Up-regulation of tau, a brain microtubule-associated protein, in lens cortical fractions of aged αA-, αB-, and αA/B-crystallin knockout mice

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Recommended Citation
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Purpose: α-Crystallin is expressed at high levels in the lens in a complex of αA- and αB-crystallin subunits in 3:1 molar ratios, and is known to maintain the solubility of unpolymerized tubulin and enhance the resistance of microtubules to depolymerization, but its effect on proteins classically associated with microtubule stability (microtubule associated proteins) in the lens is unknown. In the present study we examined the expression of the brain microtubule associated protein tau in lenses of α-crystallin gene knockout mice.

Methods: Quantitative RT-PCR, immunoblotting, cryo-immunoelectron microscopic and immunohistochemical methods were used to characterize the expression of tau in the lenses of αA-/-, αB-/-, and αA/B-/-crystallin mice.

Results: Immunoreactivity to tau, a 45-66 kDa brain microtubule associated protein that has been best characterized in neurons and neuronal pathologies, was uniquely upregulated in lens cortical fiber cells with aging and was associated with the microtubule fraction of αA-/-, αB-/-, and αA/B-/-crystallin mouse lenses, but was undetectable in wild type lenses. Quantitative RT-PCR analysis further showed an upregulation of tau transcripts in αA-/- and αA/B-/-crystallin lenses. Brain microtubule fractions served as a positive control for tau in these experiments. An increase in phosphorylation of tau was detected in αA-/- and αB-/-crystallin brain proteins.

Conclusions: Although tau aggregation and αB-crystallin expression have been shown to increase in neurodegenerative diseases, surprisingly tau expression increases in the α-crystallin knockout lenses, suggesting that αA- and αB-crystallins are important potential regulators of tau expression in lens.

The vertebrate lens is composed of a single layer of epithelial cells on its anterior surface, which divide and differentiate in the equatorial zone, and undergo elongation, and nuclei and organelle degradation [1,2]. This orderly process continues throughout life, and is thought to be responsible for maintaining a transparent lens. The lens fiber cells have a limited ability to turnover proteins as the lens ages. α-Crystallin, a major protein of lens fiber cells, is a member of the small heat shock protein family of chaperones, and is isolated from a major protein of lens fiber cells, is a member of the small heat shock protein family of chaperones, and is isolated from a major protein of lens fiber cells with aging and was associated with the microtubule fraction of αA-/-, αB-/-, and αA/B-/-crystallin mouse lenses, but was undetectable in wild type lenses. The role of α-crystallin in proteasomal targeting has also been shown during repeated cycling through the chaperone systems, allowing recognition of the incompletely folded protein by an E3 ubiquitin ligase and subsequent targeting of the misfolded protein for degradation by the proteasome [16,17]. The role of αB-crystallin in proteasomal targeting has also been shown during normal cellular processes, as illustrated by degradation of cyclin D1 during the cell cycle [17].

The brain microtubule associated protein tau, known to stabilize the microtubule cytoskeleton and promote microtubule assembly, is a group of 45-66 kDa proteins encoded by alternative splicing of a single gene [18]. There are six predominant tau isoforms in human brain containing 352-441 amino acids [19]. The tau proteins are unstructured in solution but bind in an ordered conformation along the microtubule protofilaments to stabilize and/or strengthen interactions between adjacent protofilament and increase microtubule integrity [20]. Aggregation of tau into paired helical filaments via hyperphosphorylation is the main component of neurofibrillary tangles found in the brains of Alzheimer disease (AD) patients [21-23]. Tau is thought to be associated with the primary pathology and neuronal damage in tauopathies.
like frontotemporal dementia (FTDP) [24-26]. The molecular chaperone/stress-inducible small heat shock protein αB-crystallin is known to be upregulated in astrocytes associated with senile plaques and cerebral amyloid angiopathy in AD [27,28]. αB-Crystallin is also associated with Lewy body disease, Parkinson disease, multiple sclerosis, FTDP and other neurodegenerative disorders [28-30]. The specific role of αB-crystallin in these disorders is unknown. Investigators have detected an early defining feature of Alzheimer disease, the Aβ peptides in the cytosol of the lens fiber cells, and suggested that the Aβ-mediated aggregation of lens proteins contributes to increased light scattering in the lens [31,32]. Indeed, several studies suggest protein-protein interactions between αB-crystallin and Aβ [33,34]. αB-Crystallin has also been shown to inhibit the aggregation of small Aβ peptides into mature fibrils in vitro [35]. Furthermore, studies by Frederikse et al. [36] on microtubules and kinesin-based transport in the lens suggest several fundamental parallels between lens and neuronal vesicle trafficking and cell biology, and suggest that Aβ-related vesicle trafficking disease mechanisms may be shared by lens and brain.

The microtubule cytoskeleton is a very dynamic network that plays a crucial role in key biologic processes such as cell division and intracellular traffic [37]. Microtubule dynamic instability and functions are regulated by microtubule-associated proteins (MAPs). αB-Crystallin enhances microtubule resistance to depolymerization, and maintains tubulin levels in vivo in muscle cells [38,39]. Our previous studies with αA-, αB- and αA/B-crystallin gene knockout mice suggested that the depletion of these chaperones is associated with changes in cell cycle parameters, and more specifically, a microtubule instability phenotype [40-43]. We therefore investigated the expression of the brain MAP tau, known to enhance microtubule stability, in microtubule fractions from αA- and αB-crystallin gene knockout mice in the current work. Surprisingly, in the αA- and αB-crystallin gene knockout mice we found that the tau is uniquely upregulated in the differentiated lens cortical fiber cells, which have a limited ability to turnover protein as the lens ages. Our results demonstrate that the lens is an important organ that can capture some of the systemic pathology involved in protein aggregation disorders that affect the brain, and suggest that the α-crystallin knockout mice may be a good model to study disease mechanisms involving protein aggregation in the brain.

**METHODS**

**Animals and tissues:** Wild type and αA+/−, αB+/−, and αA/B+/− knockout mice were used in this study. Wild type mice were the 129SvEv strain from Taconic Farms (Hudson, New York). The crystallin knockout mice αA+/−, αB/HSPB2−/− (termed αB−/− in this study) and αA/αB/HSPB2−/− (termed αA/B−/− in this study) were kindly provided by E. Wawrousek (National Eye Institute, NIH, Bethesda, MD) [44,45]. αB-Crystallin has also been termed HSPB5 [46]. Mice were inbred and all animal protocols were in accordance with the institutional policy on the use of animals in research [40,41]. Mice were used at 1 to 12 months of age. At the appropriate age, animals were killed by CO2 inhalation. Whole eyes were dissected, fixed and sectioned for immunofluorescent detection of tau. Whole lenses were dissected and epithelial and cortical fractions (Figure 1A) were isolated under a microscope, and were used to prepare microtubules and microtubule associated proteins as described previously [42,43]. Lens cortical fractions were used for RNA isolation. Brains collected from the mice of each genotype were also used.

**Antibodies:** The following tau antibodies were used for immunoblot, immunofluorescence and cryo-immuno electron microscopic (em) analysis: (1) KAM-MA305 (Stressgen, Ann Arbor, MI).

**Figure 1. Preparation of microtubule associated proteins. A:** Dissection of mouse lens epithelial and cortical fractions used for the isolation of microtubule associated proteins (MAPs). Wild type, αA+/−, αB+/−, and αA/B+/− lenses were dissected into epithelial (blue) and cortical fiber cell (red) fractions. Microtubules were reconstituted from lens cortical fiber cell lysates and MAPs were extracted as described under Experimental Procedures. B and C: Electron micrographs of mouse lens cortical fiber microtubules that were used to dissociate the MAPs. Microtubules were negatively stained and examined. B shows wild type microtubules and C shows αB−/− microtubules. The scale bar is equal to 50 nm.
Arbor, MI) at 1:1000 dilution for immunoblotting, and 1:100 for cryo-immuno em and immunofluorescence; (2) AT8 antibody to PHF-tau (Pierce, Rockford, IL) at 1:40 dilution for immunoblotting and immunofluorescence, and 1:5 for cryoimmuno electron microscopy [47]; (3) TAU-5 (Lab Vision, Fremont, CA) at 1:200 dilution for immunoblotting and 1:5 for cryoimmuno electron microscopy [48-50]; (4) pS422 (Biosource, Carlsbad, CA) at 1:1000 dilution [48].

**Quantitative reverse transcriptase polymerase chain reaction (RT-PCR):** Total RNA from wild type and knockout (αA-/-, αB-/-, and αA/B-/-) was isolated from mouse lens cortical fractions and mouse brain fractions by the Qiagen kit. One μg of total RNA was used for each sample to prepare cDNA. The RNA was treated with DNase and first strand cDNA synthesis was performed using a kit from Invitrogen using Oligo(dT) as primer. Primers were designed and synthesized by Integrated DNA Technologies (Coralville, IA). Their concentrations optimized using the manufacturer’s recommendations. The forward and reverse primers used for mouse tau gene are shown in Figure 2. To optimize the primers, reverse-transcriptase polymerase chain reaction (RT-PCR) was performed and products were run on 1.5% agarose gels to ascertain that a single band of the correct size was obtained. Real time-PCR was performed using a supermix containing the fluorescent dye SYBR green (BioRad, Hercules, CA) as described previously [51]. The increase in fluorescence was detected using the iCycler (BioRad). The relative quantification of tau gene expression was performed using the standard curve method according to manufacturer’s instructions (BioRad). For comparison between wild type and knockout samples, a standard curve of cycle thresholds for several serial dilutions of RNA sample was established and then used to calculate the relative abundance levels of mRNA (mRNA). The expression level of each tau mRNA was determined relative to GAPDH of the same sample. All RT-PCR reactions were performed in triplicate, and three independent experiments were performed on lens and brain samples.

**Isolation of microtubule associated proteins:** Microtubules were reconstituted from freshly dissected mouse lens epithelial and cortical fractions (Figure 1) [42,52]. Briefly, lens cortical fraction pooled from 16 to 20 mouse lenses was homogenized in 100 µl PME buffer (80 mM PIPES, 1 mM MgCl₂, 1 mM EGTA, pH 6.8) at 4 °C, followed by centrifugation at 30,000g for 15 min and the resulting supernatants were centrifuged at 100,000g for 60 min in a Beckman Optima TLX ultracentrifuge. Taxol (20 µM) and GTP (1 mM) were added to the supernatants, and incubated for 15 min at 37 °C, followed by centrifugation at 30,000g for 15 min at 37 °C through a sucrose cushion to pellet the microtubules. Microtubule associated proteins were extracted by washing the pellets in 0.35 M NaCl, and centrifugation at 30,000g for 25 min. This procedure has been shown to extract the MAPs in the supernatant [52]. Microtubules were examined by electron microscopy on negatively stained microtubule pellets as described previously [42]. To analyze tau expression in the brain, wild type and αA-/-, αB-/-, or αA/B-/- brains were homogenized in PME buffer.

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**Figure 2. Transcripts for mouse tau.** The three major tau transcripts were designated Tau-2, Tau-4, and Tau-6. The Ensembl transcript ENSMUST00000018555 was used to design gene-specific primers. Exons are indicated by vertical lines. Horizontal arrows indicate the sites for sequence-specific primers. The following nucleotides correspond to the primers: Exon 2 forward primer: nucleotide +12 to +35; Exon 4 forward primer: nucleotide +431 to +456; Exon 6 forward primer: nucleotide +905 to +928; Exon 7 reverse primer: antisense of nucleotide +1268 to +1245.
buffer and microtubule associated proteins were isolated as described above for lens tissue.

**Gel electrophoresis and immunoblot analysis:** Supernatants containing MAPs were analyzed by SDS-PAGE and immunoblotting, using several commercially available antibodies to tau described above. Proteins were separated using SDS-PAGE as described previously [42,53,54]. Samples were heated to 95 °C in sample buffer for SDS-PAGE. In experiments on brain tissue, better visualization of proteins in immunoblots was obtained when temperature was lowered to 85 °C. To examine the expression of tau in MAP fractions of wild type and knockout lens cortical fiber cells, equal amount

Figure 3. Immunoblot analysis of tau in mouse lens. Lens cortical fiber cell MAPs from 8-month-old wild type, αA−/− and αB−/− mice were prepared and the proteins were separated by SDS-PAGE. Tissue from 16 to 20 lenses was pooled for each preparation. Samples were heated to 95 °C in SDS-PAGE sample buffer to detect tau in lens samples. Immunoblot analysis was performed with the monoclonal antibody KAM-MA305 to full length brain tau protein and the Tau-5 antibody which reacts with nonphosphorylated as well as phosphorylated forms of tau. A: Antibody used was KAM-MA305 to full length brain tau. Left panel, Wild type (WT) and αA−/− MAPs. Tau was undetectable in the wild type lenses, but tau immunoreactive proteins were detected in the 45-66 kDa range in the αA−/− and αB−/− lens fractions. Note that the αA−/− tau immunoreactive bands included a prominent 100 kDa band; middle panel, WT and αB−/− MAPs. Tau immunoreactivity was undetectable in the wild type MAPs from cortical fiber cells, but higher molecular weight crosslinked bands (asterisks) crossreacted with the antibody in the αB−/− MAPs. Right panel, Microtubule associated proteins were isolated from 3-month-old mouse brains and analyzed by SDS-PAGE and immunoblotting. Note that the major immunoreactive bands were detected at 45-66 kDa, whereas minor bands were detected at about 100 kDa. B: Human cells-34 lysates were analyzed with the KAM-MA305 antibody. Tau immunoreactive bands at about 100 and 200 kDa bands were prominent in these cells. C: Antibody used was the Tau-5 antibody which recognizes both nonphosphorylated and phosphorylated forms of tau; left panel, Immunoreactivity of wild type and αA/B−/− lens fractions. Although no immunoreactive bands were detected in the wild type lens, a broad band was observed in the αA/B−/− lens; right panel, wild type brain fractions run on the same gel with a similar broad band showing immunoreactivity to this antibody.
of total protein was loaded from each sample. Immune complexes were detected using a horseradish peroxidase labeled secondary antibody and luminol (Santa Cruz Biotechnology, Santa Cruz, CA). The detected proteins were quantified with an Alpha Innotech photoimaging system (San Leandro, CA) [42].

**Immunolabeling and confocal microscopy of the microtubule associated protein tau:** To examine tau immunolabeling in the lens, wild type and α-crystallin knockout eyes were fixed and mounted in glycol methacrylate [41,43]. Three micron sections were cut in the mid-sagittal plane [41]. Non-specific binding was blocked by incubation with normal goat serum for 30 min. To visualize the distribution of tau, tissues were incubated overnight with a 1:100 dilution of an antibody to tau, and an Alexa-568-conjugated secondary antibody (1:200). Immunofluorescence and confocal microscopy were performed in the red channel of a Zeiss 510 confocal microscope. Differential interference contrast (DIC) was used to examine morphology in the blue channel of the confocal microscope [41].

**Cryoinmuno electron microscopic analysis:** Lens sections were examined by cryo-immuno electron microscopy. Sections (50-80 nm) were treated with primary antibodies to tau KAM-MA305 (Stressgen) and Tau-5 (Laboratory Vision) and anti-mouse IgG conjugated with 18 nm gold particles as secondary antibody (Sigma, St. Louis, MO). Specimens were stained with uranyl acetate and examined in a 1200EX transmission electron microscope as described previously [55,56].

**UVB irradiation of lenses:** Wild type 129SvEv mice were irradiated with FS20 UVB lamps emitting 290-320 nm radiation (maximum output at 310 nm) at a fluence rate of 7.8 W/m² for 60 min, with a total fluence of 28 kJ/m² at the cornea [57]. Twenty-four h later, mice were sacrificed and eyes were dissected and sectioned. Tau expression was visualized in control and UVB-exposed lenses by immunofluorescence analysis using antibodies to tau, and sectioned were examined by confocal microscopy.

**RESULTS**

Previous studies on gene knockout models of αA- and αB-crystallin suggested a decrease in lens microtubule stability [42]. As a first step to verify this observation, we examined the expression of the tau, a brain microtubule-stabilizing protein, in the lens. Lens cortical fractions were dissected (Figure 1A) and microtubule fractions were isolated (Figure 1B,C). More clusters appear to be associated with αB⁺⁻ microtubules than wild type as described previously [42].

**Expression of tau protein in lens fiber cells:** Lens microtubule fractions containing microtubules and MAPs were examined for tau protein expression by immunoblotting using an antibody made against full-length brain tau protein. Tau immunoreactivity was undetectable in the wild type lens MAP fractions. Tau immunoreactive bands at the expected mobilities were observed in αA⁺⁻ lens cortical fiber cells of mice 6 months of age and older (Figure 3) A distinct band of slower electrophoretic mobility corresponding to a molecular mass of 100 kDa was also detected in the αA⁺⁻ lens fractions. In addition, a group of significantly weaker bands of about 200 kDa were detected in the αA⁺⁻ lens fractions (Figure 3). The αB⁺⁻ lens fractions also demonstrated immunoreactivity to the tau antibody in the 45-66 kDa range. Two bands having lower electrophoretic mobilities, and about 100 kDa and 200 kDa mass crossreacted with the tau antibody in the αB⁺⁻ lens fractions. We used brain MAP preparations as positive control for the antibody against tau that was run together with the lens samples for comparison (Figure 3). Mouse brain MAPs showed strong tau immunoreactivity with distinct 45-66 kDa proteins, suggesting that the 45-66 kDa bands in the lens samples are unlikely to be nonspecific artifacts that cross react with the tau antibody. In addition, the brain fractions expressed a weaker tau immunoreactive band at 100 kDa. We also used a positive antibody control, human cells-34. These cells are known to produce tau in abundance. Immunoreactive bands to tau were observed both in the 45-66 kDa as well

![Figure 4. Immunofluorescence analysis of tau in the lens. Anti-tau immunostaining in lens sections from 6-month-old-mice. Red=tau; blue=DIC. A: Wild type lens. B: αA/β⁺⁻ - lens. C: αB⁺⁻ - lens. D: Absence of immunofluorescence in the lens specimen of a 6-month-old αA/β⁺⁻ - lens probed with non-immune mouse IgG. The scale bar is equal to 25 µm. E: Dark field image of a 6-month-old wild type mouse lens. F: Dark field image of a 6-month-old αA/β⁺⁻ - lens. Note that the opacity occupies three quarters of the lens. Note also that the outer cortex of the αA/β⁺⁻ - lens remains relatively clear.](Image 315x169 to 558x512)
as about 100 and 200 kDa molecular mass range. To further establish that the proteins detected with the KAM antibody were indeed tau, a previously well characterized tau antibody (Tau-5) against brain tau was also used. This antibody which detects both non-phosphorylated and phosphorylated tau proteins detected a strong broad band in the 45-66 kDa range in the αA/B+/− but not the wild type lens, and in the wild type brain (Figure 3C). Similar results were obtained with lens fractions of αA+/− and αB+/− lenses (data not shown).

The distribution of tau immunoreactive proteins was examined by immunofluorescence and confocal microscopy. Lens sections from wild type and αA/αB+/− mice were treated with an antibody to tau and examined by confocal microscopy. Figure 4 shows a strong increase in immunofluorescence of the αB+/− lenses as compared with wild type lenses (Figure 4A-C). Figure 4C also shows that the distribution of tau in the cortical fiber cells of αB+/− lenses appears to follow the lens fiber cell length. Replacement of the primary antibody with a non-immune mouse IgG and normal rabbit serum did not result in immunofluorescence staining (Figure 4D). These data suggest that our results are not attributable to non-specific staining artifact. Although tau immunoreactivity was observed in newly formed cortical fiber cells (Figure 4B,C), it was not detectable in the inner nuclear fibers, where where lenses of αA/αB+/− mice develop nuclear cataract (Figure 4E,F). To further confirm the increase in expression of tau in the αB+/− mouse lenses, cryo-immunoelectron microscopy was employed. Figure 5A,B show cryo-immunolabeling of tau in wild type and αB+/− mouse lens cortical fiber cells, respectively. Very sparse background labeling was observed in the wild type lens cortical fiber cells with the KAM antibody. In contrast, the αB−/− lens cortical fibers showed abundant labeling, with clusters of immunogold particles in electron dense regions. Figure 5C,D show the immunolabeling of lens cortical fibers with the Tau-5 antibody. A vast increase in labeling was observed in the αB−/− lenses whereas wild type lenses showed no labeling. These data confirm the upregulation of tau in the αB−/− lens cortical fiber cells.

A developmental time course for the appearance of tau immunoreactive proteins in mouse lens cortical fiber cells was performed. Figure 6 shows the effect of mouse age on expression of tau immunoreactivity in αA+/−, αB+/−, and αA/αB+/− lenses. In lenses of mice less than 4 months old, no tau isoforms (45-66 kDa) were detectable by immunoblot analysis. Tau expression stabilized in lens fractions derived from mice older than 6 months of age. These results indicate that the change in tau immunoreactivity in lens cortical fiber cells of α-crystallin gene knockout mice occurs around six months of age. A parallel study in lens epithelial cells with age showed that the expression of tau immunoreactivity was very weak in wild type or αA+/−, αB+/−, and αA/αB+/− epithelial fractions (data not shown). Taken together, the immunoblot, immunofluorescence and cryo immunoelectron microscopic analysis indicated that the loss of chaperones αA- and αB-crystallins resulted in enhancement of tau immunoreactive proteins in the differentiated lens cortical fiber cells.

Transcriptional activity of tau in the lens: We next examined the expression of tau transcripts in the lenses of wild type and α-crystallin gene knockout mice. Tau is encoded via transcription of a single gene by alternative splicing [18,23]. We designed primers specific to sequences in tau gene encoding the major tau peptides (Figure 2).
of tau gene in the lens was confirmed by RT-PCR (Figure 7). A 187 bp fragment was amplified using a pair of gene-specific primers designed to anneal to sequences associated with the Tau-2 transcript (Figure 2). This was the major tau transcript detected in the lens, and corresponded to expression of the smallest mouse tau peptide with 372 amino acid residues (Figure 2). Primers specific to the alternatively spliced Tau-4 transcript encoding the medium-length (432 residues) tau protein amplified a 127 bp PCR product in the brain (Figure 8) but not in the lens (Figure 7), suggesting that this transcript is not expressed in the lens. Similarly, a 364 bp PCR product was amplified from primers sequences designed to amplify the transcript encoding the longest tau peptide (733 residues) in the lens (Figure 7). Quantitative RT-PCR reactions were performed to assess the effect of αA- and αB-crystallin gene knockout on tau gene expression. GAPDH gene expression was used as a control. This analysis confirmed the expression of the Tau-2 transcript in the wild type lenses, and showed that this transcript was uniquely upregulated by 2.5 fold in the αA−/− lenses and a nearly fivefold in αA/B−/− lenses, but not in αB−/− lenses (Figure 7B). In contrast, the Tau-4 transcript was undetectable in lenses of any genotype. Although the Tau-6 transcript encoding the largest tau peptide was undetectable in the wild type and αB−/− lenses, it was strongly upregulated in the αA−/− and αA/B−/− lenses. However, the relative abundance of the Tau-6 transcript was one tenth of the Tau-2 transcript in the αA−/− and αA/B−/− lenses. Tau transcripts were also examined in the lenses of 1 month old mice (data not shown). The tau transcripts were expressed at one fourth the levels in young (1 month old) as compared with old (6 months old) mice. The ratio of Tau-2/GAPDH transcripts was 0.3±0.05 in young lenses, increasing to 1.44±0.26 in 6 month old lenses.

Expression of tau in mouse brains: To ascertain unequivocally that tau is indeed expressed in the lens cortex, we conducted parallel studies on tau transcripts and protein expression in wild type, αA−/−, αB−/−, and αA/B−/− mouse brains. This analysis is particularly relevant because αA- and αB-crystallin are also expressed at low levels in brain [5,6]. RT-PCR analysis was performed. Data are representative of three independent experiments.

Figure 6. Effect of age on tau expression in mouse lens. Microtubule-associated proteins were isolated from cortical fiber cells of 1- to 12-month-old mice. Lenses (16-20) from each of wild type (WT), αA−/−, αB−/−, and αA/B−/− mice were pooled at every age. Proteins were analyzed by immunoblotting with KAM-305 antibody to full length brain tau, and quantified by densitometric analysis. WT (black bars); αA−/− (red bars); αB−/− (blue bars); αA/B−/− (green bars).

Figure 7. Quantitative RT-PCR analysis for tau transcripts in the lens. A: Transcripts for GAPDH, Tau-2, Tau-4, and Tau-6 were amplified from wild type, αA−/−, αB−/−, and αA/B−/− lenses using the indicated primers. The primers for mouse GAPDH were: forward primer, AAG GTG AAG GTC GGA GTC AAC G, and reverse primer, GCT CCT GGA AGA TGG TGA TGG; product size, 252 bp. B: Quantitative RT-PCR analysis was performed. Data are representative of three independent experiments.
PCR analysis using mouse tau gene primers showed the expression of all three tau transcripts in brain (Figure 8A). The relative expression of tau transcript encoding the smallest tau peptide was 10 fold higher in the brain as compared with lenses of wild type mice. The ratio of tau-2/GAPDH was 11.2±1.7 in wild type brains as compared with 1.44±0.26 in the wild type lenses. Furthermore, in contrast to the enhanced expression of tau transcripts in αA−/- and αA/B−/- lenses as compared with wild type, quantitative RT-PCR showed that there was no significant upregulation of tau transcripts in the brains of αA−/- and αB−/- mice (Figure 8B). αA/B−/- brains, unlike αA−/- or αB−/- brains showed a marked reduction in the tau transcripts (Figure 8B). We next analyzed the effect of gene knockout of αA− and αB-crystallin on the expression of tau protein in the brain. Immunoblot analysis of tau protein in brains from knock-out mice as compared with wild types revealed changes in relative distribution of the three murine tau isoforms in αA−/- and αB−/- brains (Figure 9). We next analyzed the effect of gene knockout of αA− and αB-crystallin on the expression of tau protein in the brain. Immunoblot analysis of tau protein in brains from knock-out mice as compared with wild types revealed changes in relative distribution of the three murine tau isoforms in αA−/- and αB−/- brains (Figure 9). Quantitative analysis of the immunoblots with the PHF tau antibody AT8 showed that the intensity of the smallest peptide increased threefold in the αA−/- and αB−/- brain fractions. While some variation between the different αB−/- brain samples was noted, statistical analysis showed that the increase in staining of the

![Figure 8](http://www.molvis.org/molvis/v13/a177/)

**Figure 8.** Quantitative RT-PCR analysis for tau transcripts in the brain. A: Transcripts for GAPDH, Tau-2, Tau-4, and Tau-6 were amplified from wild type, αA−/-, αB−/-, and αA/B−/- lenses. B: Quantitative RT-PCR analysis was performed. Data are representative of three independent experiments.

![Figure 9](http://www.molvis.org/molvis/v13/a177/)

**Figure 9.** Immunoblot analysis of tau in mouse brain. Brain MAPs from 8-month-old wild type, αA−/-, and αB−/- mice were prepared and the proteins were separated by SDS-PAGE. Samples were heated to 85 °C in SDS-PAGE sample buffer to get good resolution. Using the KAM antibody to full length brain tau, tau was detected as three immunoreactive bands in the wild type brain, but the smallest peptide band was undetectable in the αA−/- and αB−/- brains (Figure 9). Analysis of brain fractions showed that the phosphorylation-dependent PHF-tau antibody AT8 recognized mainly the highest molecular mass tau peptide in wild type brain fractions (Figure 9). Quantitative analysis of the immunoblots with the PHF tau antibody AT8 showed that the intensity of the smallest peptide increased threefold in the αA−/- and αB−/- brain fractions. While some variation between the different αB−/- brain samples was noted, statistical analysis showed that the increase in staining of the...
smallest peptide in the αAβ- and αBβ- brain samples was significant (p=0.01). This antibody has been shown to recognize tau protein phosphorylated at both serine 202 and threonine 205 of the longest human brain tau isoform [47]. These results suggest that gene deletion of αA- and αB-crystallin caused an increase in phosphorylation of the tau epitope Ser202/Thr205 in mouse brain. The phosphorylation-dependent antibody pS422 did not label mouse brain tau from wild type or αAβ- and αBβ- samples (data not shown). We next assessed tau staining in another mouse model for cataract by UVB irradiation of mouse lenses in vivo (Figure 10). The UVB irradiated lens showed vacuoles and opacities all over the lens surface. Positive tau staining in the UVB-irradiated lens cortex was detected by immunofluorescence analysis, suggesting that tau upregulation is not restricted to the α-crystallin knockout models.

**DISCUSSION**

The data presented in this investigation clearly establish the expression of the brain MAP tau in the lens. Tau immunoreactivity was detected in the pool of microtubule associated proteins derived from lens cortical fiber cell microtubules of aged α-crystallin knockout mouse lenses. The localization of tau immunoreactivity in the microtubule fraction is important since it demonstrates that this microtubule associated protein localizes to the same cellular fraction in the lens as in the brain [52]. That tau accumulation is significant only in the cortical fiber cells and not in epithelial cells of αA- and αB-crystallin knockout lenses is attributable to the epithelial cells being able to upregulate other mechanisms that may compensate for α-crystallin, whereas cortical fiber cells cannot [58,59]. These findings expand the previously established developmentally regulated expression of cargo vesicle transport proteins, synapsins and synaptic vesicle proteins in lens [36].

The major tau transcript (Tau-2) was expressed even in the wild type mouse lenses. The Tau-2 transcript was upregulated several fold over wild type in the αAβ- and αA/ Bβ- lenses. However, the tau protein (45-66 kDa) was in fact not detected in the wild type lenses strongly suggesting that it may be degraded in the wild type lenses. The tau protein was upregulated in α-crystallin knockout lenses in an age-dependent manner, with significant accumulation restricted to the cortical fiber cells of lenses derived from mice older than 6 months of age. Aging lens cortical fiber cells accumulate insoluble proteins, an increase in protein modifications, and a reduction in levels of reduced glutathione, and experience increased oxidative damage [60]. Our data suggest that these changes in the lens may enhance the accumulation of tau in the α-crystallin knockout lenses. Our results contradict a previous report where tau expression was reported in lens as a 36 kDa protein in crude lens soluble and insoluble fractions [36]. Since the molecular mass of tau peptides is normally reported to be 45-66 kDa, the protein at 36 kDa might be a cross-reacting protein in the crude lens preparation. While microtubule motor protein expression has been reported in young rodent lenses [61], it is not known at present whether their expression is age-dependent.

The cortical fibers where tau protein accumulated are particularly interesting because fiber cell changes in this region have been associated with other cataract models including diabetic cataract in rodents [62]. The known ability of tau to enhance microtubule stability suggests that an increase in tau expression may be a response of the lens to the reported increase in instability of the microtubule cytoskeleton in the knockout lenses [42]. Indeed, αA- and αB-crystallin have been shown to enhance the stability of microtubules, and protect tubulin from denaturation [38,42].

One explanation for the observed increase in αB-crystallin in neurodegenerative diseases may be as a failed protective mechanism to suppress tau aggregation and to keep tau proteins in a productive folding pathway. This mechanism may modulate microtubule integrity and accumulation of tau aggregates in the form of neurofibrillary tangles, and αB-crystallin may therefore play a role in the pathogenesis of tau-related diseases [63]. There are at least 6 tau protein isoforms expressed in the brain. In addition to the 45-66 kDa subunits, the αBβ- aged lens cortical fibers also expressed the high molecular weight forms of tau (100 and 200 kDa) suggesting that these might result from crosslinking and aggregation of tau in the lens in the absence of αB-crystallin. It is known that the αA- and αB-crystallin are anti-apoptotic proteins and protect cells from stress-induced death [40,64,65]. The pathogenic
potential of tau and neurofibrillary tangles has been demonstrated by dementia-associated mutations and in transgenic mouse models [66-68]. While αB-crystallin protects against cell death, the increased tau and αB-crystallin in neuronal pathologies may not be an effective protective mechanism as disease progresses and oxidative stress increases [69].

From our studies, we can propose that the mechanism of tau accumulation as a result of loss of αA- or αB-crystallin chaperones may be an enhanced tendency of tau to misfold and undergo phosphorylation. This hypothesis is lent support by the results of other studies that show that the chaperones HSP70 and HSP90 promote tau solubility and binding to microtubules in the brain, and reduce tau phosphorylation [70]. Furthermore, chaperones are thought to affect cytoskeletal organization and the lack of chaperones or mutations in cytoskeletal proteins or chaperones have been shown to result in inclusion bodies in pathological conditions [11,27,27]. Proteasome inhibition stabilizes tau inclusions in oligodendroglial cells [30,73]. Because αB-crystallin is known to target proteins for degradation by the proteasome, another possible mechanism for tau accumulation in α-crystallin gene knockout lenses is that depleting the pool of α-crystallin chaperones impairs the ability of the misfolded tau protein to be targeted to the proteasome [10,17,74]. The increase in tau immunoreactivity observed in the lenses of αA−/−, αB−/−, and αA/αB−/− mouse lenses suggests that αA- and αB-crystallin are needed to chaperone tau and assist in its degradation, and in their absence, tau accumulates in the lens.

In summary, the data in this investigation demonstrate the expression of tau transcripts in the lens; describe tau protein accumulation with lens aging in α-crystallin knockout mice; and serve as a basis for characterizing the relationship between tau and α-crystallin and serve as a basis for characterizing the relationship between tau and α-crystallin in neuronal disease models and in aged human lenses with and without neurodegeneration.

ACKNOWLEDGEMENTS

The authors thank E. Wawrousek (National Eye Institute, NIH), for providing the α-crystallin gene knockout mice, Belinda McMahan for immunohistochemical analysis, Wandy Beatty for electron microscopic analysis, and Michael Casey in the Molecular Biology Core laboratory for designing primers. This work is supported by the National Eye Institute (NIH), grants R01EY05681 to U.P.A. and the Vision Core grant EY02687, and an unrestricted grant to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, Inc.

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