Long-lived metabolic enzymes in the crystalline lens identified by pulse-labeling of mice and mass spectrometry

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Abstract The lenticular fiber cells are comprised of extremely long-lived proteins while still maintaining an active biochemical state. Dysregulation of these activities has been implicated in diseases such as age-related cataracts. However, the lenticular protein dynamics underlying health and disease is unclear. We sought to measure the global protein turnover rates in the eye using nitrogen-15 labeling of mice and mass spectrometry. We measured the $^{14}\text{N}/^{15}\text{N}$-peptide ratios of 248 lens proteins, including Crystallin, Aquaporin, Collagen and enzymes that catalyze glycolysis and oxidation/reduction reactions. Direct comparison of lens cortex versus nucleus revealed little or no $^{15}\text{N}$-protein contents in most nuclear proteins, while there were a broad range of $^{14}\text{N}/^{15}\text{N}$ ratios in cortex proteins. Unexpectedly, like Crystallins, many enzymes with relatively high abundance in nucleus were also exceedingly long-lived. The slow replacement of these enzymes in spite of young age of mice suggests their potential roles in age-related metabolic changes in the lens.

Introduction

The lens is a transparent body with an essential role in visual acuity. It consists of an outer capsule of type IV collagen-laminin membrane, the cortex of lens epithelium, and denuclearized and organelle-free fiber cells at the core. A single layer of germinal cells beneath the anterior capsule gives rise to transitional cells which differentiate into elongating fiber cells and finally the mature lenticular fiber cells forming the nuclear mass of the lens. In this process, the human lens continues to grow slowly in its weight and size throughout life (Vavvas et al., 2002; Guirou et al., 2013; Augusteyn, 2007; Bassnett, 2002). Frequently associated with aging, lens disease, such as cataracts, account for approximately half of the global blindness (the World Health Organization data). However, the underlying molecular mechanisms for most cataracts remain poorly understood (Pescosolido et al., 2016). Evidence suggest that accumulation of oxidized proteins and lipids predisposes the lens to nuclear cataract development (Reddy, 1971; Williams, 2006). Despite the slow turnover of the lens tissue, it remains a site of biochemical activity (Reddy and Glibin, 1984; Hejtmancik et al., 2015), in which the production of reducing metabolites and perhaps local enzymatic reactions within the fiber cells are important in combating oxidative stress.

Previously, radiocarbon ($^{14}\text{C}$) dating studies demonstrated that with respect of the total protein and lipid, there was little turnover at the nuclear core of lens (Lynnerup et al., 2008; Hughes et al., 2015; Nielsens et al., 2016). However, a recent single-fiber-cell transcriptome study of postnatal day 2 mice detected mRNA encoding proteins known to exist in anucleate lens fibers (Gangalam et al., 2018), implicating active protein synthesis. In order to gain insight into the protein dynamics of
lenticular fiber cells, including their structural proteins, chaperones and enzymes, we performed whole organism pulse-labeling of mice with heavy nitrogen-15 ($^{15}\text{N}$) supplied in the diet to assist distinguish newly synthesized proteins from their counterparts existing before labeling.

## Results

**The lens had extremely slow turnover of its proteome as compared to non-lens tissues in the eye**

Between 3 and 15 weeks of age, mice were fed with exclusively $^{15}\text{N}$ chow (Savas et al., 2012; Liu et al., 2018), following which the eye tissues were harvested. The entire lens was processed and then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Figure 1A–C and Methods). In total, 535 proteins were identified at 1% FDR at the protein level, of which 248 proteins showed the presence of both fully $^{14}\text{N}$ and $^{15}\text{N}$ spectra for calculating their $^{14}\text{N}/^{15}\text{N}$ ratios (MS1 ratios of the old vs. the new protein. See Methods, Figure 1D and Supplementary file 1). These ratios reflected the proportions of individual proteins being replaced with new $^{15}\text{N}$ proteins in addition to newly formed fiber layers being added to the growing lens. More than 50% of individual proteins in the lens were only detected in their $^{14}\text{N}$ forms, as compared to 1.8% and 2.8% in the vitreoretinal and sclera/choroid tissues respectively (Figure 2A and Supplementary file 1). These non-lens tissues had a majority of individual proteins completely replaced by new $^{15}\text{N}$ proteins, in contrast to the slow turnover of 1.7% of individual lens protein. While the determination of lack of $^{15}\text{N}$ peptides is subjected to MS detection sensitivity, we listed only the most abundant proteins with the total absence of $^{15}\text{N}$ labels (Figure 2B). These extremely long-lived proteins included structural proteins such as PE-binding protein 1 (PEBP-1), $\beta$-Catenin, Moesin, and enzymes that are involved in oxidoreduction such as Peroxiredoxin-2 (Prdx2), Farnesyl pyrophosphate synthase (FPS) and Aldehyde dehydrogenase (Aldh7A), and in glycolysis such as ATP-dependent 6-phosphofructokinase (ATP-PFK). For those proteins with both $^{14}\text{N}$ and $^{15}\text{N}$ peptides detected, lens proteins exhibited the most long-lived proteins with greater $^{14}\text{N}$ proportions (Figure 2C).

**The longevity of proteins associated with fiber cell differentiation**

Meanwhile, the histone variant H3.3 was among the fastest turned over proteins ($^{14}\text{N}/^{15}\text{N} = 0.2$, Figure 2D). H3.3 is associated with transcription loci in the chromosome (Szenker et al., 2011; Toyama et al., 2013) and its frequent replacement by newer H3.3 indicated active transcription and translation activities in the lens, most likely in the capsule and the cortex. By contrast, histone H3.1 and H3.2 in the heterochromatin regions that only renew during cell cycle replication (Hake and Allis, 2006) were found to be comprised of a greater proportion of older proteins ($^{14}\text{N}/^{15}\text{N} = 2.98$ and 4.02, respectively), consistent with the notion that most lens fiber cells are postmitotically differentiated and subsequently lose chromatin (Bassnett and Matacic, 1997). The overall rate of lens cell growth was estimated to be slow, with 1/5 to 1/4 of H3.2 and H3.1 being synthesized with full $^{15}\text{N}$ during the 12 week period. However, in these fiber cells that had ceased to replicate, the beaded filament proteins of Phakinin and Filensin (Blankenship et al., 2001; Wenke et al., 2016) that are implicated in cataract development (Conley et al., 2000; Jakobs et al., 2000; Carter et al., 2000) were still being actively produced ($^{14}\text{N}/^{15}\text{N}$ equals 2.02 and 1.02 respectively) (Figure 2D and Supplementary file 1).

**A wide range of crystallin $\alpha$, $\beta$ and $\gamma$ new protein synthesis**

The $\gamma$-Crystallins A/E/F/N that are localized to the nuclear lens showed the greatest proportion of their $^{14}\text{N}$-proteins ($^{14}\text{N}/^{15}\text{N} > 20$), followed by phosphoglycerate mutase 2 (Pgm2) that catalyzes glycolysis ($^{14}\text{N}/^{15}\text{N} > 20$), and cysteine protease Calpain-3 ($^{14}\text{N}/^{15}\text{N} = 18.8$) responsible for protein degradation (Figure 2D). Crystallins are the most abundant proteins in the lens (Figure 1D), and they are divided into $\alpha$, $\beta$ and $\gamma$ protein groups based on sequence homology. $\alpha$- and $\beta$- Crystallins (Crya and Cryb) are chaperone proteins for protein refolding under conditions of oxidative stress (Hejtmancik et al., 2015; Horwitz et al., 1999; Andley, 2007), and the densely packed $\gamma$-Cryallins (Cryg) contribute to reflection and hardness of the lens. Interestingly, a range of $^{14}\text{N}/^{15}\text{N}$ ratios was detected among distinct family members of each class (Figure 2D and Supplementary file 1): from 1.4 to 3.01 for $\alpha$-Crystallins, 1.16 to 9.13 for $\beta$-Crystallins, and 1.46
Figure 1. The $^{15}$N-labeling workflow for measuring the protein dynamics. (A) After weaning, C57BL/6J mice were subjected to an exclusively $^{15}$N chow diet starting at P21 for a total duration of 12 weeks. $^{15}$N was incorporated into newly synthesized proteins. (B) In LC-MS/MS, $^{14}$N- and $^{15}$N-peptides of the same sequence co-elute (left panel). Regardless of where MS/MS is triggered (arrows pointing at random positions), the MS1 peptide signal intensities between the $^{14}$N and $^{15}$N channels reflect of their relative abundance (right panel). (C) Representative proteins showed different turnover rates. (D) Individual Crystallins were analyzed for their MS1 ratios. Protein abundance (Spec. #) was plotted against the $^{14}$N/$^{15}$N MS1 ratios.
to >20 for γ-Crystallins. These results are in agreement with our previous analysis of Crystallin longevity in aged rats (Toyama et al., 2013).

The gap junctions, the water channels, and the extracellular matrices of the lens

Beside the Crystallins, cataract-linked mutations have been reported in other structural proteins (Shiels et al., 2010; Churchill and Graw, 2011), including those forming the connexin gap junction channel (Berthoud and Ngezahayo, 2017; Goodenough, 1992) and aquaporin water channel (Agre and Kozono, 2003) (A complete list of all identified proteins in Supplementary file 1 with selected examples shown in Figure 2D). In our dataset,Cx50 was among the longest-lived gap junction proteins with an \( ^{14}\text{N}/^{15}\text{N} \) ratio of 7.19, consistent with Cx50’s presence in mature fibers at the nucleus (White et al., 1992). This was in contrast with the water channel Aquaporins of Aqp0/Lim1/Mip \( (^{14}\text{N}/^{15}\text{N} = 1.34) \) (Bateman et al., 2000; Berry et al., 2000; Francis et al., 2000) and Lim2/MP19/Cataract19 \( (^{14}\text{N}/^{15}\text{N} = 3.25) \) (Pras et al., 2002). These gap junctions and Aquaporins form channels known to be critical for the passage of important small metabolites to the lens, and mutations of their genes predispose individuals to cataracts (Verkman et al., 2014; Liu et al., 2011; Chepelinsky, 2009).

Unlike the mature fibers that uniquely form the core of the lens, the outer capsule of the lens resembles other basement membranes such as the glomerular basement membrane of the kidney. Mutations in the major components of type IV Collagen cause Alport syndrome that concurrently affects the kidney, the eye including cataracts and the ear. These type IV Collagen proteins in the lens membrane matrix are produced by the adjacent epithelial cells (Arita et al., 1993). Collagen IV-\(\alpha_1\), -\(\alpha_2\) and -\(\alpha_3\) all had balanced \( ^{14}\text{N}/^{15}\text{N} \) ratios of 1.52, 1.34 and 1.34 respectively (Supplementary file 1) that had greater proportions of older \( ^{14}\text{N}\)-proteins than the majority of lens proteins, however remarkably similar to their counterparts in the kidney at 1.43, 1.43 and 1.16 respectively (not shown). By contrast, another basement membrane protein Perlecan/Hspg2 at the outer and inner surfaces that contributes to anionic charges (Danysh and Duncan, 2009) lived longer than Collagen IV \( (^{14}\text{N}/^{15}\text{N} = 4.11) \) (Figure 2D). This apparent contrast of having long-lived Perlecan may partly explain the phenomenon of the lens capsule losing its net anionic charges during aging (Winkler et al., 2001): the slow replacement Perlecans may contribute to the gradual loss of its sulfated glycosaminoglycan moieties.

Contrasting difference between the long preservation of proteins in the nucleus and a varying dynamic turnover of cortex proteins in the lens

Next, we sought to compare protein dynamics in the nucleus and in the cortex of lens. As expected, proteins extracted from the nucleus were mostly shared with their cortex counterparts (Figure 3A and B), and a majority of nuclear proteins had little or no protein turnover as determined by their \( ^{14}\text{N}/^{15}\text{N} \) ratios close to or above 100 (Figure 3B: upper limit set at 100). Meanwhile, proteins harvested from the cortex tend to have a wide range of new vs. old protein ratios (Figure 3B). This was also reflected among Crystallin isoforms (Figure 3B: highlighted), with α-Crystallins having the highest contents of \( ^{15}\text{N} \) in the cortex (Figure 3C). Although all Crystallin isoforms appeared to contain fractions of newly expressed \( ^{15}\text{N}\)-proteins in the cortex, their individual abundance at the total protein level vary substantially (Figure 3C). For instance, while α- and β-Crystallins had more balanced presence between cortex and nucleus fractions, γ-Crystallin levels in the cortex were very low (Figure 3C). When protein abundance of all proteins was compared, the cortex and the nucleus had comparable \( ^{14}\text{N} \) levels, in contrast to very low new protein contents in the nucleus (Figure 3D, and Supplementary file 1).
Figure 2. The lenticular proteins generally had longer life times than the vitreoretinal and sclera/choroid proteins. (A) Inter-tissue comparison of protein longevity showing numbers of proteins based on $^{14}\text{N}$-peptides only, $^{15}\text{N}$-peptides only, or $^{14}\text{N}/^{15}\text{N}$ ratios calculated (hatched pie). While in the lens a large number of protein had a greater proportion of $^{14}\text{N}$, other ocular tissues had a faster protein turnover with more proteins completely labeled with $^{15}\text{N}$ within 12 weeks. (B) A list of the most abundant proteins that were only detected by $^{14}\text{N}$ peptides with the absence of $^{15}\text{N}$.

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Figure 2 continued on next page
Very slow turnover of enzymes for Redox and glycolysis

The most unexpected finding of this $^{15}$N-labeling study was that metabolic enzymes in the lens were remarkably long-lived, particularly those catalyzing electron transport chain (ETC) in glycolysis and redox reactions (Figure 4 and Supplementary file 1). For instance, phosphoglycerate mutase 2 (Pgam2) that catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate had an $^{14}$N/$^{15}$N ratio of $>20$, comparable to those of γ-Crystallin. A number of other enzymes in glycolysis (such as Pgk1, 6-Phosphogluconolactone, β-Enolase and γ-Enolase) also had $^{14}$N/$^{15}$N ratios greater than that of histone H3.2 ($^{14}$N/$^{15}$N = 4.02), the benchmark protein that had ceased to renew following fiber cell differentiation. It can be inferred that these enzymes were preserved beyond the point of fiber cell differentiation, which is consistent with the notion that biochemical activity continues in mature fiber cells. Glycolysis that generates electron transport and ATP is an integral process of the overall redox reaction. Given the importance of maintaining a reduced cellular environment, redox enzymes such as glutathione S-transferases (GST) and S-synthases (GSS), and alcohol dehydrogenase class-3 (Adh5) were not only abundantly present in the lens but also long-lived with their $^{14}$N/$^{15}$N ratios between 4.45 and 5.94 after 12 weeks of labeling (Figure 2D, Figure 4 and Supplementary file 1). It is important to note that a majority of these enzymes were still preserved in the nuclear lens (Figure 4 and Supplementary file 1) with most of them having predominately $^{14}$N contents. This observation strongly indicates active enzymatic activities at the core of the lens attributable to these extremely long-lasting enzymes.

Discussion

We performed $^{15}$N-labeling of mice in conjunction with mass spectrometry-based measurement of $^{15}$N- vs. $^{14}$N-protein ratios. These ratios to a great extent reflected the turnover rates of individual proteins in the lens. The results illustrated a range of new protein synthesis activities, as well as an unexpected panel of proteins that were preserved long after terminal differentiation of the fiber cells, particularly in the nuclear lens. In this latter category, besides structural, water channel and chaperone proteins, metabolic enzymes that catalyze glycolysis and redox reactions were long-lived, and therefore may have implications for age-related cataract formation.

Although the mouse model is our convenient choice for the labeling protocol that is associated with a high cost of $^{15}$N-chow, there are notable limitations in addressing the protein basis for cataract. For instance, certain Crystallin isoforms do not express in human (Figure 3C). In addition, the choice of rather young animals between 3 and 15 weeks was not ideal for understanding the disease. Instead, the results are more relevant to lens development from post-weaning, through sexual maturity (by 4 weeks) to fully grown adult (by 12–24 weeks), which is equivalent for human age of 20–30 years (information from jax.org) (Dutta and Sengupta, 2016). However, the most common presenile cataracts have much later onset. Therefore the protein turnover indices only reveal a narrow spectrum of the changes in protein dynamics in the lens, and the observed preservation of redox enzymes in the nucleus core might not last as long as some Crystallins at old age. In addition to oxidation in cataract lens, many other biochemical changes also occur. Spontaneous conversion of L- to D-amino acids in proteins contributes to racemization in cataract lens (Hooi and Truscott, 2011), and protein isomerase activities are thought to have a role counteracting cataract development (Lyon et al., 2018; Lyon et al., 2019). Related to point that older proteins tend to accumulate post-translational modifications, our mass spec-based approach was not set up to detect all relevant modifications such as non-enzymatic deamidation of Gln and Asn sidechains that occurs more often in old age (Forsythe et al., 2019). This omission of modified peptides would have
**Figure 3.** Comparison of lens cortex and nucleus proteins by $^{14}\text{N}/^{15}\text{N}$ ratios. (A) Lens tissues from the cortex and the nucleus were separately harvested and subsequently resolved by SDS-PAGE. Prominent gel bands of Crystallins were present in both cortex and nucleus fractions, whereas in the higher molecular weight areas of the gel the cortex tissue appeared more intensely stained for its protein contents. (B) A direct comparison of 166 proteins identified in both cortex (red diamond) and nucleus (blue circle) plotted along the x-axis with their $^{14}\text{N}/^{15}\text{N}$ ratios separately plotted against...
affected the calculation of $^{14}$N/$^{15}$N ratios, particularly when the types of modifications were more prevalent in the older $^{14}$N-proteins. Although we only included other non-lens tissues such as the retinal, the sclera and the choroid as controls for having faster turnover dynamics, proteins such as laminin, collagen and fibrillin elastic fibers in these tissues were found to be long-lived (not shown). It should be noted that since we selected a relatively long duration of the labeling process (12 weeks in total), we have passed the most dynamic phase of $^{14}$N-to-$^{15}$N transition in non-lens tissues. It is however anticipated that $^{15}$N pulse-labeling, when the duration is adjusted based on the target tissue, can provide valuable information about protein dynamics, which will be particularly useful in comparing normal and disease tissues for insight on pathogenic transformation or adaptation.

Materials and methods

Stable isotope labeling in mouse (SILAM)
The general method of raising $^{15}$N-labeled mice was described previously (Savas et al., 2012; Liu et al., 2018). In brief, starting at postnatal day 21 after weaning, C57BL/6J mice were fed exclusively with a $^{15}$N-raised spirulina diet (from Cambridge Isotopes and Harlan Laboratories) for 12 consecutive weeks. At this time, the $^{15}$N-proteins in the serum was determined to be greater than 99% by mass spectrometry (Liu et al., 2018).

Harvest of the crystallin lens
Immediately after cervical dislocation of the mice, eye globes were surgically removed and then dissected for collecting the lens. For total protein extraction of the lens, the intact lens was first washed in phosphate-buffered saline, and then submerged into 100 μL of 2x concentrated SDS sample buffer containing 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue and 0.125 M Tris HCl, pH = 6.8. The lens tissue was dissolved following sonication on ice. After the solution turned completely clear, the samples were let to be further dissolved at 4°C for overnight. On the next day, the tissue homogenates from three mice were combined and were resolved by SDS-PAGE. Following staining of the gel with GelCode Blue (Thermo Fisher Scientific), the gel lanes were excised and further divided into ~1 mm$^3$-sized gel cubes. These gel cubes were subsequently subjected to a standard in-gel trypsin/lys-C digestion (Promega), reduction and iodoacetamide alkylation protocol following the manufacturer’s instruction.

Separation of cortex and nucleus tissues
Lenses retrieved from frozen stock formed clear separation between their cortex and nucleus tissues. The cortex in association with the lens capsule were fragile, whereas the nuclear core tissue remained rigid and was picked out using a pair of tissue forceps. The collected nucleus specimens (combined from three eyes) were washed three times in PBS solution before submerged in SDS sample buffer. The lens cortex (also combined from three eyes) was collected without the lens capsule, and then dissolved in SDS sample buffer. Following sonication until the tissue homogenates turned clear, the samples were loaded on an SDS-PAGE for protein separation. Proteins were subsequently digested from the gel with Trypsin/LysC as described above.
The general procedures for conducting $^{15}$N- vs. $^{14}$N-based proteomics were described previously (Liu et al., 2018; Savas et al., 2017). In brief, 3 μg of the peptides in Buffer A solution (94.785% H2O, 5% ACN, 0.125% FA) was loaded onto a nanoViper C18 trap column. The peptides were resolved following a 2 hr gradient following an increase Buffer B (99.875% ACN, 0.125% FA) concentration. Peptides were electrosprayed from the Nanospray Flex Ion Source and analyzed on the Orbitrap Fusion Tribrid mass spectrometer. MS parameters were as follows: ion transfer tube...
temp = 300˚C, Easy-IC internal mass calibration, default charge state = 2. Detector type set to Orbitrap, with 60K resolution, wide quad isolation, mass range = normal, scan range = 300–1500 m/z. Max injection time = 50 ms, AGC target = 200,000, microscans = 1, S-lens RF level = 60. Without source fragmentation, datatype = positive and centroid, MIPS was on, included charge states = 2–6 (reject unassigned). Dynamic exclusion enabled with n = 1. Precursor selection decision = most intense, top 20, isolation window = 1.6, scan range = auto normal, first mass = 110, collision energy 30%, CID, Detector type = ion trap, max injection time = 75 ms, AGC target – 10,000, inject ions for all available parallelizable time.

Spectral analysis and protein quantification

Spectral analysis was done using Integrated Proteomics Pipeline (IP2), including running ProLuCID searches against the RefSeq mouse dataset. Basic parameters of 10 ppm precursor mass tolerance and 600 ppm for fragmented ions were used. Searches were filtered with DTAselect containing one peptide per protein, at least one trypic end and unlimited missed cleavages of a minimum of 6 amino acid, with a false discovery rate (FDR) < 0.001, fixed modification of +57.02146 Da on cysteine residues, and all precursor mass within 10 ppm of expected. To estimate peptide FDRs accurately (set at <1%), target/decoy database was used containing the reversed sequences of all the proteins appended to the target database (Elias and Gygi, 2007). Searches were done for combined light and heavy peptides and Census quantified (Savas et al., 2017).

To calculate the $^{14}\text{N}/^{15}\text{N}$ peptide ion intensity, the ProLuCID results were used to reconstruct MS1 ion chromatograms in the m/z range that included both the heavy and light peptide (Liu et al., 2018; Park et al., 2008). The intensity ratios were then calculated per peptide using the reconstructed chromatogram. Peptide ratios with correlation values greater than 0.5 were used to remove poor-quality peptide ratio measurements. When more than two peptides were found for the same protein, Census removed outliers based on the Grubbs test (p value < 0.01) by calculating the SDs for the proteins. With QuantCompare, the final peptide ratios were generated. For each protein, its heavy vs. light ratios were represented by the composite of all peptide ratios identified by MS that are assigned to the protein. In cases of extremely low signals in one of the two channels, which will render extremely high or low ratio values mathematically, we arbitrarily set upper and lower limits of the protein ratios at 20 and 0.05. For the cortex vs. nucleus comparison, because of the extreme longevity of nucleus proteins we raised the ceiling to 100 for $^{14}\text{N}/^{15}\text{N}$ ratios. The final list of RefSeq protein entries were searched against the UniProtKB database to obtain a non-redundant set of proteins based on their unique gene identifiers (listed in Supplementary file 1).

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Additional files
Supplementary files
- Supplementary file 1. A complete list of proteins identified by mass spectrometry. Tables S1 to S5 show all proteins identified in whole lens, lens cortex, lens nucleus, sclera choroid and vitreoretinal respectively. The tables also include individual peptide and protein $^{14}$N/$^{15}$N ratios, as well as relative total protein amount (calculated as MS spectral count).
- Transparent reporting form

Data availability
All data generated or analysed during this study are included in the manuscript and supporting files. Source data file (Supplementary File 1) provides a complete list of proteins identified by mass spectrometry. Data has also been deposited at MassIVE under the accession number MSV000084566.

The following dataset was generated:

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References
Agre P, Kozono D. 2003. Aquaporin water channels: molecular mechanisms for human diseases. FEBS Letters [555:72–78](https://doi.org/10.1016/s0014-5793(03)01083-4), PMID: 14630322
Andley UP. 2007. Crystallins in the eye: function and pathology. Progress in Retinal and Eye Research [26:78–98](https://doi.org/10.1016/j.preteyeres.2006.10.003), PMID: 17166758
Arita T, Murata Y, Lin LR, Tsuji T, Reddy VN. 1993. Synthesis of Lens capsule in long-term culture of human Lens epithelial cells. Investigative Ophthalmology & Visual Science [34:355–362](https://doi.org/10.1093/iovs.2001.1111), PMID: 11878813
Augusteyn RC. 2007. Growth of the human eye lens. Molecular Vision [13:252–257](https://pmid.org/17356512), PMID: 17356512
Bassnett S. 2002. Lens organelle degradation. Experimental Eye Research [74:1–6](https://doi.org/10.1006/exer.2001.1111), PMID: 11878813
Bassnett S, Mataic D. 1997. Chromatin degradation in differentiating fiber cells of the eye Lens. The Journal of Cell Biology [137:37–49](https://doi.org/10.1083/jcb.137.1.37), PMID: 9105035
Bateman JB, Johannes M, Flodman P, Geyer DP, Clancy KP, Heimannz C, Kojis T, Berry R, Sparkes RS, Spence MA. 2000. A new locus for autosomal dominant cataract on chromosome 12q13. *Investigative Ophthalmology & Visual Science* **41**:2665–2670. PMID: 10937580

Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. 2000. Missense mutations in MIP underlie autosomal dominant ‘polymorphic’ and lamellar cataracts linked to 12q. *Nature Genetics* **25**:15–17. DOI: https://doi.org/10.1038/75538, PMID: 10802646

Berthoud VM, Ngezhahay A. 2017. Focus on lens connexins. *BMC Cell Biology* **18**:6. DOI: https://doi.org/10.1186/s12860-016-0116-6, PMID: 28124426

Blankenship TN, Hess JF, FitzGerald PG. 2001. Development- and differentiation-dependent reorganization of intermediate filaments in fiber cells. *Investigative Ophthalmology & Visual Science* **42**:735–742. PMID: 11222535

Carter JM, McLean WH, West S, Quinlan RA. 2000. Mapping of the human CP49 gene and identification of an intragenic polymorphic marker to allow genetic linkage analysis in autosomal dominant congenital cataract. *Biochemical and Biophysical Research Communications* **270**:432–436. DOI: https://doi.org/10.1016/S0006-291X(00)00054-7, PMID: 10753642

Chepelinsky AB. 2009. Structural function of MIP/aquaporin 0 in the eye Lens; genetic defects lead to congenital inherited cataracts. *Handbook of Experimental Pharmacology* **190**:265–297. DOI: https://doi.org/10.1007/978-3-540-79885-9_14

Churchill A, Graw J. 2011. Clinical and experimental advances in congenital and paediatric cataracts. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**:1234–1249. DOI: https://doi.org/10.1098/rstb.2010.0227

Conley YM, Erturk D, Keverline A, Mah TS, Keravala A, Barnes LR, Bruchis A, Hess JF, FitzGerald PG, Weeks DE, Ferrell RE, Gorin MB. 2000. A juvenile-onset, progressive cataract locus on chromosome 3q21-q22 is associated with a missense mutation in the beaded filament structural protein-2. *The American Journal of Human Genetics* **66**:1426–1431. DOI: https://doi.org/10.1086/308271, PMID: 10729115

Danysh BP, Duncan MK. 2009. The lens capsule. *Experimental Eye Research* **88**:151–164. DOI: https://doi.org/10.1016/j.exer.2008.08.002, PMID: 18773892

Dutta S, Sengupta P. 2016. Men and mice: relating their ages. *Life Sciences* **152**:244–248. DOI: https://doi.org/10.1016/j.lfs.2015.10.025, PMID: 26596653

Elias JE, Gygi SP. 2007. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nature Methods* **4**:207–214. DOI: https://doi.org/10.1038/nmeth1019, PMID: 17327847

Forsythe HM, Vetter CJ, Jara KA, Reardon PN, David LL, Barber EJ, Lampi KJ. 2019. Altered protein dynamics and increased aggregation of human yS-4 crystallin due to cataract-associated deamidations. *Biochemistry* **58**:4112–4124. DOI: https://doi.org/10.1021/acs.biochem.9b00593, PMID: 31490062

Francis P, Chang JJ, Yasui M, Berry V, Moore A, Wyatt MK, Wistow G, Bhattacharya, Agre P. 2000. Functional impairment of Lens aquaporin in two families with dominantly inherited cataracts. *Human Molecular Genetics* **9**:2329–2334. DOI: https://doi.org/10.1093/oxfordjournals.hmg.a018925, PMID: 11009197

Gangalum RK, Kim D, Kashyap RK, Mangul S, Zhou X, Elashoff D, Bhattacharya S, Agre P. 2018. Spatial Analysis of Single Fiber Cells of the Developing Ocular Lens Reveals Regulated Heterogeneity of Gene Expression. *iScience* **10**:66–79. DOI: https://doi.org/10.1016/j.isci.2018.11.024

Goodenoough DA. 1992. The crystalline Lens. A system networked by gap junctional intercellular communication. *Seminars in Cell Biology* **3**:49–58. DOI: https://doi.org/10.1016/1090-425X(92)90038-9, PMID: 1320431

Guirou N, Napo A, Dougonon A, Bakayoko S, Sidibé F, Sidibé MK, Conaré I, Traoré L, Traoré J. 2013. [Visual outcome of cataract surgery in adults]. *Journal Francais d’ophtalmologie* **36**:19–22. DOI: https://doi.org/10.1016/j.jfo.2012.04.016, PMID: 23218597

Hake SB, Allis CD. 2006. Histone H3 variants and their potential role in indexing mammalian genomes: the “H3 barcode hypothesis”. *PNAS* **103**:6428–6435. DOI: https://doi.org/10.1073/pnas.0608031103, PMID: 16571659

Hejtmancik JF, Riazuddin SA, McGreal R, Liu W, Cvekl A, Shiels A. 2015. Lens biology and biochemistry. *Progress in Molecular Biology and Translational Science* **134**:169–201. DOI: https://doi.org/10.1016/bse.ptms.2015.04.007, PMID: 26310155

Hooi MYS, Truscott RJW. 2011. Racemisation and human cataract. d-Ser, d-Asp/Asn and d-Thr are higher in the lifelong proteins of cataract lenses than in age-matched normal lenses. *Age* **33**:131–141. DOI: https://doi.org/10.1007/s11357-010-9171-7

Horwitz J, Bova MP, Ding LL, Haley DA, Stewart PL. 1999. Lens alpha-crystallin: function and structure. *Eye* **13 (Pt 3b)**:403–408. DOI: https://doi.org/10.1038/eye.1999.114, PMID: 10627817

Hughes JR, Levchenko VA, Blanksby SJ, Mitchell TW, Williams A, Truscott RJW. 2015. No turnover in Lens lipids for the entire human lifespan. *eLife* **4**:e06003. DOI: https://doi.org/10.7554/eLife.06003

Jakobs PM, Hess JF, FitzGerald PG, Kramer P, Weleber RG, Litt M. 2000. Autosomal-dominant congenital cataract associated with a deletion mutation in the human beaded filament protein gene BFSP2. *The American Journal of Human Genetics* **66**:1432–1436. DOI: https://doi.org/10.1086/302872, PMID: 10739768

Liu J, Xu J, Gu S, Nicholson BJ, Jiang JX. 2011. Aquaporin 0 enhances gap junction coupling via its cell adhesion function and interaction with connexin 50. *Journal of Cell Science* **124**:198–206. DOI: https://doi.org/10.1242/jcs.072652, PMID: 21172802

Liu P, Thomson BR, Khaltayan N, Feng L, Liu X, Savas JN, Quaggin SE, Jin J. 2018. Selective permeability of mouse blood-aqueous barrier as determined by 15N-heavy isotope tracing and mass spectrometry. *PNAS* **115**:9032–9037. DOI: https://doi.org/10.1073/pnas.1807982115, PMID: 30127000
Lynnerup N, Kjeldsen H, Heegaard S, Jacobsen C, Heinemeier J. 2008. Radiocarbon dating of the human eye Lens crystallines reveal proteins without carbon turnover throughout life. PLOS ONE 3:e1529. DOI: https://doi.org/10.1371/journal.pone.0001529

Lyon YA, Sabbah GM, Julian RR. 2018. Differences in α-Crystallin isomerization reveal the activity of protein isoaspartyl methyltransferase (PIMT) in the nucleus and cortex of human lenses. Experimental Eye Research 171:131–141. DOI: https://doi.org/10.1016/j.exer.2018.03.018, PMID: 29571628

Lyon YA, Collier MP, Riggs DL, Degiacomi MT, Benesch JLP, Julian RR. 2019. Structural and functional consequences of age-related isomerization in α-crystallins. Journal of Biological Chemistry 294:7546–7555. DOI: https://doi.org/10.1074/jbc.RA118.007052, PMID: 30804217

Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG, Christiansen JS, Olsen J, Ramsey CB, Brill RW, Simon M, Steffenkof SF, Steffenkof JS. 2016. Eye Lens radiocarbon reveals centuries of longevity in the Greenland shark (Somniosus microcephalus). Science 353:702–704. DOI: https://doi.org/10.1126/science.aaf1703, PMID: 27516602

Park SK, Venable JD, Xu T, Yates JR. 2008. A quantitative analysis software tool for mass spectrometry-based proteomics. Nature Methods 5:319–322. DOI: https://doi.org/10.1038/nmeth.1195, PMID: 18345006

Pescosolido N, Barbato A, Giannotti R, Komaia C, Lenarduzzi F. 2016. Age-related changes in the kinetics of human lenses: prevention of the cataract. International Journal of Ophthalmology 9:1506–1517. DOI: https://doi.org/10.18240/jo.2016.10.23, PMID: 27803872

Pras E, Levy-Nissenbaum E, Bakh A, Lahat H, Assia E, Gefen-Carmi N, Frydman M, Goldman B, Pras E. 2002. A missense mutation in the LM2 gene is associated with autosomal recessive presenile cataract in an inbred iranian Jewish family. The American Journal of Human Genetics 70:1363–1367. DOI: https://doi.org/10.1086/340318, PMID: 11917274

Reddy VN. 1971. Metabolism of glutathione in the Lens. Experimental Eye Research 11:310–328. DOI: https://doi.org/10.1016/S0014-4835(71)80043-X

Reddy VN, Giblin FJ. 1984. Metabolism and function of glutathione in the Lens. Ciba Foundation Symposium 106:65–87. DOI: https://doi.org/10.1002/9780470720875.ch5, PMID: 6568981

Savas JN, Toyama BH, Xu T, Yates JR, Hetzer MW. 2012. Extremely long-lived nuclear pore proteins in the rat brain. Science 335:942. DOI: https://doi.org/10.1126/science.1217421, PMID: 22300851

Savas JN, Wang YZ, DeNardo LA, Martinez-Bartolome S, McClatchy DB, Hark TJ, Shanks NF, Cozzolino KA, Lavallée-Adam M, Smukowski SN, Park SK, Kelly JW, Koo EH, Nakagawa T, Masliah E, Ghosh A, Yates JR. 2017. Amyloid accumulation drives Proteome-wide alterations in mouse models of Alzheimer’s Disease-like Pathology. Cell Reports 21:2614–2627. DOI: https://doi.org/10.1016/j.celrep.2017.11.009, PMID: 29186695

Shiels A, Bennett TM, Hejtmanick JF. 2010. Cat-Map: putting cataract on the map. Molecular Vision 16:2007–2015. PMID: 21042563

Szenker E, Ray-Gallet D, Almouzni G. 2011. The double face of the histone variant H3.3. Cell Research 21:421–434. DOI: https://doi.org/10.1038/cr.2011.14

Toyama BH, Savas JN, Park SK, Harris MS, Ingolia NT, Yates JR, Hetzer MW. 2013. Identification of Long-Lived proteins reveals structural stability of essential cellular structures. Cell 154:971–982. DOI: https://doi.org/10.1016/j.cell.2013.07.037

Vavvas D, Azar NF, Azar DT. 2002. Mechanisms of disease: cataracts. Ophthalmology Clinics of North America 15:49–60. DOI: https://doi.org/10.1016/S0896-1549(01)00015-3, PMID: 12064081

Verkman AS, Anderson MO, Papadopoulos MC. 2014. Aquaporins: important but elusive drug targets. Nature Reviews Drug Discovery 13:259–277. DOI: https://doi.org/10.1038/nrd4226, PMID: 24625825

Wenke JL, McDonald WH, Schey KL. 2016. Spatially directed proteomics of the human Lens outer cortex reveals an intermediate filament switch associated with the remodeling zone. Investigative Ophthalmology & Visual Science 57:4108–4114. DOI: https://doi.org/10.1167/iovs.16-19791

White TW, Bruzzone R, Goodenough DA, Paul DL. 1992. Mouse Cx50, a functional member of the connexin family of gap junction proteins, is the Lens fiber protein MP70. Molecular Biology of the Cell 3:711–720. DOI: https://doi.org/10.1091/mbc.3.7.711, PMID: 1325220

Williams DL. 2006. Oxidation, antioxidants and cataract formation: a literature review. Veterinary Ophthalmology 9:292–298. DOI: https://doi.org/10.1111/j.1463-5224.2006.00498.x, PMID: 16939456

Winkler J, Wirbelauer C, Frank V, Laqua H. 2001. Quantitative distribution of glycosaminoglycans in young and senile (cataractous) anterior Lens capsules. Experimental Eye Research 72:311–318. DOI: https://doi.org/10.1006/exer.2000.0952, PMID: 11180980