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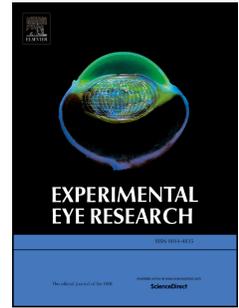
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Small molecules, both dietary and endogenous, influence the onset of lens cataracts

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ABSTRACT

How the lens ages successfully is a lesson in biological adaptation and the emergent properties of its complement of cells and proteins. This living tissue contains some of the oldest proteins in our bodies and yet they remain functional for decades, despite exposure to UV light, to reactive oxygen species and all the other hazards to protein function. This remarkable feat is achieved by a shrewd investment in very stable proteins as lens crystallins, by providing a reservoir of ATP-independent protein chaperones unequalled by any other tissue and by an oxidation-resistant environment. In addition, glutathione, a free radical scavenger, is present in mM concentrations and the plasma membranes contain oxidation-resistant sphingolipids, so what compromises lens function as it ages? In this review, we examine the role of small molecules in the prevention or causation of cataracts, including those associated with diet, metabolic pathways and drug therapy (steroids).

INTRODUCTION

A central question of great current interest in cataract research is whether there is a role for small molecules coming either from the diet or as part of cellular metabolism in the maintenance the eye lens in a healthy, optically clear and functional state. A review on the nutritional modulation of cataracts appeared in 2014 (Weikel et al., 2014), which focused on familiar dietary components. This current review examines recent exciting developments in sterol-based small molecule biochemistry in the lens and what led to these findings in order to focus attention on the opportunity to deliver small molecules to prevent and treat lens cataract.

PHYSIOLOGY AND BIOCHEMISTRY OF THE LENS

First and foremost, the fundamental feature of the lens is that it is one of the most optically transparent tissues in the body. This is achieved by a series of distinctive cell biological events to deliver this essential property for its function. These include the orchestrated spatial organisation of the differentiating lens fibre cells to minimize light scatter, the expression of stable proteins so that concentrations of up to 800 mg/ml protein have been reported in some vertebrate lenses (Mirarefi et al., 2010) to refract light onto the retina, the development of a graded refractive index system to minimize spherical aberration (Land, 2012) and lastly the removal of all organelles that would otherwise scatter light, e.g., nuclei, mitochondria and endoplasmic reticulum (Bassnett et al., 2011). As only the first few outermost layers of cells in the lens retain their organelles, all the other differentiated fiber cells that comprise the bulk of the lens are unable to synthesize new proteins (Lieska et al., 1992) despite the presence of mRNAs (Jaworski and Wistow, 1996). Nevertheless, as the lens ages, lens proteins are subjected to continuous and also significant post-translational modifications (Truscott and Friedrich, 2015) that accumulate as the lens cells age leading to the loss of protein function (Zhu et al., 2010b).

This presents a biological paradox for the lens. It must age successfully and avoid pathological consequences, i.e., cataracts, but without replenishing its original protein complement (Lynnerup et al., 2008; Zhu et al., 2010b). A possible replenishment by a soluble protein exchange process (Shestopalov and Bassnett, 2003) has yet to be fully assessed (Stewart et al., 2013). The lens fiber cells have biochemical mechanisms to ensure that proteins synthesized early in life resist becoming misfolded with time and the eventual aggregation and cataract formation (Truscott and Friedrich, 2016). Also, transporters ensure that avaricious proteases lying in wait in the fiber cells are denied the calcium ions needed for their activity (Duncan and Jacob, 1984). Other membrane proteins such as the aquaporins regulate water flow in and out of the lens cells, as occurs during accommodation (Gerometta et al., 2007) and in a pH dependent manner (Nemeth-Cahalan et al., 2004). This activity of the aquaporins relies on the lipid environment that they occupy (Laganowsky et al., 2014; Tong et al., 2013).

SUSTAINING THE SOLUBILITY OF LENS PROTEINS

Lens cells have a large reservoir of particular protein chaperones called α -crystallins (Horwitz, 1992). These are members of the small heat shock protein family (Garrido et al., 2012). The α -crystallins can suppress the aggregation of off-pathway intermediates in refolding human γ C-, γ D- and γ S-crystallins (Acosta-Sampson and King, 2010) and so are thought to be a major factor in securing lens function. The paradox, however, is that in the centre of the lens, there is very little full length α A- or α B-crystallin (Grey and Schey, 2009; Truscott and Friedrich, 2015). The truncated products in combination with other age-related post-translational modifications such as deamidation can preserve some chaperone activity (Asomugha et al., 2011), but some peptides derived from these lenticular chaperones have compromised activity (Kannan et al., 2013; Santhoshkumar et al., 2011). So, while the chaperone reservoir is one of the biochemical mechanisms maintaining lens transparency over the lifetime of the tissue, it is not without ageing effects.

Another mechanism is the innate stability of the proteins responsible for the refractive properties of the lens (Slingsby et al., 2013). There are often structurally related to enzymes

(Bloemendal et al., 2004; Piatigorsky et al., 1994; Wistow, 2012) and were also called crystallins (Morner, 1894), but they are quite distinct from the α -crystallin protein chaperones. In mammalian lenses these are the β - and γ -crystallin protein families (Slingsby et al., 2013). The combination, therefore, of the most abundant lens proteins being very stable proteins and the presence of the largest reservoir of protein chaperones in the vertebrate body being located in the lens loads the die in favour of sustained protein function throughout the lifetime of the lens (Slingsby et al., 2013), although not all the mechanistic details are currently known.

As with other protein chaperones, α -crystallins also assist in the assembly/disassembly of protein complexes and also influence phospholipid head group organization (Zhu et al., 2010a). They are also nucleotide-independent protein chaperones, a distinct advantage in the low oxygen tension environment of the lens (Barbazetto et al., 2004; Beebe et al., 2014) where ATP is replenished by glycolysis (Cheng et al., 1991). The lens α -crystallins comprise two proteins, α A- and α B-crystallin. They form large, dynamic protein complexes (Hochberg and Benesch, 2014). The applications of advanced physical techniques such as ion mobility mass spectrometry (Hilton et al., 2013; Hochberg and Benesch, 2014; Hochberg et al., 2014) and nuclear magnetic spectroscopy (Roos et al., 2015) have produced important new insights into oligomer dynamics, subunit organization and therefore the structure-function relationship.

ANTIOXIDANTS AND OTHER OXIDATION LIMITING MECHANISMS IN THE LENS

Besides the switching off of DNA transcription and protein synthesis (Jaworski and Wistow, 1996; Lieska et al., 1992; Lynnerup et al., 2008; Stewart et al., 2013) and a reduction in metabolism to a maintenance level (Dovrat et al., 1986; Dovrat et al., 1984; Scharf et al., 1987; Zhu et al., 2010b), a third biochemical process occurs to assist the lens in preventing premature protein denaturation, namely the presence of antioxidants in or near the lens. The anterior side of the lens is washed with the aqueous humor, a selected ultrafiltrate of the blood. In animals such as humans that operate in the light rather than the dark, the aqueous humor and the lens epithelial cells (but not the fiber cells) contain millimolar concentrations of ascorbate due to an ascorbate transporter in the ciliary epithelium (Helbig et al., 1989). In the lens cells there are

millimolar concentrations of the tripeptide glutathione (GSH). High-resolution magic angle spinning proton NMR spectroscopy of ocular tissues revealed that GSH is present at much higher concentrations in the lens (Kryczka et al., 2014). GSH can react with free radicals and other oxidants generated by the formation of singlet oxygen ($^1\text{O}_2$) from the interaction between ultraviolet photons and tryptophan residues on the proteins (Ortwerth et al., 2009) and can also react with the oxidized form of ascorbate, dehydroascorbate, to regenerate ascorbate (Sasaki et al., 1995). The resulting oxidized glutathione (GSSG) is reduced back to GSH by NADPH, produced by residual metabolic activity (the pentose phosphate shunt) in the lens (Ganea and Harding, 2006). An important question is where GSH comes from (Srinivas, 2014). There is evidence for transporters for glutamate and cysteine in the differentiated fiber cells in the lens (Lim et al., 2013), thereby offering a continuing source of GSH. Whether this extends to the nuclear region of the lens remains to be seen. It is possible that like other biochemical entities in the lens, GSH in the nuclear region is generated during the undifferentiated, epithelial stage of lens cells and is therefore “old”, particularly as GSH exchange between nuclear and cortical regions is compromised by the barrier that develops in older human lenses (Sweeney and Truscott, 1998). This would imply that there would be a loss of GSH with aging in this critical region of the lens (Srinivas, 2014; Truscott, 2005).

The lens plasma membranes are a further barrier to oxidation. Those membranes in the centre (nucleus) of the lens are less permeable to oxygen than those in the lens cortex and ageing decreases further that permeability to oxygen (Raguz et al., 2015; Subczynski et al., 2012). The nuclear membranes of the human lens are rich in sphingomyelin and dihydro-sphingomyelin that can help protect cholesterol against free-radical mediated oxidation (Sargis and Subbaiah, 2006). Nonetheless, despite these protective mechanisms, 7-ketocholesterol accumulates in ageing human lenses (Rodriguez et al., 2014) and 7β -hydroxycholesterol, 7-ketocholesterol, 5α , 6α -epoxycholestanol, 20α -hydroxycholesterol and 25-hydroxycholesterol are all found in human cataract (Girao et al., 1998). So, these mechanisms minimize, but don't prevent lipid oxidation events in the lens. Oxidation of the gap junction proteins may alter the passage of

small molecules (<1,000 Da) between the cells of the lens (Berthoud and Beyer, 2009), although this may be relatively, non-selective.

LENS PROTEIN MODIFICATIONS

Despite large concentrations of antioxidants in the lens system, extensive posttranslational modifications (PTMs) of lens proteins occur with aging (Truscott and Friedrich, 2015). The most common PTM, however, does not come from interaction with oxidants. Instead, it is a purely chemical process whereby asparagine and glutamine residues undergo deamidation to form aspartate, isoaspartate and glutamate residues (Robinson et al., 2005). The other PTMs represent many types of oxidation reactions. Some of these are due to UV light ($^1\text{O}_2$) and result in oxidation of tryptophan, tyrosine, cysteine, methionine and histidine residues (Andley et al., 1985; Goosey et al., 1980; Ortwerth et al., 1998) and can induce disulphide bond cleavage (Wang and Wen, 2010), loss of chaperone activity (Mafia et al., 2008) to promote protein aggregation. Others involve dehydroascorbate-induced modifications (Dickerson et al., 1995; Linetsky et al., 2008; Nemet and Monnier, 2011) and include α -crystallin protein chaperones (Dickerson et al., 1995). Proteins on long storage (not just in the lens) undergo the Maillard reaction between their free lysine and arginine amino groups and aldehyde and ketone groups coming from monosaccharides and oxidized lipids. These rearrange to form Amadori products that can form crosslinks between proteins and are prevalent in patients with diabetes (Smuda et al., 2015).

Another process that alters the lens proteins is proteolysis. Using a variety of methods, including imaging mass spectrometry, extensive C-truncation has been shown to occur of the α -crystallins (Grey and Schey, 2009). For α A-crystallin in rats, this process has already begun by the stage of weaning (Stella et al., 2010). It continues with aging. α A-crystallin truncated forms from residues 1-100 are restricted to the nuclear zone of the lens. At weaning, the full length α A-crystallin is found throughout the cortical region and in the undifferentiated outermost epithelial cell layer, but not in the nuclear region (Stella et al., 2010). As rats age, full length α A-crystallin is only found in the newest epithelial cell layer (Anderson et al., 2015). The reason for

C-truncation is multi-factorial. The other crystallins as well as cytoskeletal and integral membrane proteins are all proteolysed during the ageing process (Korlimbinis et al., 2009; Lampi et al., 2014; Sandilands et al., 1995; Truscott and Friedrich, 2016). It may be due to the activity of calcium-dependent proteases such as calpain, alternative enzymatic capabilities of other lens proteins, or by purely chemical processes that occur over the long period of life.

Less is known about other compounds arising from the diet itself, intermediates in normal cellular metabolism or from modifications of endogenous or exogenous substances by the gut microbiota, that may have an impact on the lens cell system. While it has been suggested that fatty acids in the diet do not influence the lipid composition of the lens (Nealon et al., 2008), both sterols and flavonoids in the diet can prevent or even reverse cataract in animal and *ex vivo* human models (Weikel et al., 2014). One of the impacts of modern -omics is that investigators are returning to a close examination of the metabolome, the summation of all the known physiological pathways as well as pathways of the organism's associated microbiota. The analytical approaches have revealed previously unidentified small molecules that were not detected in the heyday of pathway research.

DIETARY FACTORS ASSOCIATED WITH ALTERED LENS CATARACT RISKS

A. Compounds with antioxidant properties

The diet provides many sources of the antioxidant ascorbic acid, particularly in fruits and vegetables. However, it alone does not necessarily prevent lens cataracts. Introduction of a human ascorbate transporter to increase ascorbate concentrations in the aqueous humor of mice, led to a more rapid onset of lens cataracts (Fan et al., 2006), likely by elevated glycation as a result of ascorbic acid oxidation products (Linetsky et al., 2014; Linetsky et al., 2008). It is possible that the increased ascorbate in aqueous humor also increased dehydroascorbate and outstripped the capacity of the GSSG-NADH regenerative cycle. Other plant phytochemicals that are part of the diet have shown preventive activities in models of oxidative stress. Ellagic acid inhibited the formation of cataracts induced by selenite in Wistar rats (Sakthivel et al.,

2008) and prevented alterations in lens proteins (Sakthivel et al., 2011). The polyphenols in *Moringa oleifera* also prevented cataract formation in selenite-treated rat pups (Sasikala et al., 2010). Diabetic cataracts in streptozotocin-induced diabetic rats were prevented by soy isoflavones (Lu et al., 2008a; Lu et al., 2008b) although it was suspected that isoflavones were increasing insulin secretion/susceptibility rather than having antioxidant activity. It's worth noting that Chacko et al. have shown that isoflavones are PPAR- γ agonists (Chacko et al., 2007; Chacko et al., 2005). Soy isoflavones also inhibited cataract in galactose-induced cataracts (Huang et al., 2007). However, in a model of age-related senile cataracts, the Ihara Cataractous Rat strain f (ICR/f), where insulin or its action were not deficient, soy isoflavones were not preventive and even accelerated the early onset of cataracts (Floyd et al., 2011). This result appears to confirm an earlier report using the Royal College of Surgeons Pink Eyed Rat, that less than 1% of animals on a soy-free AIN76A diet developed lens cataracts with aging, as opposed to 29% of those on a lab chow diet containing copious amounts of soy (Hess et al., 1985). Grape seed proanthocyanidins were shown to delay the late stages of development of cataracts in ICR/f rats (Yamakoshi et al., 2002) and the selenite model (Zhang and Hu, 2012).

Another dietary antioxidant, vitamin E, prevents lens cataract in selenite-induced cataracts (Mathew et al., 2003). Other studies suggest a role for vitamin E in prevention of cataracts in other rodent models (Haque and Gilani, 2005; Kojima et al., 2002; MacDonald-Wicks and Garg, 2003) but not in humans (Olmedilla et al., 2003). A meta-analysis of epidemiological data revealed that dietary, but not supplemental vitamin E reduced the risk of age-related cataracts (Zhang and Hu, 2012).

In contrast, some dietary supplements and drugs increase the risk of lens cataracts by absorbing incident light at non-UV wavelengths and generating $^1\text{O}_2$ and other oxidation products through their photophore properties. Examples are intermediates in porphyrin synthesis and metabolism (Roberts and Dillon, 1987), compounds in dietary supplements such as hypericin in St John's wort (Schey et al., 2000) and drugs such as ciprofloxacin (Zhao et al., 2010).

B. Lipids and fatty acids

A feature of the maturing lens is gradual association of the α -crystallins with membrane components (Borchman and Tang, 1996; Cobb and Petrash, 2002; Grami et al., 2005). It therefore is important to understand how the lens lipid composition changes with age. In humans, the relative amounts of sphingolipids increase with aging (Huang et al., 2007). However, this may be more a reflection of the disappearance of the other major membrane lipids, the phosphatidylcholines and phosphatidylethanolamines (Li et al., 1987; Zigman et al., 1984). Although not proven, long chain unsaturated fatty acids released from PCs and PEs may be the source of energy needed to maintain lens function over a lifetime, although the low levels of butyrate measured in the lens (Kryczka et al., 2014) and the spatial restriction for fatty acid oxidation to the lens epithelium and outer cortex because of the lack of mitochondria (Bassnett et al., 2011) challenges this concept. Based on the $^{14}\text{C}/^{12}\text{C}$ isotopic ratio of the lens lipid matching that of the ratio of their year of birth, lipid replacement in lens membranes is minimal (Hughes et al., 2015). Nonetheless, the changing lipid composition, paralleled by the establishment of membrane domains in the aging lens nucleus (Raguz et al., 2015), may alter the distribution of the crystallins (and other proteins) within the differentiated, old fiber cell. With the increase in sphingolipids in the nuclear lens membranes, α -crystallins then bind more strongly to these membranes (Grami et al., 2005) and in doing so may recruit other lens proteins and thereby increase total light scattering, again an ageing signature for the lens (Michael and Bron, 2011).

C. Sterols and other small molecules

Two recent studies have revealed the role of compounds in the cholesterol biosynthesis and metabolism pathways. In the first (Zhao et al., 2015), clever analysis of genetic mutations of two family members with frank lens cataracts revealed a mutation in the active site of lanosterol synthase (LSS), the enzyme responsible for the conversion of a linear unsaturated hydrocarbon, 2,3-oxidosqualene, into the steroid ring conformation of mammalian sterols, steroids and bile acids. Examination of a further 150 subjects with lens cataracts identified a

second mutation in the active site of LSS (Zhao et al., 2015). Expression of the two mutant LSS demonstrated that both mutations led to loss of LSS activity. However, this was not the first finding concerning the association of loss of LSS activity and cataracts. It had been previously shown that in the Shumiya rat that cataracts were associated with deletion of exon 4 of LSS (Mori et al., 2006). In addition, drug candidates for inhibiting cholesterol synthesis, each inhibitors of LSS, caused the formation of lens cataracts (Fouchet et al., 2008; Funk and Landes, 2005; Pyrah et al., 2001). A mechanism of action was proposed by (Cenedella et al., 2004) where inhibition of lanosterol synthesis (and therefore products downstream of it) resulted in a “stiffer” membrane, thereby altering the way in which proteins associate with the membranes. The α -crystallins become attached to the lipid membrane (Borchman and Tang, 1996; Grami et al., 2005; Maddala and Rao, 2005) and their association with lens membranes coincides with decreased lens compliance (Heys et al., 2007). The α -crystallins influence the head group organization of the phospholipids (Zhu et al., 2010a). Other lens proteins also become associated as the membranes age (Truscott et al., 2011) and it is thought that this will also contribute to the complexity of the protein complement on the membranes in aged lenses. Zhao et al. (2015) found that applying lanosterol, but not cholesterol, to the eyes of dogs with age related (non-diabetic) cataract, cells transfected with mutant α A-crystallin and to *ex vivo* cataractous rabbit lens resulted in solubilization of the cataracts and solubilisation of the protein aggregates. In the latter case, lanosterol-induced solubilization obeyed first order kinetics with a $T_{1/2}$ of 4 h. Excitingly, a nanoparticle preparation of lanosterol when applied as eye drops to one eye of a dog with bilateral cataracts for 6 weeks led to a marked dissolution of that cataract. This remarkable study suggests that dietary-derived small molecules may not only reduce the risk of lens cataracts, but may even be used to effect a therapeutic reversal of cataracts.

A second study, recently published in *Science* (Makley et al., 2015), took a completely different approach but arrived nevertheless at cholesterol-related small molecules to help reverse cataract caused by α -crystallin mutations. From a relative small screen (~2,500) of bioactives, two oxysterols were eventually identified (5 α -cholestan-3 β -ol-6-one and 5-cholesten-3 β ,25-

diol) that reversed cataract caused by R120G α B-crystallin and R49C α A-crystallin. Molecular modeling suggested that these molecules bind at the dimer interface of the crystallin domain for α B-crystallin.

Steroids are associated with cataracts – use of prednisone to treat lupus, glucocorticoid injections for Addison's disease and other conditions – are they displacing “beneficial” oxysterols? Of course, the Zhao paper (Zhao et al., 2015) identified mutations in lanosterol synthase as the genetic basis of inherited cataract in humans, an observation made also for the Shumiya rat cataract model (Mori et al., 2006). This quite clearly establishes lanosterol and cholesterol metabolism as key factors in preventing cataract, complementing the observation that patients with Smith-Lemli-Opitz syndrome (Cotlier and Rice, 1971; Fitzky et al., 1998), mevalonic aciduria (Hoffmann et al., 1986; Schafer et al., 1992) and cerebrotendinous xanthomatosis (Chen et al., 1996; Yoshinaga et al., 2014) and those affected by Coats' Disease with ocular deposits of cholesterol (Beby et al., 2005; Chang et al., 1984) also present with cataract. The debate over the increased risk or otherwise of cataract associated with statin use continues (Desai et al., 2014; Kostis and Dobrzynski, 2015). There are many documented changes in lipid composition that accompany cataract in human lenses (Borchman and Yappert, 2010). Dietary linoleic acid levels are correlated with increased nuclear cataract (Lu et al., 2007), although dietary fatty acids do not influence the lipid composition of the rat lens (Nealon et al., 2008), the inability to synthesise ether lipids in the plasmalogen biosynthesis pathway is still linked to cataract in mice (Gorgas et al., 2006; Liegel et al., 2011; Park et al., 2014) and humans (Buchert et al., 2014; Liegel et al., 2013). Nevertheless, lipids and sterols are clearly linked to cataractogenesis and its prevention.

Comparing the three sterols (Figs. 2A-C) that have been shown to improve mutant α A-crystallin solubility or clarify pre-existing lens cataracts with three well known examples of xenobiotics (therapeutic agents and a botanical) (Figs. 2D-F) whose use leads to cataract formation, a common structural feature in the former but not the latter is the presence of a hydrophobic side chain. Thus, prednisone, while having a steroid ring nucleus, lacks the side chain of the

sterols. While this would appear to advocate for a beneficial effect of a more familiar sterol, cholesterol, a previous study showed cholesterol led to a more tightly packed bilayer than lanosterol (Cournia et al., 2007). Accordingly, lanosterol-enriched membranes had a lower melting temperature (increased fluidity). In contrast, hypericin increased membrane stiffness (Chaloupka et al., 1999).

FUTURE DIRECTIONS

Lipids and their exchange through the lens over short (week) timescales compared to protein exchange and turnover in the membrane and soluble fractions are clearly areas of interest. This is because of the possibility of pharmacological alternatives to surgery for the treatment and prevention of cataract in humans and in animals. The lens also offers insight into the ageing process itself and the biochemical and cell biological processes that accompany the ageing of the lens and the consequences for its proteins and lipids. In the lens, time is measured in decades and therefore that parameter is an area of the biochemical and cell biological time scale that is rarely considered let alone studied, but is key to understanding how to age successfully. The recent reports that certain sterols prevent and even reverse lens cataract will encourage a more thorough understanding of the role of the changing lipid environment with aging on the primary function of the lens, to remain clear and pass observed light to the rest of the visual apparatus. Finally, delivery of these sterols or lipid domain modifiers to the lens environment, particularly orally via the diet, will remain a challenge.

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FIGURE LEGENDS

Fig. 1. A summary of the major ageing effects on lens proteins and plasma membranes. The recent discovery of the effects of selected sterols (Makley et al., 2015; Zhao et al., 2015) on lens transparency and the data from ^{14}C distribution in the soluble protein fraction of human lenses (Stewart et al., 2013) suggest the protein and sterol content of the lens nucleus exchanges with the cortex.

Fig. 2. Chemical structures of small molecules that influence the solubilities of lens proteins. The sterols, lanosterol (A), 5α -cholestan- 3β -ol-6-one (B) and 5-cholesten- 3β ,25-diol (C), have recently been shown (Makley et al., 2015; Zhao et al., 2015) to solubilize mutant α A-crystallin and proteins in lens cataracts. In contrast, the therapeutic agents, prednisone (D) and ciprofloxacin (E) and the botanical, hypericin (F), are associated clinically with the formation of lens cataract.

BIBLIOGRAPHY

- Acosta-Sampson, L., and J. King. 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.
- Anderson, D.M., K.A. Floyd, S. Barnes, J.M. Clark, J.I. Clark, H. McHaourab, and K.L. Schey. 2015. A method to prevent protein delocalization in imaging mass spectrometry of non-adherent tissues: application to small vertebrate lens imaging. *Analytical and bioanalytical chemistry.* 407:2311-2320.
- Andley, U.P., S.F. Chapman, and L.J. Chylack. 1985. Fluorescence studies on tryptophan and sulfhydryl group changes of bovine lens crystallins in a photodynamic system. *Curr Eye Res.* 4:831-842.
- Asomugha, C.O., R. Gupta, and O.P. Srivastava. 2011. Structural and functional roles of deamidation of N146 and/or truncation of NH2- or COOH-termini in human alphaB-crystallin. *Mol Vis.* 17:2407-2420.
- Barbazetto, I.A., J. Liang, S. Chang, L. Zheng, A. Spector, and J.P. Dillon. 2004. Oxygen tension in the rabbit lens and vitreous before and after vitrectomy. *Exp Eye Res.* 78:917-924.
- Bassnett, S., Y. Shi, and G.F. Vrensen. 2011. Biological glass: structural determinants of eye lens transparency. *Philos Trans R Soc Lond B Biol Sci.* 366:1250-1264.
- Beby, F., O. Roche, C. Burillon, and P. Denis. 2005. Coats' disease and bilateral cataract in a child with Turner syndrome: a case report. *Graefes Arch Clin Exp Ophthalmol.* 243:1291-1293.
- Beebe, D.C., Y.B. Shui, C.J. Siegfried, N.M. Holekamp, and F. Bai. 2014. Preserve the (intraocular) environment: the importance of maintaining normal oxygen gradients in the eye. *Jpn J Ophthalmol.* 58:225-231.
- Berthoud, V.M., and E.C. Beyer. 2009. Oxidative stress, lens gap junctions, and cataracts. *Antioxidants & redox signaling.* 11:339-353.
- Bloemendal, H., W. de Jong, R. Jaenicke, N.H. Lubsen, C. Slingsby, and A. Tardieu. 2004. Ageing and vision: structure, stability and function of lens crystallins. *Prog Biophys Mol Biol.* 86:407-485.
- Borchman, D., and D. Tang. 1996. Binding capacity of alpha-crystallin to bovine lens lipids. *Exp Eye Res.* 63:407-410.
- Borchman, D., and M.C. Yappert. 2010. Lipids and the ocular lens. *Journal of lipid research.* 51:2473-2488.
- Buchert, R., H. Tawamie, C. Smith, S. Uebe, A.M. Innes, B. Al Hallak, A.B. Ekici, H. Sticht, B. Schwarze, R.E. Lamont, J.S. Parboosingh, F.P. Bernier, and R. Abou Jamra. 2014. A peroxisomal disorder of severe intellectual disability, epilepsy, and cataracts due to fatty acyl-CoA reductase 1 deficiency. *Am J Hum Genet.* 95:602-610.
- Cenedella, R.J., R. Jacob, D. Borchman, D. Tang, A.R. Neely, A. Samadi, R.P. Mason, and P. Sexton. 2004. Direct perturbation of lens membrane structure may contribute to cataracts caused by U18666A, an oxidosqualene cyclase inhibitor. *Journal of lipid research.* 45:1232-1241.
- Chacko, B.K., R.T. Chandler, T.L. D'Alessandro, A. Mundhekar, N.K. Khoo, N. Botting, S. Barnes, and R.P. Patel. 2007. Anti-inflammatory effects of isoflavones are dependent on flow and human endothelial cell PPARgamma. *The Journal of nutrition.* 137:351-356.

- Chacko, B.K., R.T. Chandler, A. Mundhekar, N. Khoo, H.M. Pruitt, D.F. Kucik, D.A. Parks, C.G. Kevil, S. Barnes, and R.P. Patel. 2005. Revealing anti-inflammatory mechanisms of soy isoflavones by flow: modulation of leukocyte-endothelial cell interactions. *Am J Physiol Heart Circ Physiol.* 289:H908-915.
- Chang, M.M., I.W. McLean, and J.C. Merritt. 1984. Coats' disease: a study of 62 histologically confirmed cases. *J Pediatr Ophthalmol Strabismus.* 21:163-168.
- Chen, W., S. Kubota, Y. Nishimura, S. Nozaki, S. Yamashita, T. Nakagawa, K. Kameda-Takemura, M. Menju, Y. Matsuzawa, I. Bjorkhem, G. Eggertsen, and Y. Seyama. 1996. Genetic analysis of a Japanese cerebrotendinous xanthomatosis family: identification of a novel mutation in the adrenodoxin binding region of the CYP 27 gene. *Biochim Biophys Acta.* 1317:119-126.
- Cheng, H.M., J. Xiong, G. Tanaka, C. Chang, A.A. Asterlin, and J.B. Aguayo. 1991. Analysis of concurrent glucose consumption by the hexose monophosphate shunt, glycolysis, and the polyol pathway in the crystalline lens. *Exp Eye Res.* 53:363-366.
- Cobb, B.A., and J.M. Petrash. 2002. alpha-Crystallin Chaperone-like Activity and Membrane Binding in Age-Related Cataracts. *Biochemistry.* 41:483-490.
- Cotlier, E., and P. Rice. 1971. Cataracts in the Smith-Lemli-Opitz syndrome. *Am J Ophthalmol.* 72:955-959.
- Desai, C.S., S.S. Martin, and R.S. Blumenthal. 2014. Non-cardiovascular effects associated with statins. *BMJ (Clinical research ed.).* 349:g3743.
- Dickerson, J.E., Jr., M.F. Lou, and R.W. Gracy. 1995. Ascorbic acid mediated alteration of alpha-crystallin secondary structure. *Curr Eye Res.* 14:163-166.
- Dovrat, A., J. Scharf, L. Eisenbach, and D. Gershon. 1986. G6PD molecules devoid of catalytic activity are present in the nucleus of the rat lens. *Exp Eye Res.* 42:489-496.
- Dovrat, A., J. Scharf, and D. Gershon. 1984. Glyceraldehyde 3-phosphate dehydrogenase activity in rat and human lenses and the fate of enzyme molecules in the aging lens. *Mechanisms of ageing and development.* 28:187-191.
- Duncan, G., and T.J. Jacob. 1984. Calcium and the physiology of cataract. *Ciba Foundation symposium.* 106:132-152.
- Fan, X., L.W. Reneker, M.E. Obrenovich, C. Strauch, R. Cheng, S.M. Jarvis, B.J. Ortwerth, and V.M. Monnier. 2006. Vitamin C mediates chemical aging of lens crystallins by the Maillard reaction in a humanized mouse model. *Proc Natl Acad Sci U S A.* 103:16912-16917.
- Fitzky, B.U., M. Witsch-Baumgartner, M. Erdel, J.N. Lee, Y.K. Paik, H. Glossmann, G. Utermann, and F.F. Moebius. 1998. Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. *Proc Natl Acad Sci U S A.* 95:8181-8186.
- Floyd, K.A., D.R. Stella, C.C. Wang, S. Laurentz, G.P. McCabe, O.P. Srivastava, and S. Barnes. 2011. Genistein and genistein-containing dietary supplements accelerate the early stages of cataractogenesis in the male ICR/f rat. *Exp Eye Res.* 92:120-127.
- Fouchet, M.H., F. Donche, C. Martin, A. Bouillot, C. Junot, A.B. Boullay, F. Potvain, S.D. Magny, H. Coste, M. Walker, M. Issandou, and N. Dodic. 2008. Design and evaluation of a novel series of 2,3-oxidosqualene cyclase inhibitors with low systemic exposure, relationship between pharmacokinetic properties and ocular toxicity. *Bioorganic & medicinal chemistry.* 16:6218-6232.

- Funk, J., and C. Landes. 2005. Histopathologic findings after treatment with different oxidosqualene cyclase (OSC) inhibitors in hamsters and dogs. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie*. 57:29-38.
- Ganea, E., and J.J. Harding. 2006. Glutathione-related enzymes and the eye. *Curr Eye Res*. 31:1-11.
- Garrido, C., C. Paul, R. Seigneuric, and H.H. Kampinga. 2012. The small heat shock proteins family: the long forgotten chaperones. *Int J Biochem Cell Biol*. 44:1588-1592.
- Gerometta, R., A.C. Zamudio, D.P. Escobar, and O.A. Candia. 2007. Volume change of the ocular lens during accommodation. *Am J Physiol Cell Physiol*. 293:C797-804.
- Girao, H., M.C. Mota, J. Ramalho, and P. Pereira. 1998. Cholesterol oxides accumulate in human cataracts. *Exp Eye Res*. 66:645-652.
- Goosey, J.D., J.S. Zigler, Jr., and J.H. Kinoshita. 1980. Cross-linking of lens crystallins in a photodynamic system: a process mediated by singlet oxygen. *Science*. 208:1278-1280.
- Gorgas, K., A. Teigler, D. Komljenovic, and W.W. Just. 2006. The ether lipid-deficient mouse: tracking down plasmalogen functions. *Biochim Biophys Acta*. 1763:1511-1526.
- Grami, V., Y. Marrero, L. Huang, D. Tang, M.C. Yappert, and D. Borchman. 2005. alpha-Crystallin binding in vitro to lipids from clear human lenses. *Exp Eye Res*. 81:138-146.
- Grey, A.C., and K.L. Schey. 2009. Age-related changes in the spatial distribution of human lens alpha-crystallin products by MALDI imaging mass spectrometry. *Invest Ophthalmol Vis Sci*. 50:4319-4329.
- Haque, S.E., and K.M. Gilani. 2005. Effect of ambroxol, spirulina and vitamin-E in naphthalene induced cataract in female rats. *Indian journal of physiology and pharmacology*. 49:57-64.
- Helbig, H., C. Korbmacher, J. Wohlfarth, S. Berweck, D. Kuhner, and M. Wiederholt. 1989. Electrogenic Na⁺-ascorbate cotransport in cultured bovine pigmented ciliary epithelial cells. *Am J Physiol*. 256:C44-49.
- Hess, H.H., J.J. Knapka, D.A. Newsome, I.V. Westney, and L. Wartofsky. 1985. Dietary prevention of cataracts in the pink-eyed RCS rat. *Laboratory animal science*. 35:47-53.
- Heys, K.R., M.G. Friedrich, and R.J. Truscott. 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.
- Hilton, G.R., G.K. Hochberg, A. Laganowsky, S.I. McGinnigle, A.J. Baldwin, and J.L. Benesch. 2013. C-terminal interactions mediate the quaternary dynamics of alphaB-crystallin. *Philos Trans R Soc Lond B Biol Sci*. 368:20110405.
- Hochberg, G.K., and J.L. Benesch. 2014. Dynamical structure of alphaB-crystallin. *Prog Biophys Mol Biol*. 115:11-20.
- Hochberg, G.K., H. Ecroyd, C. Liu, D. Cox, D. Cascio, M.R. Sawaya, M.P. Collier, J. Stroud, J.A. Carver, A.J. Baldwin, C.V. Robinson, D.S. Eisenberg, J.L. Benesch, and A. Laganowsky. 2014. The structured core domain of alphaB-crystallin can prevent amyloid fibrillation and associated toxicity. *Proc Natl Acad Sci U S A*. 111:E1562-1570.
- Hoffmann, G., K.M. Gibson, I.K. Brandt, P.I. Bader, R.S. Wappner, and L. Sweetman. 1986. Mevalonic aciduria--an inborn error of cholesterol and nonsterol isoprene biosynthesis. *N Engl J Med*. 314:1610-1614.

- Horwitz, J. 1992. Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci U S A*. 89:10449-10453.
- Huang, R., F. Shi, T. Lei, Y. Song, C.L. Hughes, and G. Liu. 2007. Effect of the isoflavone genistein against galactose-induced cataracts in rats. *Experimental biology and medicine (Maywood, N.J.)*. 232:118-125.
- Hughes, J.R., V.A. Levchenko, S.J. Blanksby, T.W. Mitchell, A. Williams, and R.J. Truscott. 2015. No turnover in lens lipids for the entire human lifespan. *eLife*. 4.
- Jaworski, C., and G. Wistow. 1996. LP2, a differentiation-associated lipid-binding protein expressed in bovine lens. *Biochem J*. 320 (Pt 1):49-54.
- Kannan, R., P. Santhoshkumar, B.P. Mooney, and K.K. Sharma. 2013. The alphaA66-80 peptide interacts with soluble alpha-crystallin and induces its aggregation and precipitation: a contribution to age-related cataract formation. *Biochemistry*. 52:3638-3650.
- Kojima, M., Y.B. Shui, H. Murano, M. Nagata, O. Hockwin, K. Sasaki, and N. Takahashi. 2002. Low vitamin E level as a subliminal risk factor in a rat model of prednisolone-induced cataract. *Invest Ophthalmol Vis Sci*. 43:1116-1120.
- Korlimbinis, A., Y. Berry, D. Thibault, K.L. Schey, and R.J. Truscott. 2009. Protein aging: truncation of aquaporin 0 in human lens regions is a continuous age-dependent process. *Exp Eye Res*. 88:966-973.
- Kostis, J.B., and J.M. Dobrzynski. 2015. Response to letter on "statins use and risk of cataracts: firm conclusions are still far off". *Journal of cardiovascular pharmacology and therapeutics*. 20:346-347.
- Kryczka, T., E. Wylegala, D. Dobrowolski, and A. Midelfart. 2014. NMR spectroscopy of human eye tissues: a new insight into ocular biochemistry. *TheScientificWorldJournal*. 2014:546192.
- Laganowsky, A., E. Reading, T.M. Allison, M.B. Ulmschneider, M.T. Degiacomi, A.J. Baldwin, and C.V. Robinson. 2014. Membrane proteins bind lipids selectively to modulate their structure and function. *Nature*. 510:172-175.
- Lampi, K.J., P.A. Wilmarth, M.R. Murray, and L.L. David. 2014. Lens beta-crystallins: the role of deamidation and related modifications in aging and cataract. *Prog Biophys Mol Biol*. 115:21-31.
- Land, M.F. 2012. The evolution of lenses. *Ophthalmic Physiol Opt*. 32:449-460.
- Li, L.K., L. So, and A. Spector. 1987. Age-dependent changes in the distribution and concentration of human lens cholesterol and phospholipids. *Biochim Biophys Acta*. 917:112-120.
- Liegel, R., B. Chang, R. Dubielzig, and D.J. Sidjanin. 2011. Blind sterile 2 (bs2), a hypomorphic mutation in Agps, results in cataracts and male sterility in mice. *Molecular genetics and metabolism*. 103:51-59.
- Liegel, R.P., M.T. Handley, A. Ronchetti, S. Brown, L. Langemeyer, A. Linford, B. Chang, D.J. Morris-Rosendahl, S. Carpanini, R. Posmyk, V. Harthill, E. Sheridan, G.M. Abdel-Salam, P.A. Terhal, F. Faravelli, P. Accorsi, L. Giordano, L. Pinelli, B. Hartmann, A.D. Ebert, F.A. Barr, I.A. Aligianis, and D.J. Sidjanin. 2013. Loss-of-function mutations in TBC1D20 cause cataracts and male infertility in blind sterile mice and Warburg micro syndrome in humans. *Am J Hum Genet*. 93:1001-1014.

- Lieska, N., K. Krotzer, and H.Y. Yang. 1992. A reassessment of protein synthesis by lens nuclear fiber cells. *Exp Eye Res.* 54:807-811.
- Lim, J.C., L. Lam, B. Li, and P.J. Donaldson. 2013. Molecular identification and cellular localization of a potential transport system involved in cystine/cysteine uptake in human lenses. *Exp Eye Res.* 116:219-226.
- Linetsky, M., C.T. Raghavan, K. Johar, X. Fan, V.M. Monnier, A.R. Vasavada, and R.H. Nagaraj. 2014. UVA light-excited kynurenines oxidize ascorbate and modify lens proteins through the formation of advanced glycation end products: implications for human lens aging and cataract formation. *J Biol Chem.* 289:17111-17123.
- Linetsky, M., E. Shipova, R. Cheng, and B.J. Ortwerth. 2008. Glycation by ascorbic acid oxidation products leads to the aggregation of lens proteins. *Biochim Biophys Acta.* 1782:22-34.
- Lu, M., A. Taylor, L.T. Chylack, Jr., G. Rogers, S.E. Hankinson, W.C. Willett, and P.F. Jacques. 2007. Dietary linolenic acid intake is positively associated with five-year change in eye lens nuclear density. *Journal of the American College of Nutrition.* 26:133-140.
- Lu, M.P., R. Wang, X. Song, R. Chibbar, X. Wang, L. Wu, and Q.H. Meng. 2008a. Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. *Nutrition research (New York, N.Y.).* 28:464-471.
- Lu, M.P., R. Wang, X. Song, X. Wang, L. Wu, and Q.H. Meng. 2008b. Modulation of methylglyoxal and glutathione by soybean isoflavones in mild streptozotocin-induced diabetic rats. *Nutrition, metabolism, and cardiovascular diseases : NMCD.* 18:618-623.
- Lynnerup, N., H. Kjeldsen, S. Heegaard, C. Jacobsen, and J. Heinemeier. 2008. Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. *PLoS ONE.* 3:e1529.
- MacDonald-Wicks, L.K., and M.L. Garg. 2003. Vitamin E supplementation in the mitigation of carbon tetrachloride induced oxidative stress in rats. *The Journal of nutritional biochemistry.* 14:211-218.
- Maddala, R., and V.P. Rao. 2005. alpha-Crystallin localizes to the leading edges of migrating lens epithelial cells. *Exp Cell Res.* 306:203-215.
- Mafia, K., R. Gupta, M. Kirk, L. Wilson, O.P. Srivastava, and S. Barnes. 2008. UV-A-induced structural and functional changes in human lens deamidated alphaB-crystallin. *Mol Vis.* 14:234-248.
- Makley, L.N., K.A. McMenimem, B.T. DeVree, J.W. Goldman, B.N. McGlasson, P. Rajogopal, B.M. Duniyak, T.J. McQuade, A.D. Thompson, R. Sunahara, R.E. Klevit, U.A. Andley, and J.E. Gestwicki. 2015. Pharmacological Chaperone for alpha-crystallin partially restores transparency in cataract models. *Science.* in press.
- Mathew, J.P., V.C. Thomas, and I. Thomas. 2003. Selenite cataract and its attenuation by vitamin E in Wistar rats. *Indian journal of ophthalmology.* 51:161-170.
- Michael, R., and A.J. Bron. 2011. The ageing lens and cataract: a model of normal and pathological ageing. *Philos Trans R Soc Lond B Biol Sci.* 366:1278-1292.
- Mirarefi, A.Y., S. Boutet, S. Ramakrishnan, A.J. Kiss, C.H. Cheng, A.L. Devries, I.K. Robinson, and C.F. Zukoski. 2010. Small-angle X-ray scattering studies of the intact eye lens: effect of crystallin composition and concentration on microstructure. *Biochim Biophys Acta.* 1800:556-564.

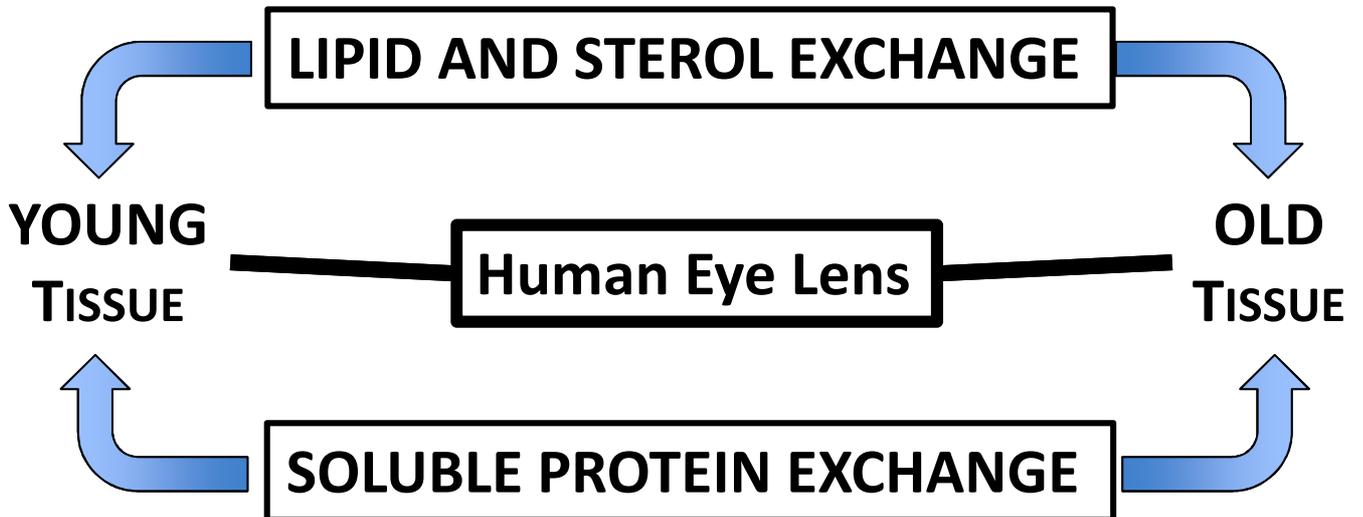
- Mori, M., G. Li, I. Abe, J. Nakayama, Z. Guo, J. Sawashita, T. Ugawa, S. Nishizono, T. Serikawa, K. Higuchi, and S. Shumiya. 2006. Lanosterol synthase mutations cause cholesterol deficiency-associated cataracts in the Shumiya cataract rat. *J Clin Invest.* 116:395-404.
- Morner, C. 1894. Untersuchung der Proteinsubstanzen in den leichtbrechenden Medien des Auges I. *Hoppe-Seyl.* 18:61-106.
- Nealon, J.R., S.J. Blanksby, S.K. Abbott, A.J. Hulbert, T.W. Mitchell, and R.J. Truscott. 2008. Phospholipid composition of the rat lens is independent of diet. *Exp Eye Res.* 87:502-514.
- Nemet, I., and V.M. Monnier. 2011. Vitamin C degradation products and pathways in the human lens. *J Biol Chem.* 286:37128-37136.
- Nemeth-Cahalan, K.L., K. Kalman, and J.E. Hall. 2004. Molecular basis of pH and Ca²⁺ regulation of aquaporin water permeability. *The Journal of general physiology.* 123:573-580.
- Olmedilla, B., F. Granado, I. Blanco, and M. Vaquero. 2003. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. *Nutrition (Burbank, Los Angeles County, Calif.).* 19:21-24.
- Ortwerth, B.J., J. Bhattacharyya, and E. Shipova. 2009. Tryptophan metabolites from young human lenses and the photooxidation of ascorbic acid by UVA light. *Invest Ophthalmol Vis Sci.* 50:3311-3319.
- Ortwerth, B.J., T.A. Casserly, and P.R. Olesen. 1998. Singlet oxygen production correlates with his and Trp destruction in brunescient cataract water-insoluble proteins. *Exp Eye Res.* 67:377-380.
- Park, A.K., R.P. Liegel, A. Ronchetti, A.D. Ebert, A. Geurts, and D.J. Sidjanin. 2014. Targeted disruption of Tbc1d20 with zinc-finger nucleases causes cataracts and testicular abnormalities in mice. *BMC genetics.* 15:135.
- Piatigorsky, J., M. Kantorow, R. Gopal-Srivastava, and S.I. Tomarev. 1994. Recruitment of enzymes and stress proteins as lens crystallins. *Exs.* 71:241-250.
- Pyrah, I.T., A. Kalinowski, D. Jackson, W. Davies, S. Davis, A. Aldridge, and P. Greaves. 2001. Toxicologic lesions associated with two related inhibitors of oxidosqualene cyclase in the dog and mouse. *Toxicologic pathology.* 29:174-179.
- Raguz, M., L. Mainali, W.J. O'Brien, and W.K. Subczynski. 2015. Lipid domains in intact fiber-cell plasma membranes isolated from cortical and nuclear regions of human eye lenses of donors from different age groups. *Exp Eye Res.* 132:78-90.
- Roberts, J.E., and J. Dillon. 1987. In vitro studies on the photosensitized oxidation of lens proteins by porphyrins. *Photochemistry and photobiology.* 46:683-688.
- Robinson, N.E., K.J. Lampi, R.T. McIver, R.H. Williams, W.C. Muster, G. Kruppa, and A.B. Robinson. 2005. Quantitative measurement of deamidation in lens betaB2-crystallin and peptides by direct electrospray injection and fragmentation in a Fourier transform mass spectrometer. *Mol Vis.* 11:1211-1219.
- Rodriguez, I.R., M.E. Clark, J.W. Lee, and C.A. Curcio. 2014. 7-ketocholesterol accumulates in ocular tissues as a consequence of aging and is present in high levels in drusen. *Exp Eye Res.* 128:151-155.

- Roos, M., S. Link, J. Balbach, A. Krushelnitsky, and K. Saalwachter. 2015. NMR-detected brownian dynamics of alphaB-crystallin over a wide range of concentrations. *Biophys J.* 108:98-106.
- Sakthivel, M., R. Elanchezian, E. Ramesh, M. Isai, C.N. Jesudasan, P.A. Thomas, and P. Geraldine. 2008. Prevention of selenite-induced cataractogenesis in Wistar rats by the polyphenol, ellagic acid. *Exp Eye Res.* 86:251-259.
- Sakthivel, M., P. Geraldine, and P.A. Thomas. 2011. Alterations in the lenticular protein profile in experimental selenite-induced cataractogenesis and prevention by ellagic acid. *Graefes Arch Clin Exp Ophthalmol.* 249:1201-1210.
- Sandilands, A., A.R. Prescott, A.M. Hutcheson, R.A. Quinlan, J.T. Casselman, and P.G. FitzGerald. 1995. Filensin is proteolytically processed during lens fiber cell differentiation by multiple independent pathways. *Eur. J. Cell Biol.* 67:238-253.
- Santhoshkumar, P., M. Raju, and K.K. Sharma. 2011. alphaA-crystallin peptide SDRDKFVIFLDVKHF accumulating in aging lens impairs the function of alpha-crystallin and induces lens protein aggregation. *PLoS One.* 6:e19291.
- Sargis, R.M., and P.V. Subbaiah. 2006. Protection of membrane cholesterol by sphingomyelin against free radical-mediated oxidation. *Free radical biology & medicine.* 40:2092-2102.
- Sasaki, H., F.J. Giblin, B.S. Winkler, B. Chakrapani, V. Leverenz, and C.C. Shu. 1995. A protective role for glutathione-dependent reduction of dehydroascorbic acid in lens epithelium. *Invest Ophthalmol Vis Sci.* 36:1804-1817.
- Sasikala, V., B.N. Rooban, S.G. Priya, V. Sahasranamam, and A. Abraham. 2010. Moringa oleifera prevents selenite-induced cataractogenesis in rat pups. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics.* 26:441-447.
- Schafer, B.L., R.W. Bishop, V.J. Kratunis, S.S. Kalinowski, S.T. Mosley, K.M. Gibson, and R.D. Tanaka. 1992. Molecular cloning of human mevalonate kinase and identification of a missense mutation in the genetic disease mevalonic aciduria. *J Biol Chem.* 267:13229-13238.
- Scharf, J., A. Dovrat, and D. Gershon. 1987. Defective superoxide-dismutase molecules accumulate with age in human lenses. *Graefes Arch Clin Exp Ophthalmol.* 225:133-136.
- Schey, K.L., S. Patat, C.F. Chignell, M. Datillo, R.H. Wang, and J.E. Roberts. 2000. Photooxidation of lens alpha-crystallin by hypericin (active ingredient in St. John's Wort). *Photochemistry and photobiology.* 72:200-203.
- Shestopalov, V.I., and S. Bassnett. 2003. Development of a macromolecular diffusion pathway in the lens. *J Cell Sci.* 116:4191-4199.
- Slingsby, C., G.J. Wistow, and A.R. Clark. 2013. Evolution of crystallins for a role in the vertebrate eye lens. *Protein Sci.* 22:367-380.
- Smuda, M., C. Henning, C.T. Raghavan, K. Johar, A.R. Vasavada, R.H. Nagaraj, and M.A. Glomb. 2015. Comprehensive analysis of maillard protein modifications in human lenses: effect of age and cataract. *Biochemistry.* 54:2500-2507.
- Srinivas, M. 2014. Delivery of glutathione to the lens nucleus. *Journal of ophthalmic & vision research.* 9:148-149.
- Stella, D.R., K.A. Floyd, A.C. Grey, M.B. Renfrow, K.L. Schey, and S. Barnes. 2010. Tissue localization and solubilities of alphaA-crystallin and its numerous C-terminal truncation

- products in pre- and postcataractous ICR/f rat lenses. *Invest Ophthalmol Vis Sci.* 51:5153-5161.
- Stewart, D.N., J. Lango, K.P. Nambiar, M.J. Falso, P.G. Fitzgerald, D.M. Rocke, B.D. Hammock, and B.A. Buchholz. 2013. Carbon turnover in the water-soluble protein of the adult human lens. *Mol Vis.* 19:463-475.
- Subczynski, W.K., M. Raguz, J. Widomska, L. Mainali, and A. Konovalov. 2012. Functions of cholesterol and the cholesterol bilayer domain specific to the fiber-cell plasma membrane of the eye lens. *J Membr Biol.* 245:51-68.
- Sweeney, M.H., and R.J. Truscott. 1998. An impediment to glutathione diffusion in older normal human lenses: a possible precondition for nuclear cataract. *Exp Eye Res.* 67:587-595.
- Tong, J., J.T. Canty, M.M. Briggs, and T.J. McIntosh. 2013. The water permeability of lens aquaporin-0 depends on its lipid bilayer environment. *Exp Eye Res.* 113:32-40.
- Truscott, R.J. 2005. Age-related nuclear cataract-oxidation is the key. *Exp Eye Res.* 80:709-725.
- Truscott, R.J., S. Comte-Walters, Z. Ablonczy, J.H. Schwacke, Y. Berry, A. Korlimbinis, M.G. Friedrich, and K.L. Schey. 2011. Tight binding of proteins to membranes from older human cells. *Age (Dordrecht, Netherlands).* 33:543-554.
- Truscott, R.J., and M.G. Friedrich. 2015. The etiology of human age-related cataract. Proteins don't last forever. *Biochim Biophys Acta.*
- Truscott, R.J., and M.G. Friedrich. 2016. The etiology of human age-related cataract. Proteins don't last forever. *Biochim Biophys Acta.* 1860:192-198.
- Wang, S.S., and W.S. Wen. 2010. Examining the influence of ultraviolet C irradiation on recombinant human gammaD-crystallin. *Mol Vis.* 16:2777-2790.
- Weikel, K.A., C. Garber, A. Baburins, and A. Taylor. 2014. Nutritional modulation of cataract. *Nutrition reviews.* 72:30-47.
- Wistow, G. 2012. The human crystallin gene families. *Human genomics.* 6:26.
- Yamakoshi, J., M. Saito, S. Kataoka, and S. Tokutake. 2002. Procyanidin-rich extract from grape seeds prevents cataract formation in hereditary cataractous (ICR/f) rats. *Journal of agricultural and food chemistry.* 50:4983-4988.
- Yoshinaga, T., Y. Sekijima, S. Koyama, K. Maruyama, T. Yoshida, T. Kato, and S. Ikeda. 2014. Clinical and radiological findings of a cerebrotendinous xanthomatosis patient with a novel p.A335V mutation in the CYP27A1 gene. *Internal medicine (Tokyo, Japan).* 53:2725-2729.
- Zhang, X., and Y. Hu. 2012. Inhibitory effects of grape seed proanthocyanidin extract on selenite-induced cataract formation and possible mechanism. *Journal of Huazhong University of Science and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban.* 32:613-619.
- Zhao, B., C.F. Chignell, M. Rammal, F. Smith, M.G. Hamilton, U.P. Andley, and J.E. Roberts. 2010. Detection and prevention of ocular phototoxicity of ciprofloxacin and other fluoroquinolone antibiotics. *Photochemistry and photobiology.* 86:798-805.
- Zhao, L., X.J. Chen, J. Zhu, Y.B. Xi, X. Yang, L.D. Hu, H. Ouyang, S.H. Patel, X. Jin, D. Lin, F. Wu, K. Flagg, H. Cai, G. Li, G. Cao, Y. Lin, D. Chen, C. Wen, C. Chung, Y. Wang, A. Qiu, E. Yeh, W. Wang, X. Hu, S. Grob, R. Abagyan, Z. Su, H.C. Tjondro, X.J. Zhao, H. Luo, R. Hou, J.J. Perry,

- W. Gao, I. Kozak, D. Granet, Y. Li, X. Sun, J. Wang, L. Zhang, Y. Liu, Y.B. Yan, and K. Zhang. 2015. Lanosterol reverses protein aggregation in cataracts. *Nature*. 523:607-611.
- Zhu, X., K. Gaus, Y. Lu, A. Magenau, R.J. Truscott, and T.W. Mitchell. 2010a. alpha- and beta-crystallins modulate the head group order of human lens membranes during aging. *Invest Ophthalmol Vis Sci*. 51:5162-5167.
- Zhu, X., A. Korlimbinis, and R.J. Truscott. 2010b. Age-dependent denaturation of enzymes in the human lens: a paradigm for organismic aging? *Rejuvenation research*. 13:553-560.
- Zigman, S., T. Paxhia, G. Marinetti, and S. Girsch. 1984. Lipids of human lens fiber cell membranes. *Curr Eye Res*. 3:887-896.

HUMAN LENS AGEING



SPHINGOLIPID



PHOSPHATIDYLCHOLINE

PHOSPHATIDYLETHANOLAMINE

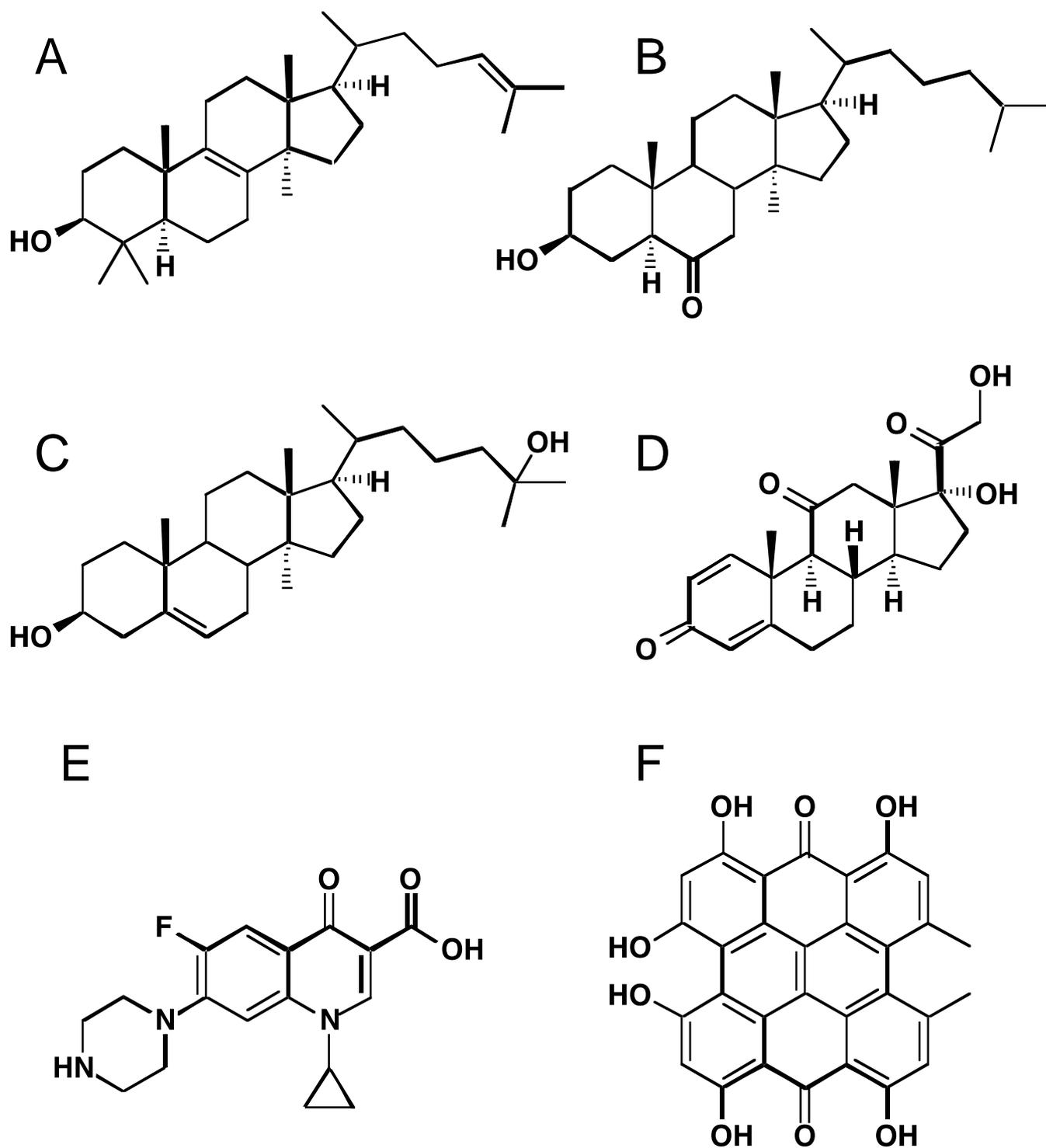


PROTEIN POSTTRANSLATIONAL
MODIFICATION



PROTEIN ACCUMULATION ON
PLASMA MEMBRANES

Fig. 1 Barnes and Quinlan



Barnes and Quinlan Fig. 2