradation machinery necessary for removing and replacing damaged proteins, a major requirement of the crystallins is superior solubility and long-term stability of their native conformations.

Members of the α - and $\beta\gamma$ -crystallin families are the major soluble proteins of the lens (Figure 1b–d and Table 1). The β - and γ -crystallins comprise duplicated domains that share double Greek key β -sheet protein folds. The γ C-, γ D-, and γ S-crystallins are monomeric, whereas the homologous β A- and β B-crystallin family members form oligomers. The atomic structures of many of these proteins have been solved by Slingsby and colleagues [6–8]. The coexpression of these closely related but not identical proteins may be a mechanism to inhibit crystallization at the high protein concentrations that occur in the lens.

α -Crystallin, a polydisperse protein complex composed of α A and α B subunits, is a member of the small heat shock protein family. It is an ATP-independent chaperone that efficiently binds to damaged or partially unfolded proteins, sequestering them to prevent widespread protein aggregation. The heterogeneous complexes contain 10–15 dimers organized into hollow, spherical assemblies (Figure 1b). Partial structures of the subunits have recently been determined [9,10].

Cataract, which is the opacification of the lens, results in the scattering of visible light as it passes through the lens and subsequently degrades visual acuity. Severe cataracts interfere with a wide range of tasks and lead to increases in automobile accidents, falls, and social problems. Cataract incidence in the US population remains very low in youth and mid-adulthood but increases sharply beginning around age 50 years (Figure 2) [11].

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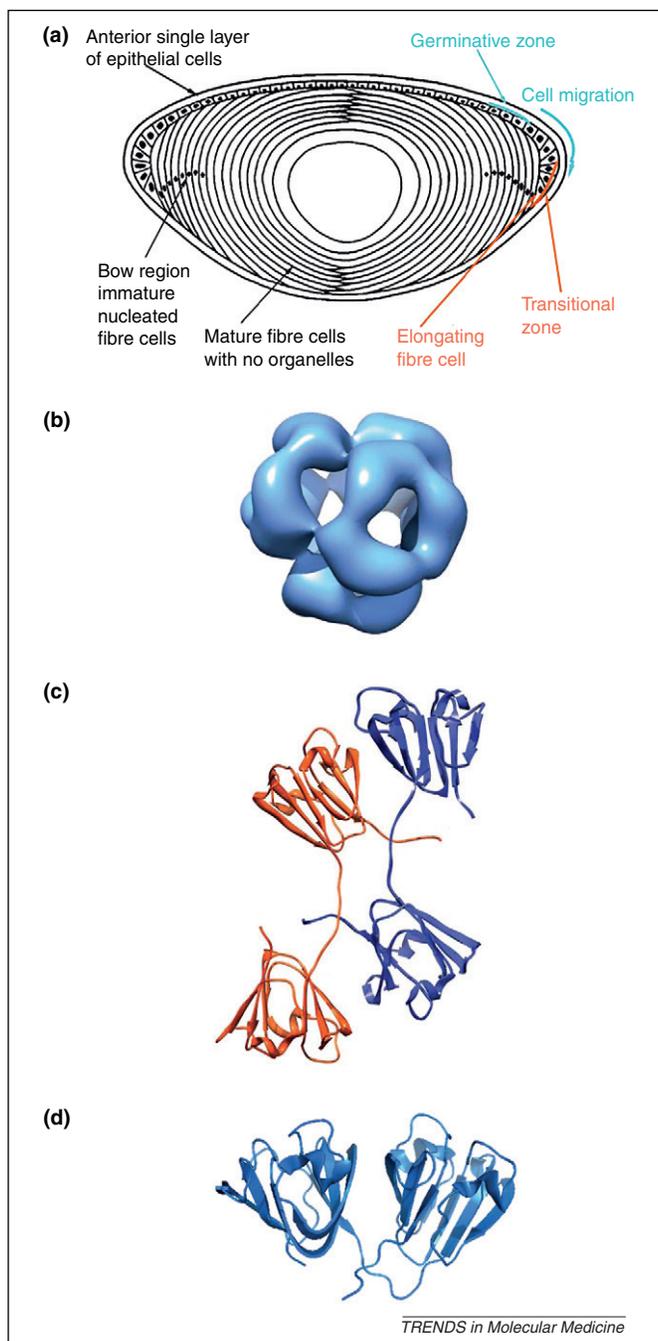


Figure 1. Structure of the lens and its major soluble proteins. (a) Cross-section of the human lens highlighting the epithelial layer, immature and mature fiber cells. Reprinted with permission from [95]. Structures of the major soluble crystallins in human lenses: (b) cryo-electron microscopy (cryo-EM) structure of human α B-crystallin representative of the α -crystallin small heat shock protein (EMD-1776) [115]; (c) crystal structure of human β B2-crystallin representative of the β -crystallins (PDB ID 1YTQ) [116]; and (d) crystal structure of human γ D-crystallin representative of the γ -crystallins (PDB ID 1HK0) [6]. Structures depicted in (b–d) were created using the program Chimera (<http://www.cgl.ucsf.edu/chimera>).

There are currently no widespread pharmacological treatments to prevent cataract or delay its onset. In the US alone, an estimated \$6 billion is spent each year on cataract surgery, the primary treatment, and related medical expenses [12]. Cataract surgery involves removing the opaque lens and replacing it with an artificial intraocular lens implant that is placed inside the natural lens capsule, which is not removed. Cataract surgery has a high success rate; however, this procedure is not without risk with

Table 1. The major crystallins present in the human lens

Protein	Size (Da)	Residues	ΔG^a	Gene	Chromosomal location	Refs ^b
α A	19 909	173	27	CRYAA	21q22.3	[86]
α B	20 159	175	21	CRYAB	11q23.1	[86]
β A1	23 191	198	–	CRYBA1	17q11.2	
β A2 ^c	21 964	196	–	CRYBA2	2q35	[113]
β A3	25 150	215	58	CRYBA1 ^d	17q11.2	[114]
β A4	22 243	195	–	CRYBA4	22q12.1	
β B1	27 892	251	67	CRYBB1	22q12.1	[86]
β B2	23 249	204	49	CRYBB2	22q11.23	[86]
β B3	24 230	211	–	CRYBB3	22q11.23	
γ C	20 747	173	36	CRYGC	2q33.3	[86]
γ D	20 607	173	69.4	CRYGD	2q33.3	[86]
γ S	20 875	177	43.9	CRYGS	3q27.3	[86]

^a ΔG given in kJ/mol calculated from thermal or chemical denaturation.

^bReferences refer to source of ΔG values, except in the case of β A2.

^cNo protein detection in newborn human lenses.

^d β A3-crystallin is translated from an initiation codon upstream from that of β A1.

posterior capsular opacification the most common complication [13]. This condition results from the ectopic growth of remaining epithelial cells on the posterior lens capsule. They eventually invade the visual axis, causing the capsule to wrinkle, which in turn scatters light. In many cases a second procedure is required to restore vision.

Cataract prevalence is increasing in the developing world. In India, for example, significantly more people develop cataract at an earlier age than in the US [14]. The INDEYE Feasibility Study conducted in several villages in Northern India found 57% of individuals over age 50 years were affected with cataract [15], and these findings are in agreement with an earlier study of South Indian populations [16]. Surgery is often not an option due to the lack of accessible facilities, doctors, or funds. Although not directly life-threatening, cataract disease has major medical, economic, and social impacts on individuals, families, and society as a whole.

As the world population ages and lifespans increase, nonsurgical solutions are needed to prevent or delay cataract formation. A seemingly modest 10-year delay in age of onset could reduce surgeries and associated costs by 50% [14]. Additionally, such treatments could reduce both stress on affected individuals and families and accidents caused by impaired vision. Development of these therapies would benefit from a deeper understanding of the mechanism of cataract formation.

Molecular mechanisms of cataract formation

Characterization of the material removed from cataractous lenses reveals multiple species of lens proteins, many of which comprise high molecular weight forms that require denaturation by sodium dodecyl sulfate, urea, or guanidinium hydrochloride for solubilization. We will refer to this state as 'aggregated'. The aggregation or polymerization of lens proteins into high molecular weight complexes accounts for the light scattering and opacity of cataractous lenses and the loss of visual acuity. Unlike other protein aggregation diseases, such as Alzheimer's, Huntington's, or Parkinson's, in which the pathology may be due to soluble oligomers that precede higher molecular weight

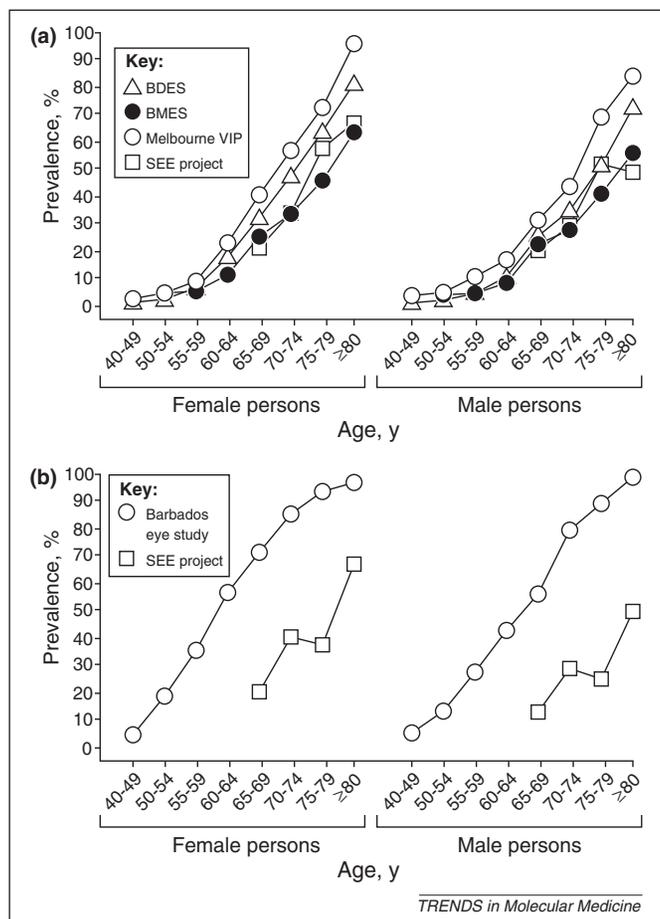


Figure 2. The prevalence of cataract in male and female populations. (a) Prevalence (per 100 people) in white individuals derived from four population-based studies. BDES, Beaver Dam Eye Study; BMES, Blue Mountains Eye Study; Melbourne VIP, Melbourne Visual Impairment Project; SEE Project, Salisbury Eye Evaluation Project. (b) Prevalence (per 100 people) in black individuals from two population-based studies. Reproduced with permission from [11].

aggregates and plaque formation, in the case of cataract the higher order aggregated state can account for the pathology.

Proteomic analyses of lens proteins have identified several covalent modifications that are associated with damage, including deamidation, oxidation, glycation, and truncation [17–21]. Deamidation is one of the most prevalent damages to the crystallins [22], introducing a negative charge to the protein by transforming glutamine residues to glutamate. Asparagine is also susceptible to deamidation, and both residues are modified in cataractous aggregates [23]. Several oxidation sites have been identified on the crystallins, targeting tryptophan, cysteine, and methionine residues. Hains and Truscott found that Trp oxidation was increased in cataractous lenses at several sites [17]. In the case of γ -crystallins, the four conserved tryptophans are all within the buried core and their oxidation to the less aromatic kynurenine might significantly destabilize the native state. Specific cysteine residues on β/γ -crystallins were found to be oxidized in aged cataractous lenses [18]. Up to 50% of methionine residues have been found to be oxidized in advanced stage cataract [24] and residue-specific identification was achieved for the water-insoluble fraction of aged normal

lenses [25]. This suggests that Met oxidation may precede the large-scale aggregation of cataract, although to some extent it may be part of the normal aging process.

It seems reasonable that some of this covalent damage destabilizes the native state of the lens proteins, leading to aggregation. Lampi and coworkers found that deamidation reduced the stability of both β A3- and β B1-crystallin and increased their aggregation propensity compared with the wild-type proteins [26–28]. Deamidation in the domain interface decreased both the thermodynamic and kinetic stability of human γ D-crystallin, suggesting that this covalent change may contribute to protein unfolding and aggregation [29].

Truncated proteins and peptide segments from crystallins have also been identified in water-insoluble fractions from cataractous lenses [25]. These truncation events may cause more pronounced protein destabilization, as suggested by experiments demonstrating that the presence of an oxidized crystallin peptide increased aggregation of both β - and γ -crystallins [30,31]. Studies on the properties of peptides isolated from lenses show that they may also negatively impact the chaperone function of α -crystallin [32,33]. Because the aggregated state in many cases is formed from a partially unfolded crystallin protein, these molecules may be particularly susceptible to covalent damage. Determining which insults are the source of protein destabilization and which are consequences is an important problem needing attention.

Destabilization resulting from the lifelong accumulation of covalent modifications/damages may drive partial protein unfolding, leading to the formation of intermediate conformations that expose previously buried hydrophobic residues. Protein unfolding within the lens environment may arise from diverse damages, resulting in a variety of perturbed conformations. Extensive unfolding may occur, such as that depicted in Figures 3 and 4, but it is equally likely that covalent modifications and damages could promote local unfolding or exposure of hydrophobic patches. The nature of polypeptide chain conformations within cataractous aggregates is not known.

α -Crystallin, the major chaperone system of the mature lens fiber cells, recognizes exposed conformational features in partially unfolded species and sequesters these misfolded/unfolded conformers from one another [34], reducing the availability of aggregation-prone species. As the finite α -crystallin population is saturated with damaged, unfolded β/γ -crystallins, it too contributes to the growing aggregates and eventually to light scattering [35,36]. With increasing age, α -crystallin is lost from the soluble fraction of lens proteins [37]. Without active α -crystallin, further damaged protein may no longer be protected from aggregation. Additionally, some modifications and mutations might alter or reduce recognition of perturbed conformations by α -crystallins, possibly rendering these proteins ‘invisible’ to the chaperone (K.L. Moreau and J.A. King, unpublished).

Cataracts have diverse morphologies, including nuclear and cortical. At present, epidemiological evidence does not resolve whether these morphologies represent different etiologies or biochemical processes. Thus, we consider them collectively in the discussion below. In this review, we treat

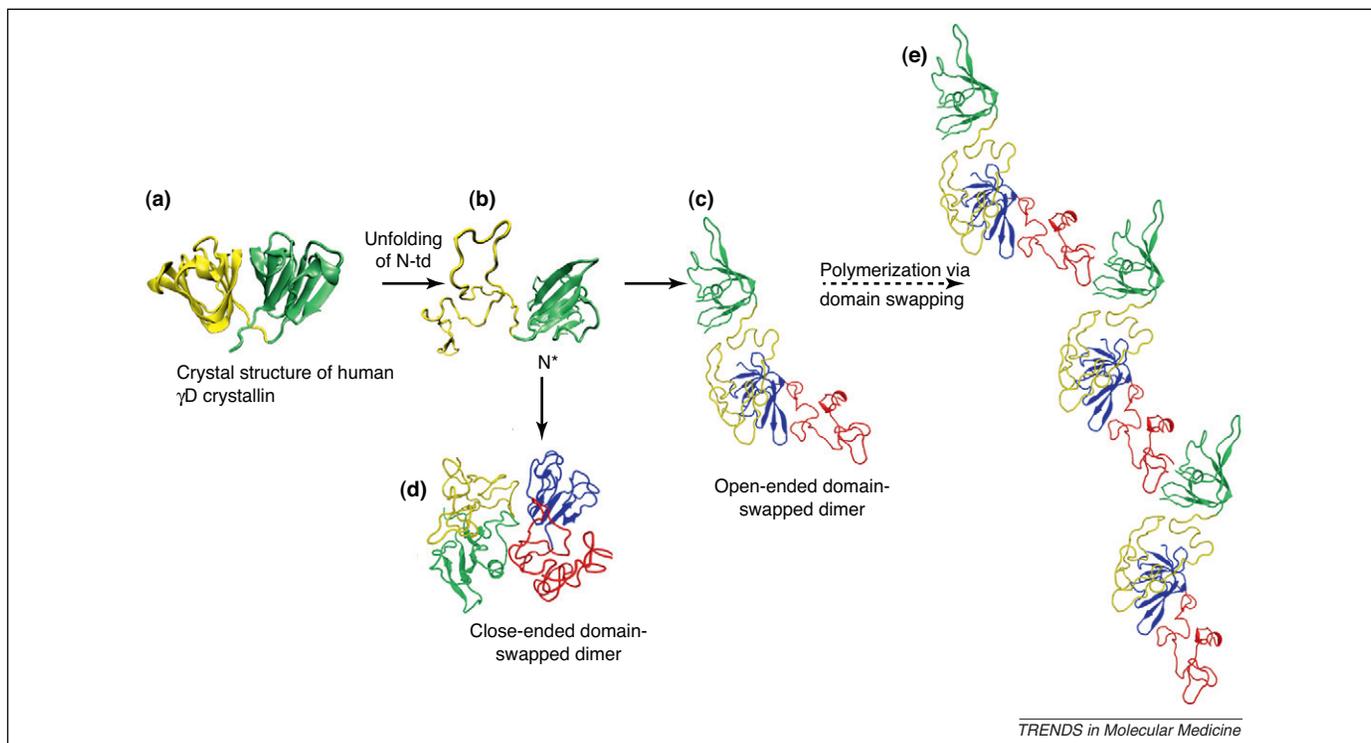


Figure 3. Computational simulation of human γ D-crystallin polymerization. (a) Crystal structure of human γ D-crystallin. (b) Simulated monomeric aggregation precursor, often referred to as N* in the general mechanism of protein aggregation in the literature. (c) Simulated structure of open-ended domain-swapped dimer. (d) Simulated structure of close-ended domain-swapped dimer. (e) Model of human γ D-crystallin hexamer formed via domain swapping. Figure and legend reproduced with permission from [57].

age-related cataract as a protein aggregation/deposition disease based on the loss of crystallin stability and their subsequent propensity to partially or fully unfold. In this model, a population of partially unfolded, non-native molecules, perhaps resulting from accumulated covalent damages, aggregates, resulting in refractive index changes, light scatter, and impaired vision. The initial insult may not be directed at the crystallins but at systems that maintain the redox, ionic, and other aspects of the physiological environment of the lens. Below we discuss possible etiologic agents, including UV radiation, reactive oxygen species within the lens, heavy metals, and reactive sugars.

A second model hinges on the solubility of the crystallins in their native state, as opposed to stability and conformational changes induced by damage. Loss of solubility and precipitation due to aberrant interactions of the native crystallins leads to separation into protein-rich and protein-poor regions within the lens [38]. This phase separation disrupts the short-range order, resulting in pockets of varying refractive index and light scatter. This model is supported by the fact that altered solubility and crystallization propensity cause hereditary cataract in certain cases [39–42], and phase separation as a cause of cataract has been reviewed [38]. However, phase separation cannot account for the irreversible association of the crystallin aggregates recovered from cataractous tissue.

Although some pathologies reflect a polymerized form of the native state of a protein, such as sickle cell disease, off-pathway aggregation reactions generally derive from protein conformations that are either partially or completely unfolded [43]. Examples include prion diseases [44], the amyloid fibrils formed from transthyretin intermediates [45], and the ER aggregates in the liver formed from

α_1 -antitrypsin [46]. Similarly, the amyloid fibers formed in Alzheimer's, Parkinson's, and Huntington's diseases derive from precursor conformations that are perturbed with regard to their functional conformation [44]. X-ray crystallography, nuclear magnetic resonance (NMR), and infrared (IR) studies have established that the repeating units in amyloid fibers have the same conformation [47–49]. In cases where such structures have not been determined, there is a tendency to assume that the resulting polymer is not the product of repeating interactions. In fact, it is much more likely that the interactions are specific and repeating, as in domain-swapping or loop/sheet insertion models [50]. Additional structural studies are required to reveal the underlying polypeptide chain conformations and interactions within the aggregated cataractous state.

An important difference between cataractous aggregates and those present in other aggregation diseases is their microscopic morphology. In general, large aggregates, such as extracellular amyloid plaques in the brains of Alzheimer's patients and Lewy bodies in neurons of Parkinson's patients, can be readily observed by appropriate light or electron microscopy techniques. By contrast, such aggregates are not readily visible in cataractous human lenses, although a textured cytoplasm was distinguishable and determined to be adequate to scatter light [51]. Similar findings were reported in the OXYS rat (a strain exhibiting increased oxidative stress), where the cytoplasm appeared highly textured with both globular and fibrillar patterns [52].

The detailed mechanism of the protein aggregation pathway within the lens is difficult to elucidate, owing to a lack of cell culture techniques for lens fiber cells and the necessarily end-point observations on cataractous

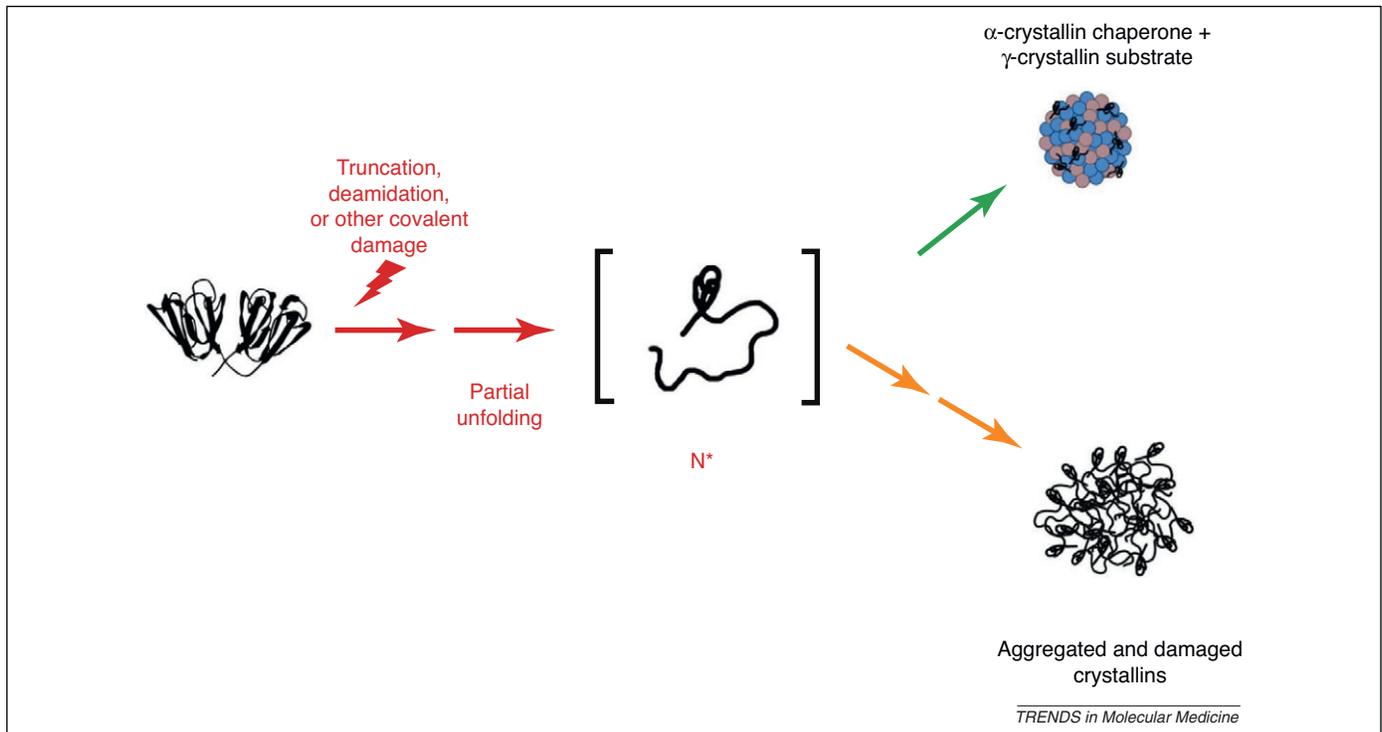


Figure 4. Cataract viewed as a protein aggregation disease. Crystallin proteins, particularly in the lens nucleus, are present from birth and over time accumulate covalent modifications and damages resulting from proteolytic activity, nonenzymatic modifications, and oxidative damage. This leads to destabilization and partial unfolding of the polypeptide chains and a population of aggregation-prone intermediates. In young lenses, α -crystallin effectively recognizes and sequesters these destabilized intermediates (upper pathway). However, with age α -crystallin complexes become saturated with substrate and lens proteins are able to aggregate, resulting in light scatter and loss of visual acuity (lower pathway). Reprinted with permission from [54].

lenses. The crystallins are extraordinarily soluble and do not aggregate from their native states. Kosinski-Collins and King [53] described the *in vitro* aggregation of partially folded human γ D-crystallin, one of the major γ -crystallins in the lens nucleus. Upon denaturation in 5 M guanidinium hydrochloride (GuHCl) and subsequent dilution with buffer to initiate refolding, a robust aggregation reaction competed with productive refolding of the protein. The reaction occurred within minutes after dilution at 37 °C and over hours formed large filamentous aggregates visualized by atomic force microscopy. The aggregates had Trp fluorescence emission spectra that were intermediate between the native and denatured states, indicating that they may be composed of partially folded polypeptide chains [53].

These experiments have identified a partially folded precursor in which the C-terminal domain (C-td) is native-like but the N-terminal domain (N-td) is not fully folded [53]. Under these conditions, the aggregation-prone regions appeared to be within the C-td [54]. Furthermore, the isolated, aggregation-prone C-td of human γ D-crystallin can recruit the non-aggregating isolated N-td into off-pathway aggregates (L. Acosta-Sampson and J. King, unpublished). Not surprisingly, this partially folded precursor is a substrate of α -crystallin [54]. Recently, a similar *in vitro* aggregation reaction was observed for β A3-crystallin, and refolding in the presence of β B1-crystallin diverted protein molecules off the aggregation pathway [55]. This is in agreement with earlier studies on the thermal denaturation of β A3-crystallin [56].

Although direct determination of the conformation in the aggregated state has been elusive, molecular dynamics

calculations by Zhou and colleagues of the IBM supercomputing group suggest a domain-swapped interaction between partially unfolded crystallin chains [57]. This is illustrated in Figure 3. For γ - and β -crystallins, which all have two homologous domains, such an interaction could propagate into a large polymeric structure.

Another aggregation pathway for human γ D- and γ C-crystallins is the formation of amyloid fibrils upon incubation at pH 3 [58,59]. The C-td was shown to be critical for nucleation and extension of amyloid fibrils [60]. Bovine α -, β -, and γ -crystallins also form amyloid fibrils under specific denaturing conditions and the murine γ B^{noP} mutant formed amyloid-like structures *in vivo* [61,62]. Although the bulk of the aggregated protein removed from cataractous lenses is not in the amyloid form, it remains possible that amyloid fibers participate in initiation or early stages of cataract.

Despite the fact that the molecular details governing the *in vitro* aggregation of these crystallins are unresolved, they may serve as a relevant model of the protein misfolding and aggregation observed in cataract. Figure 4 depicts an overall model of cataract formation based on a population of proteins with partially unfolded conformations that preferentially aggregate. Although this aggregation is suppressed when α -crystallin is available, this function may be lost in older adults [37].

Etiology of cataract

Congenital and juvenile cataract

There are several well-documented cases of inherited cataract that either manifest as congenital or juvenile, usually developing within the first decade of life. These may affect

protein structure and packing within fiber cells, as well as proper differentiation or organization of lens fiber cells. Although these account for a small fraction of the overall incidence of cataract, they support the biochemical evidence that the state of crystallins is important in cataract formation.

Mutations in α -, β -, and γ -crystallin genes are responsible for a large percentage of early-onset cataracts. Several mutations have been characterized, including Arg14Cys, Pro23Thr, Arg36Ser, Arg58His, and Trp156Stop in human γ D-crystallin. The four substitution mutations had various physiochemical effects, and dramatic decreases in solubility were observed without major changes to the native structure of the protein. For both surface substitutions Arg36Ser and Arg58His, an increase in crystallization propensity was observed, and the juvenile cataracts were found to be actual crystals formed in the lens [40,41]. Pande *et al.* [41] showed that the loss of surface arginine residues reduced the energy barrier for initiation of crystallization. However, this phenotype is rare, and the cataracts commonly occurring in older patients are not crystals. The introduction of a cysteine residue in the Arg14Cys substitution resulted in inappropriate intermolecular disulfide bond formation and protein precipitation [42]. The Pro23Thr mutant protein has been the subject of much research and recent NMR studies suggest local residue-specific conformational changes that may contribute to the cataract phenotype [63,64]. For the truncation Trp156Stop, the final β -strand of motif 4 and its preceding loop region are removed. Given that *in vitro* refolding probably begins with motif 4 [29,65], this mutant is likely folding deficient such that the aggregation pathway dominates. Additional experiments are in agreement with this model, and even purification from inclusion bodies yielded very little protein [66].

Mutations in the other major γ -crystallins, as well as in the α - and β -crystallins, are also associated with inherited cataract. The Thr5Pro substitution in human γ C-crystallin affected stability and crystallin–crystallin interactions [67–69]. The human γ S-crystallin mutant Gly18Val lowered the transition midpoint of unfolding in GuHCl and reduced stability under conditions of heat denaturation [70]. Certain mutations may also affect the lens on a cellular level, such as the 5 bp duplication in human γ C-crystallin that resulted in severe fiber cell defects [71] and the single substitution in mouse γ D-crystallin that resulted in abnormal fiber cell development along with extensive protein aggregation [72]. Several mutations in the β - and α -crystallins also segregate with the cataract phenotype [73]. Although not exhaustive, this list illustrates the detrimental effects that even single amino acid substitutions may have on lens structure and function.

As noted above, because successful protocols for culturing lens fibers are lacking, it is difficult to assess how closely *in vitro* aggregation models match the *in situ* aggregation process. Some confidence has come from studies of single amino acid substitutions that cause early-onset cataract in mice [72,74,75]. Introducing these substitutions into the homologous human proteins destabilized them and enhanced aggregation [76]. These experiments suggest that aggregation *in vitro* may capture features of the aggregation reactions within the lens.

In addition to the crystallin genes, mutations have been identified in a variety of genes expressing other major lens proteins. These include gap junction components, aquaporins, cytoskeletal proteins, transcription factors, and growth factors. A recent review by Churchill and Graw [73] provides a comprehensive list of cataract-causing mutations in both humans and mice. However, as noted in the next sections, the majority of human cataracts are not associated with germline mutations.

Age-related cataract

As shown in Figure 2, cataract prevalence, although very rare in youth and young adults, increases sharply with age. The etiology of age-related cataract is complex and multiple factors are likely to be involved. Mature onset cataracts are presumably formed from wild-type proteins whose conformations have been perturbed, in many cases by covalent damage. Some of the agents capable of inducing cataract have been identified in animal models and from epidemiological studies. These include heavy metals, UV radiation, and other treatments that induce the formation of reactive oxygen species. Diabetes is linked with an increased risk of early cataract development [77], by mechanisms discussed below. Although variable in phenotype and onset, studies suggest that there may be a genetic influence on the development of age-related cataract and linkage studies have identified genomic regions of interest [78].

Oxidative damage is thought to be a major contributor to cataract [24]. Given the low oxygen tension in the lens [79], the physiological source of the reactive oxygen species is not clear, although these low levels coupled with the photooxidation of UV filters may be sufficient to cause damage. High levels of glutathione protect the lens proteins from oxidation, and significant reductions of this redox molecule in the nucleus with age are thought to contribute to cataract development and progression [24]. Sodium selenite swiftly and reproducibly induces cataract formation in rats, an effect that is presumed to act at least partially by inducing oxidative damage [80]. Initial oxidative insults significantly alter lens epithelial cell function and dysregulation of calcium content and activation of calpains appear to play a significant role in the selenite cataract model [81]. Additional evidence for oxidative damage in cataract is the increased levels of lipid peroxidation. Products of lipid peroxidation are dramatically increased in selenite-induced cataract relative to normal lenses [82,83], and evidence of lipid peroxidation was observed in the OXYS rat [52]. As described in the next section, there is evidence that various forms of antioxidant treatment may be efficacious in preventing or delaying the onset of cataract disease.

Epidemiological research suggests that UV light, as well as other electromagnetic radiation, is associated with cataract formation [84–86]. An increase in oxidizing species may occur as a result of UV irradiation [87,88]. Although the cornea is the major absorber of UV radiation reaching the eye, UVA and the longest wavelengths of UVB radiation are absorbed by the lens and have been shown to damage lens proteins both *in vivo* and *in vitro* [87,88]. Small molecule UV filters, such as 3-hydroxykynurenine,

confer protection upon lens proteins [89] and the crystallins themselves possess mechanisms to efficiently disperse absorbed energy [90,91]. Reduced availability of free UV filters may decrease protection, and UV exposure may actually cause these small molecules to act as sensitizers that enhance protein damage [89]. Analyses of irradiated guinea pig lenses suggest that photooxidation may be responsible for a variety of deleterious effects on the lens nucleus, including loss of water-soluble protein, protein disulfide bond formation, and cytoskeletal and membrane alterations [87]. In addition to UV radiation, occupational exposure to intense light and heat is highly linked to glassblower's cataract, an observation confirmed by animal studies [86].

Dysregulation of calcium ion content within the lens is also implicated in cataract formation via activation of calpain proteases. Although they play a role in normal lens development and function [92,93], their inappropriate over-activation could lead to cataract formation. Calpain 2, in particular, is strongly suggested to play a role in some forms of human cataract [93]. Selenite-treated animals that rapidly develop nuclear cataract were shown to have elevated intracellular calcium levels compared with untreated controls [94], and this in turn could activate calpains.

Premature cataract development is one of the earliest secondary complications of diabetes. The uncontrolled increase of glucose levels in the lens can lead directly to protein glycation, which is detrimental to α -crystallin chaperone activity [95]. High glucose concentrations also activate the polyol pathway [77]. Activation of aldose reductase, the first enzyme of this pathway, results in production of high levels of sorbitol which alters tonicity in the lens, leading to the swelling and disruption of fiber cells. The altered lens environment may also increase free radical formation and further oxidative damage [77]. Although aldose reductase seems to be an attractive candidate for small molecule inhibition, several compounds have produced discouraging results in clinical trials, and side effects and toxicity posed major problems. There are currently no trials of aldose reductase inhibitors aimed at cataract prevention or treatment registered with the US FDA.

Prevention or retardation of cataract disease

Estimates of future cataract surgeries are projected to be 20–25 million by the year 2020 [96]. Although cataract prevention would be ideal, treatments that could retard cataract initiation or growth would have a broad impact, both socially and economically. Because some species of small molecules can reach the lens using ophthalmic solutions, some of the complexities that hinder conventional therapeutic development might be side-stepped.

An active area of investigation is in the use of phytochemical antioxidant treatments in the form of both crude plant extracts and single isolated compounds. The flavonoid fraction of broccoli was found to reduce the severity of selenite cataract in rats, maintaining antioxidant activity and calcium ion content at near normal levels [97]. Treatment with the flavonoid fraction of the shrub *Vitex negundo* prevented cataract formation in the majority of selenite-treated animals [82]. Intraperitoneal injections of aqueous garlic extract had a similar effect on cataract formation

[98]. Curcumin, a phenolic compound derived from turmeric, also has anticataract activity, but only when administered before sodium selenite [99]. Tetramethylpyrazine, purified from the Chinese herbal medicine *Ligusticum wallichii franchet*, reduced the severity of selenite cataract and restores the activity of free-radical scavenging enzymes [94]. The possibility of antioxidant flavonoid-based therapies for diabetic cataract in particular has been recently reviewed [100].

Other small molecules such as aspirin and pantethine have been shown to inhibit cataract formation both *in vitro* and *in vivo*, although data from clinical trials have been less favorable. Aspirin is believed to acetylate residues prone to glycation; however, data obtained from clinical trials were inconclusive [101]. Similarly, pantethine lowered the phase separation temperature of lens protein homogenates and delayed cataract formation in animal models, but clinical trials were unsuccessful [101]. The dipeptide N-acetyl carnosine was reported to improve visual acuity and glare sensitivity in human subjects diagnosed with various stages of cataract disease [102]. It is available as a topical ophthalmic supplement, despite lacking US FDA approval, and there is conflicting evidence for its function [103]. Varma and colleagues found that caffeine maintained the transparency of lenses from selenite-treated animals [104] and reduced both lens opacity and damage to ion pumps caused by UV-B radiation [105]. Two small molecule compounds, each with the dual functions of chelating redox metals and quenching free radicals, were shown to delay cataract formation in both diabetic and γ -irradiated rats [106]. It should be noted that many of the molecules and extracts described above may act on the epithelial cell layer, inhibiting initial events whose cascade results in cataract. A number of small molecule inhibitors of aldose reductase and calpains have been investigated as treatments for diabetic and other cataract cases, respectively [77,93].

A complementary target for anticataract drugs may be inhibition of the protein aggregation pathway that stems from destabilized, partially unfolded polypeptide chains. Recent studies have shown that plant-derived small molecules including curcumin (described above), and (–)epigallocatechin 3-galate, inhibited amyloid formation and effectively disaggregated preformed amyloid fibers [107,108]. Gong and colleagues described the chemical chaperone activity of sodium 4-phenylbutyrate and its ability to rescue the insolubility phenotype of truncated human γ D-crystallin in cell culture [109]. Sodium citrate stabilized wild-type and mutant human γ D-crystallin in the presence of denaturant, suggesting that it bound an intermediate along the unfolding pathway [110].

Our laboratory has recently undertaken a small molecule screen to identify inhibitors of the aggregation pathway that competes with productive refolding of human γ D-crystallin, characterized by Kosinski-Collins and King [53]. Screening libraries include known bioactives, commercially available small molecules, and natural product extracts. Active inhibitors may function by blocking protein addition to the growing aggregate, disaggregating preformed species, or stabilizing refolding-competent conformations (or the native state) of crystallins.

In addition to direct interference with the protein aggregation pathway, a second strategy is to modulate chaperone activity to promote active protein refolding. Whereas the chaperone content of the lens nucleus is mainly α -crystallin, which does not actively refold protein substrates, the lens cortex and epithelium contain ATP-dependent chaperones including the group II chaperonin CCT. CCT is required for the productive folding of both actin and tubulin, and its archaeal homolog Mm-cpn binds and actively refolds human γ D-crystallin *in vitro* [111]. Small molecule modulation of chaperonin activity in the lens cortex may be a viable option to prevent cortical cataract, which comprises approximately 20% of senile cataract cases [112]. A similar screen to that described above could identify molecules with the proposed activity.

Concluding remarks and future perspectives

The factors that contribute to cataract disease are complex and still under investigation. Pharmacological treatments to delay or prevent cataract development and subsequent blindness would result in increased quality of life as well as reduced economic burdens. The combined efforts of the *in vitro* study of protein modifications and aggregation pathways associated with cataract and the *in vivo* evaluation of small molecule therapeutics in animal models of cataract will certainly uncover novel therapeutic possibilities.

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References

- Augusteyn, R.C. (2010) On the growth and internal structure of the human lens. *Exp. Eye Res.* 90, 643–654
- Michael, R. *et al.* (2003) Changes in the refractive index of lens fibre membranes during maturation – impact on lens transparency. *Exp. Eye Res.* 77, 93–99
- Bassnett, S. (2002) Lens organelle degradation. *Exp. Eye Res.* 74, 1–6
- Delaye, M. and Tardieu, A. (1983) Short-range order of crystallin proteins accounts for eye lens transparency. *Nature* 302, 415–417
- Oyster, C.W. (ed.) (1999) *The Human Eye: Structure and Function*, Sinauer Associates, Inc.
- Basak, A. *et al.* (2003) High-resolution X-ray crystal structures of human gammaD crystallin (1.25 Å) and the R58H mutant (1.15 Å) associated with aculeiform cataract. *J. Mol. Biol.* 328, 1137–1147
- Purkiss, A.G. *et al.* (2002) The X-ray crystal structure of human gamma S-crystallin C-terminal domain. *J. Biol. Chem.* 277, 4199–4205
- Van Montfort, R.L. *et al.* (2003) Crystal structure of truncated human betaB1-crystallin. *Protein Sci.* 12, 2606–2612
- Bagneris, C. *et al.* (2009) Crystal structures of alpha-crystallin domain dimers of alphaB-crystallin and Hsp20. *J. Mol. Biol.* 392, 1242–1252
- Laganowsky, A. *et al.* (2010) Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function. *Protein Sci.* 19, 1031–1043
- Congdon, N. *et al.* (2004) Prevalence of cataract and pseudophakia/aphakia among adults in the United States. *Arch. Ophthalmol.* 122, 487–494
- Prevent Blindness America, N.E.I. (2008) *Vision Problems in the U.S.: Prevalence of Adult Vision Impairment and Age-related Eye Disease in America*, (update to the 4th edn), Published in conjunction with the National Eye Institute, pp. 22–25
- Ashwin, P.T. *et al.* (2009) Advances in cataract surgery. *Clin. Exp. Optom.* 92, 333–342
- Brian, G. and Taylor, H. (2001) Cataract blindness – challenges for the 21st century. *Bull. World Health Organ.* 79, 249–256
- Murthy, G.V. *et al.* (2007) Prevalence of lens opacities in North India: the INDEYE feasibility study. *Invest. Ophthalmol. Vis. Sci.* 48, 88–95
- Nirmalan, P.K. *et al.* (2003) Lens opacities in a rural population of southern India: the Aravind Comprehensive Eye Study. *Invest. Ophthalmol. Vis. Sci.* 44, 4639–4643
- Hains, P.G. and Truscott, R.J. (2007) Post-translational modifications in the nuclear region of young, aged, and cataract human lenses. *J. Proteome Res.* 6, 3935–3943
- Hains, P.G. and Truscott, R.J. (2008) Proteomic analysis of the oxidation of cysteine residues in human age-related nuclear cataract lenses. *Biochim. Biophys. Acta* 1784, 1959–1964
- Lampi, K.J. *et al.* (1998) Age-related changes in human lens crystallins identified by two-dimensional electrophoresis and mass spectrometry. *Exp. Eye Res.* 67, 31–43
- Ma, Z. *et al.* (1998) Age-related changes in human lens crystallins identified by HPLC and mass spectrometry. *Exp. Eye Res.* 67, 21–30
- Zhang, Z. *et al.* (2003) Human beta-crystallins modified by backbone cleavage, deamidation and oxidation are prone to associate. *Exp. Eye Res.* 77, 259–272
- Wilmarth, P.A. *et al.* (2006) Age-related changes in human crystallins determined from comparative analysis of post-translational modifications in young and aged lens: does deamidation contribute to crystallin insolubility? *J. Proteome Res.* 5, 2554–2566
- Hains, P.G. and Truscott, R.J. (2010) Age-dependent deamidation of lifelong proteins in the human lens. *Invest. Ophthalmol. Vis. Sci.* 51, 3107–3114
- Truscott, R.J. (2005) Age-related nuclear cataract-oxidation is the key. *Exp. Eye Res.* 80, 709–725
- Hanson, S.R. *et al.* (2000) The major *in vivo* modifications of the human water-insoluble lens crystallins are disulfide bonds, deamidation, methionine oxidation and backbone cleavage. *Exp. Eye Res.* 71, 195–207
- Kim, Y.H. *et al.* (2002) Deamidation, but not truncation, decreases the urea stability of a lens structural protein, betaB1-crystallin. *Biochemistry* 41, 14076–14084
- Lampi, K.J. *et al.* (2006) Deamidation in human lens betaB2-crystallin destabilizes the dimer. *Biochemistry* 45, 3146–3153
- Takata, T. *et al.* (2008) Deamidation destabilizes and triggers aggregation of a lens protein, betaA3-crystallin. *Protein Sci.* 17, 1565–1575
- Flaugh, S.L. *et al.* (2006) Glutamine deamidation destabilizes human gammaD-crystallin and lowers the kinetic barrier to unfolding. *J. Biol. Chem.* 281, 30782–30793
- Udupa, E.G. and Sharma, K.K. (2005) Effect of oxidized betaB3-crystallin peptide on lens betaL-crystallin: interaction with betaB2-crystallin. *Invest. Ophthalmol. Vis. Sci.* 46, 2514–2521
- Udupa, P.E. and Sharma, K.K. (2005) Effect of oxidized betaB3-crystallin peptide (152–166) on thermal aggregation of bovine lens gamma-crystallins: identification of peptide interacting sites. *Exp. Eye Res.* 80, 185–196
- Santhoshkumar, P. *et al.* (2011) α A-Crystallin peptide SDRDKFVIFLDVKHF accumulating in aging lens impairs the function of alpha-crystallin and induces lens protein aggregation. *PLoS ONE* 6, e19291
- Santhoshkumar, P. *et al.* (2008) Significance of interactions of low molecular weight crystallin fragments in lens aging and cataract formation. *J. Biol. Chem.* 283, 8477–8485
- Bloemendal, H. *et al.* (2004) Ageing and vision: structure, stability and function of lens crystallins. *Prog. Biophys. Mol. Biol.* 86, 407–485
- Carver, J.A. *et al.* (1996) Age-related changes in bovine alpha-crystallin and high-molecular-weight protein. *Exp. Eye Res.* 63, 639–647
- Horwitz, J. (1993) The function of alpha-crystallin. *Invest. Ophthalmol. Vis. Sci.* 34, 10–22
- Heys, K.R. *et al.* (2007) Presbyopia and heat: changes associated with aging of the human lens suggest a functional role for the small heat shock protein, alpha-crystallin, in maintaining lens flexibility. *Aging Cell* 6, 807–815
- Benedek, G.B. (1997) Cataract as a protein condensation disease: the Proctor Lecture. *Invest. Ophthalmol. Vis. Sci.* 38, 1911–1921
- Evans, P. *et al.* (2004) The P23T cataract mutation causes loss of solubility of folded gammaD-crystallin. *J. Mol. Biol.* 343, 435–444

- 40 Kmoch, S. *et al.* (2000) Link between a novel human gammaD-crystallin allele and a unique cataract phenotype explained by protein crystallography. *Hum. Mol. Genet.* 9, 1779–1786
- 41 Pande, A. *et al.* (2001) Crystal cataracts: human genetic cataract caused by protein crystallization. *Proc. Natl. Acad. Sci. U.S.A.* 98, 6116–6120
- 42 Pande, A. *et al.* (2000) Molecular basis of a progressive juvenile-onset hereditary cataract. *Proc. Natl. Acad. Sci. U.S.A.* 97, 1993–1998
- 43 Jaenicke, R. and Seckler, R. (1997) Protein misassembly in vitro. *Adv. Protein Chem.* 50, 1–59
- 44 Moreno-Gonzalez, I. and Soto, C. (2011) Misfolded protein aggregates: mechanisms, structures and potential for disease transmission. *Semin. Cell Dev. Biol.* 22, 482–487
- 45 Colon, W. and Kelly, J.W. (1992) Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. *Biochemistry* 31, 8654–8660
- 46 Lomas, D.A. *et al.* (1992) The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature* 357, 605–607
- 47 Bayro, M.J. *et al.* (2010) High-resolution MAS NMR analysis of PI3-SH3 amyloid fibrils: backbone conformation and implications for protofilament assembly and structure. *Biochemistry* 49, 7474–7484
- 48 Nelson, R. and Eisenberg, D. (2006) Recent atomic models of amyloid fibril structure. *Curr. Opin. Struct. Biol.* 16, 260–265
- 49 Petty, S.A. and Decatur, S.M. (2005) Intersheet rearrangement of polypeptides during nucleation of [beta]-sheet aggregates. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14272–14277
- 50 Bennett, M.J. *et al.* (2006) Deposition diseases and 3D domain swapping. *Structure* 14, 811–824
- 51 Metlapally, S. *et al.* (2008) Analysis of nuclear fiber cell cytoplasmic texture in advanced cataractous lenses from Indian subjects using Debye-Bueche theory. *Exp. Eye Res.* 86, 434–444
- 52 Marsili, S. *et al.* (2004) Cataract formation in a strain of rats selected for high oxidative stress. *Exp. Eye Res.* 79, 595–612
- 53 Kosinski-Collins, M.S. and King, J. (2003) In vitro unfolding, refolding, and polymerization of human gammaD crystallin, a protein involved in cataract formation. *Protein Sci.* 12, 480–490
- 54 Acosta-Sampson, L. and King, J. (2010) Partially folded aggregation intermediates of human gammaD-, gammaC-, and gammaS-crystallin are recognized and bound by human alphaB-crystallin chaperone. *J. Mol. Biol.* 401, 134–152
- 55 Wang, S. *et al.* (2011) The benefits of being beta-crystallin heteromers: betaB1-crystallin protects betaA3-crystallin against aggregation during co-refolding. *Biochemistry* 50, 10451–10461
- 56 Takata, T. *et al.* (2009) Deamidation alters interactions of beta-crystallins in hetero-oligomers. *Mol. Vis.* 15, 241–249
- 57 Das, P. *et al.* (2011) Aggregation of gamma-crystallins associated with human cataracts via domain swapping at the C-terminal beta-strands. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10514–10519
- 58 Papanikolopoulou, K. *et al.* (2008) Formation of amyloid fibrils in vitro by human gammaD-crystallin and its isolated domains. *Mol. Vis.* 14, 81–89
- 59 Wang, Y. *et al.* (2010) Formation of amyloid fibrils in vitro from partially unfolded intermediates of human C-crystallin. *Invest. Ophthalmol. Vis. Sci.* 51, 672–678
- 60 Moran, S.D. *et al.* (2012) Two-dimensional IR spectroscopy and segmental ¹³C labeling reveals the domain structure of human gammaD-crystallin amyloid fibrils. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3329–3334
- 61 Meehan, S. *et al.* (2004) Amyloid fibril formation by lens crystallin proteins and its implications for cataract formation. *J. Biol. Chem.* 279, 3413–3419
- 62 Sandilands, A. *et al.* (2002) Altered aggregation properties of mutant gamma-crystallins cause inherited cataract. *EMBO J.* 21, 6005–6014
- 63 Banerjee, P.R. *et al.* (2011) Increased hydrophobicity and decreased backbone flexibility explain the lower solubility of a cataract-linked mutant of gammaD-crystallin. *J. Mol. Biol.* 412, 647–659
- 64 Jung, J. *et al.* (2009) The structure of the cataract-causing P23T mutant of human gammaD-crystallin exhibits distinctive local conformational and dynamic changes. *Biochemistry* 48, 2597–2609
- 65 Kong, F. and King, J. (2011) Contributions of aromatic pairs to the folding and stability of long-lived human gammaD-crystallin. *Protein Sci.* 20, 513–528
- 66 Talla, V. *et al.* (2008) Visualization of in situ intracellular aggregation of two cataract-associated human gamma-crystallin mutants: lose a tail, lose transparency. *Invest. Ophthalmol. Vis. Sci.* 49, 3483–3490
- 67 Fu, L. and Liang, J.J. (2003) Alteration of protein-protein interactions of congenital cataract crystallin mutants. *Invest. Ophthalmol. Vis. Sci.* 44, 1155–1159
- 68 Fu, L. and Liang, J.J.N. (2002) Conformational change and destabilization of cataract gammaC-crystallin T5P mutant. *FEBS Lett.* 513, 213–216
- 69 Liu, B.F. *et al.* (2008) Protein-protein interactions involving congenital cataract T5P gammaC-crystallin mutant: a confocal fluorescence microscopy study. *Exp. Eye Res.* 87, 515–520
- 70 Ma, Z. *et al.* (2009) The G18 V CRYGS mutation associated with human cataracts increases gammaS-crystallin sensitivity to thermal and chemical stress. *Biochemistry* 48, 7334–7341
- 71 Ma, Z. *et al.* (2011) Overexpression of human gammaC-crystallin 5 bp duplication disrupts lens morphology in transgenic mice. *Invest. Ophthalmol. Vis. Sci.* 52, 5369–5375
- 72 Wang, K. *et al.* (2007) γ D-Crystallin associated protein aggregation and lens fiber cell denucleation. *Invest. Ophthalmol. Vis. Sci.* 48, 3719–3728
- 73 Churchill, A. and Graw, J. (2011) Clinical and experimental advances in congenital and paediatric cataracts. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* 366, 1234–1249
- 74 Lee, S. *et al.* (2010) A single destabilizing mutation (F9S) promotes concerted unfolding of an entire globular domain in gammaS-crystallin. *J. Mol. Biol.* 399, 320–330
- 75 Sinha, D. *et al.* (2001) A temperature-sensitive mutation of Crygs in the murine Opj cataract. *J. Biol. Chem.* 276, 9308–9315
- 76 Moreau, K.L. and King, J. (2009) Hydrophobic core mutations associated with cataract development in mice destabilize human D-crystallin. *J. Biol. Chem.* 284, 33285–33295
- 77 Pollreisz, A. and Schmidt-Erfurth, U. (2010) Diabetic cataract-pathogenesis, epidemiology and treatment. *J. Ophthalmol.* 2010, 608751
- 78 Shiels, A. and Hejtmancik, J.F. (2007) Genetic origins of cataract. *Arch. Ophthalmol.* 125, 165–173
- 79 Barbazetto, I.A. *et al.* (2004) Oxygen tension in the rabbit lens and vitreous before and after vitrectomy. *Exp. Eye Res.* 78, 917–924
- 80 Kyselova, Z. (2010) Different experimental approaches in modelling cataractogenesis: an overview of selenite-induced nuclear cataract in rats. *Interdisciplinary Toxicol.* 3, 3–14
- 81 Shearer, T.R. *et al.* (1997) Selenite nuclear cataract: review of the model. *Mol. Vis.* 3, 8
- 82 Rooban, B.N. *et al.* (2009) *Vitex negundo* attenuates calpain activation and cataractogenesis in selenite models. *Exp. Eye Res.* 88, 575–582
- 83 Sasikala, V. *et al.* (2010) *Moringa oleifera* prevents selenite-induced cataractogenesis in rat pups. *J. Ocul. Pharmacol. Ther.* 26, 441–447
- 84 Delcourt, C. *et al.* (2000) Light exposure and the risk of cortical, nuclear, and posterior subcapsular cataracts: the Pathologies Oculaires Liees a l'Age (POLA) study. *Arch. Ophthalmol.* 118, 385–392
- 85 McCarty, C.A. and Taylor, H.R. (2002) A review of the epidemiologic evidence linking ultraviolet radiation and cataracts. *Dev. Ophthalmol.* 35, 21–31
- 86 Wang, Y. and King, J.A. (2010) Cataract as a protein-aggregation disease. In *Protein Misfolding Diseases: Current and Emerging Principles and Therapies* (Ramirez-Alvarado, M. *et al.*, eds), pp. 487–515, John Wiley & Sons, Inc.
- 87 Giblin, F.J. *et al.* (2002) UVA light in vivo reaches the nucleus of the guinea pig lens and produces deleterious, oxidative effects. *Exp. Eye Res.* 75, 445–458
- 88 Simpanya, M.F. *et al.* (2008) Measurement of lens protein aggregation in vivo using dynamic light scattering in a guinea pig/UVA model for nuclear cataract. *Photochem. Photobiol.* 84, 1589–1595
- 89 Mizdrak, J. *et al.* (2008) Tryptophan-derived ultraviolet filter compounds covalently bound to lens proteins are photosensitizers of oxidative damage. *Free Radic. Biol. Med.* 44, 1108–1119
- 90 Chen, J. *et al.* (2009) Mechanism of the very efficient quenching of tryptophan fluorescence in human gammaD- and gammaS-crystallins: the gamma-crystallin fold may have evolved to protect tryptophan residues from ultraviolet photodamage. *Biochemistry* 48, 3708–3716

- 91 Chen, J. *et al.* (2006) Mechanism of the highly efficient quenching of tryptophan fluorescence in human gammaD-crystallin. *Biochemistry* 45, 11552–11563
- 92 De Maria, A. *et al.* (2009) Calpain expression and activity during lens fiber cell differentiation. *J. Biol. Chem.* 284, 13542–13550
- 93 Biswas, S. *et al.* (2004) Calpains: targets of cataract prevention? *Trends Mol. Med.* 10, 78–84
- 94 Li, N. *et al.* (2011) Protective effects and mechanism of tetramethylpyrazine against lens opacification induced by sodium selenite in rats. *Exp. Eye Res.* 93, 98–102
- 95 Sharma, K.K. and Santhoshkumar, P. (2009) Lens aging: effects of crystallins. *Biochim. Biophys. Acta* 1790, 1095–1108
- 96 Taylor, H.R. (2000) Cataract: how much surgery do we have to do? *Br. J. Ophthalmol.* 84, 1–2
- 97 Vibin, M. *et al.* (2010) Broccoli regulates protein alterations and cataractogenesis in selenite models. *Curr. Eye Res.* 35, 99–107
- 98 Javadzadeh, A. *et al.* (2009) Preventive effect of onion juice on selenite-induced experimental cataract. *Indian J. Ophthalmol.* 57, 185–189
- 99 Manikandan, R. *et al.* (2010) Effect of curcumin on selenite-induced cataractogenesis in Wistar rat pups. *Curr. Eye Res.* 35, 122–129
- 100 Stefek, M. (2011) Natural flavonoids as potential multifunctional agents in prevention of diabetic cataract. *Interdisciplinary Toxicol.* 4, 69–77
- 101 Harding, J.J. (2001) Can drugs or micronutrients prevent cataract? *Drugs Aging* 18, 473–486
- 102 Babizhayev, M.A. *et al.* (2002) Efficacy of N-acetylcarnosine in the treatment of cataracts. *Drugs R D* 3, 87–103
- 103 Ha, J.W. *et al.* (2010) Ability of N-acetylcarnosine to protect lens crystallins from oxidation and oxidative damage by radical probe mass spectrometry (RP-MS). *Rapid Commun. Mass Spectrom.* 24, 2900–2908
- 104 Varma, S.D. *et al.* (2010) Inhibition of selenite-induced cataract by caffeine. *Acta Ophthalmol.* 88, e245–e249
- 105 Varma, S.D. *et al.* (2008) UV-B-induced damage to the lens in vitro: prevention by caffeine. *J. Ocul. Pharmacol. Ther.* 24, 439–444
- 106 Randazzo, J. *et al.* (2011) Orally active multi-functional antioxidants delay cataract formation in streptozotocin (type 1) diabetic and gamma-irradiated rats. *PLoS ONE* 6, e18980
- 107 Ferreira, N. *et al.* (2011) Natural polyphenols inhibit different steps of the process of transthyretin (TTR) amyloid fibril formation. *FEBS Lett.* 585, 2424–2430
- 108 Meng, F. *et al.* (2010) The flavanol (–)-epigallocatechin 3-gallate inhibits amyloid formation by islet amyloid polypeptide, disaggregates amyloid fibrils, and protects cultured cells against IAPP-induced toxicity. *Biochemistry* 49, 8127–8133
- 109 Gong, B. *et al.* (2010) Sodium 4-phenylbutyrate ameliorates the effects of cataract-causing mutant gammaD-crystallin in cultured cells. *Mol. Vis.* 16, 997–1003
- 110 Goulet, D.R. *et al.* (2011) Inhibition of unfolding and aggregation of lens protein human gamma D crystallin by sodium citrate. *Exp. Eye Res.* 93, 371–381
- 111 Knee, K.M. *et al.* (2011) The group II chaperonin Mm-Cpn binds and refolds human gammaD crystallin. *Protein Sci.* 20, 30–41
- 112 Mitchell, P. *et al.* (1997) Prevalence of cataract in Australia: the Blue Mountains eye study. *Ophthalmology* 104, 581–588
- 113 Lampi, K.J. *et al.* (1997) Sequence analysis of betaA3, betaB3, and betaA4 crystallins completes the identification of the major proteins in young human lens. *J. Biol. Chem.* 272, 2268–2275
- 114 Takata, T. *et al.* (2007) Deamidation alters the structure and decreases the stability of human lens betaA3-crystallin. *Biochemistry* 46, 8861–8871
- 115 Peschek, J. *et al.* (2009) The eye lens chaperone alpha-crystallin forms defined globular assemblies. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13272–13277
- 116 Smith, M.A. *et al.* (2007) Mutation of interfaces in domain-swapped human betaB2-crystallin. *Protein Sci.* 16, 615–625