Published in final edited form as:
Invest Ophthalmol Vis Sci. 2001 January ; 42(1): 188–193.

Identification and Spatial Analysis of Metallothioneins Expressed by the Adult Human Lens

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Abstract

Purpose—Metallothioneins (MTs) are a large family of proteins involved in multiple protective pathways including binding of toxic metals, free radical scavenging, and oxidative stress. Increased expression of the *MT IIA* gene in age-related cataractous relative to normal human lenses, suggesting a role for MTs in the maintenance of lens transparency, has previously been detected. The MT family consists of many closely related isoforms grouped into four classes (I–IV). As a first step toward defining the function of MTs in the lens, the range and expression patterns of those MT isoforms expressed by the adult human lens were established.

Methods—Normal human lenses were microdissected into epithelia and fiber cells. Primers specific for individual MT isoforms were designed. MT transcripts were monitored in whole lenses, epithelia, and fibers by RT-PCR and confirmed to be authentic by sequencing. MT protein levels were evaluated by immunoblotting and by immunostaining.

Results—Transcripts encoding MT classes I and II but not III or IV were detected in adult human lenses. In addition to MT IIA, five other MT transcripts were identified including IE, IF, IG, IH, and IL. MT IIA was detected almost exclusively in the lens epithelium, whereas the class I isoforms were detected at high levels in both lens epithelia and fibers. MT protein was detected almost exclusively in the lens epithelium.

Conclusions—The present report establishes the spectrum of MTs expressed by the adult human lens, defines their spatial expression patterns in lens epithelia and fibers, and demonstrates that MT protein is abundantly present in the lens epithelium.

The vertebrate eye lens contains multiple protective and regulatory pathways to maintain its transparent function. Insult to these pathways is believed to result in cataract formation. Insulting agents include toxic metals, free radical formation, and oxidative stress.[1–7] We are interested in identifying those components of the human lens that protect against these insults.

One component, likely to play a major role in lens protection are the metallothioneins (MTs), which are a large family of closely related isoforms that have been implicated in numerous detoxification and protective pathways.[8] We have previously detected increased levels of MT IIA transcripts in pooled, age-related cataractous relative to normal human lenses by RT-PCR differential display,[9] indicating a potential role for at least one member of the MT family in the maintenance of lens transparency.

MTs are 6- to 7-kDa metal binding proteins that are induced upon exposure to heavy metals and participate in heavy metal regulation and detoxification.[8] They are known to bind Zn, Cu,

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Co, Cd, Hg, Pb, Ag, Bi, Sn, Ni, and Au.[8] Recent studies suggest that detoxification and regulation of heavy metals is not the only or even the primary function of MTs.[10] In addition to their metal binding properties, they are also free radical scavengers,[11] whose synthesis is activated by steroids,[12,13] carcinogens,[14] chemicals that induce oxidative stress,[15] X-irradiation, and UV-induced DNA damage.[16] They have been reported to function as regulators of copper homeostasis,[17–19] as zinc donors for the zinc-dependent transcription factors,[20,21] as mediators of redox balance,[22] and as protectors against reactive oxygen intermediates.[23–25]

The MT family is composed of multiple, separately encoded isoforms[26] that are grouped into four physically distinct classes called I–IV.[10] MTs I and II are the most widely expressed and are regulated coordinately in all tissues.[27,28] MT III is brain specific,[29] and MT IV is expressed mainly in squamous epithelium.[30] In humans, single genes located on chromosome 16 and designated *IIA*, *III*, and *IV* encode MT isoforms II–IV, whereas there are 13 separately encoded MT I isoforms.[30]

Given the large number of MTs and their different expression patterns, we have explored the range and spatial expression patterns of specific MTs expressed by the adult human lens as a first step toward elucidating their potential lens functions. We demonstrate that at least six different MT transcripts are expressed by the lens that exhibit different patterns of expression in the lens epithelium compared with the lens fibers. We also demonstrate that MT protein is primarily present in the lens epithelium.

Methods

Microdissection of Human Lenses

Normal human lenses from donors with no known history of metal exposure were obtained within 24 to 60 hours postmortem and inspected under a dissecting scope to be free of opacity. The lens epithelium was surgically dissected away from the rest of the lens, and contaminating fiber cells were removed as previously described.[9] Carefully washed surgical grade instruments and metal free solutions were used in all dissection and preservation procedures. The average age of lenses used in this study was 57 years, the age range was 38 to 84 years, and the donors were 69% male donors. Where indicated, four to eight lenses or lens subcomponents were pooled for each experiment. This research was conducted under IRB exemption 4.

Design of Oligonucleotides for RT-PCR

Oligonucleotides specific for individual MT isoforms were designed using the BLAST program and GenBank data (National Library of Medicine, Bethesda, Maryland). The GenBank accession numbers are MT IA (K01383), MT IB (M13484), MT IE (M10942), MT IF (M10943), MT IG (J03910), MT IH (X64834), MT IL (AJ011,772), MT IIA (X00504), MT III (M93311), and MT IV (U07807). The sequences were MT IA (5′-GCCTCTCAACTTCTTGCTTG and 5′-GAC ATCAGGCACAGCAGCTG), MT IB (5′-GCCCTGACTTCTCATATCTTG and 5′-GG CACTTCTCTGATGAGCCTT), MT IE (5′-GCTCCAGCATCCCCTTTGCT and 5′-CA CATCAGGCACAGCAGCTG), MT IF (5′-GCTTCTCTCTTGGAAAGTCC and 5′-GG CATCAGTCGCAGCAGCTG), MT IG (5′-GCCTCTTCCCTTCTCGCTTG and 5′-GACATCAGGCGCAGCAGCTG), MT IH (5′-GAACTCCAGTCTCACCTCGG and 5′-GACATCAGGCACAGCAGCTG), MT IL (5′-GACTGCCTCTTCGCCTCTCC and 5′-CACATCAGGCACAGCAGCTG), MT IIA (5′-AAGTCCCAGCGAACCCGCGT and 5′-CAGCAGCTGCACTTGTCCGACGC), MT III (5′-GTTGCTTGGAGAAGCCCGTTCA and 5′-GCATAGGTGGCACTGAGCCA), and MT IV (5′-GCCGTGACAGCACTGGAGCCT and 5′-GATTCATGCACTGTACAGACAC). The expected product sizes were as follows: MT IA, 237 bp; MT IB, 229 bp; MT IE, 211 bp; MT

IF, 226 bp; MT IG, 234 bp; MT IH, 213 bp; MT IL, 226 bp; MT IIA, 236 bp; MT III, 318 bp; and MT IV, 294 bp. Control oligonucleotides were also designed to examine GAPDH, MIP26, and SPARC. The GenBank accession numbers are GAPDH (NM_002046), MIP26 (U36308), and SPARC (J03040). The sequences were GAPDH (5′-TCCACCACCCTGTTGCTGTA and 5′-TGTTCCAGTATGATTCCACCC), SPARC (5′-CCTGAGGCTGT AACTGAGAGAAAG and 5′-GTGGGAGGGGAAACAAGAAGATAA), and MIP26 (5′-GCTTGGCCCTGGCTACACTGGT and 5′-TGAGCCGGGGGAAGAGAAGAA). The expected product sizes were GAPDH, 840 bp; SPARC, 419 bp; and MIP26, 538 bp. Oligonucleotides were designed to cross intron–exon boundaries to distinguish between DNA and RNA.

Reverse Transcription–Polymerase Chain Reaction

Total RNA was prepared from pooled whole lenses, lens epithelium, and lens fibers by phenol-guanidinium isothiocyanate extraction.[9] RT-PCR was performed with indicated amounts of RNA using the one-step system according to the manufacturer (GIBCO-BRL, Gaithersburg, MD). As control, reactions were also performed in the presence and absence of reverse transcriptase using amplitaq polymerase (Perkin-Elmer Cetus, Foster City, CA). Products were separated by gel electrophoresis on 1.2% agarose gels and visualized by ethidium bromide staining.

RT-PCR Using Universal Recognition Primers

RT-PCR was also performed using primers designed to recognize all MT I and MT II isoforms. The conditions for this procedure were identical with the above procedure. The sequences of the MT universal primers are (5′-ATGGACCCCAACTGCTCCTG and 5′-GCAGCAGCTCTTCTTGCAGG). The expected product size for these primers is 102 bp.

Cloning of PCR Products

Specific RT-PCR products were excised and purified using the QIA-quick gel extraction kit (Qiagen, Valencia, CA), and cloned into Topo TA vectors as specified by the manufacturer (Invitrogen, Carsbad, CA).

Identification of Specific Transcripts

Transcripts were sequenced using automated fluorescent cycle DNA sequencing on an Applied Biosystems 373a DNA sequencer using Applied Biosystems sequencing software (Perkin Elmer/Applied Bio-systems, Foster City, CA). Sequences were analyzed using the BLAST algorithm with GenBank data (National Library of Medicine). Subsequent alignments were performed using the MegAlign program contained in Lasergene software (DNASTAR, Inc., Madison, WI).

Western Blot Analysis

Indicated amounts of protein were denatured by boiling in 10% SDS buffer (10% w/v SDS; 0.5 M Tris-HCL, pH 6.8, 5% [v/v] 2-mercaptoethanol; 5% [v/v] glycerol), resolved by electrophoresis on 12% SDS-polyacrylamide gels and transferred (60 V for a half hour in 12 mM Tris-HCL, 96 mM glycine, 15% methanol) to nitrocellulose filters. The resulting blots were blocked overnight in Superblock (Pierce, Rock-ford, IL) according to the manufacturer's directions. The blot was washed three times with PBS over 15 minutes, incubated with a 1:200 dilution of mouse anti-MT antibody (StressGen, Victoria, British Columbia, Canada), and washed three times in PBS containing 0.1% Tween 20. Immunoreactive MT was visualized using ECL Western blotting reagents (Amersham-Pharmacia, Piscataway, NJ) as specified by the manufacturer. Identical procedures were carried out with purified rabbit liver MT (Sigma, St. Louis, MO) as control.

Immunostaining

An 18-year-old female human lens (<24 hours postmortem) was fixed in 4% paraformaldehyde in PBS overnight, followed by cryoprotection overnight in 30% sucrose in PBS before embedding. Frozen sections (14 μm) were prepared and air-dried. Sections were blocked for 1 hour at room temperature in DMEM, 10% fetal calf serum, 1% goat serum, and 0.1% Triton X-100. Sections were incubated overnight with a 1:400 dilution of anti-MT antibody (DAKO, Carpinteria, CA) in blocking solution at 4°C. After five washes with 0.1% Tween 20 in PBS, sections were incubated with streptavidin-conjugated secondary antibody (Vector Laboratories, Burlingame, CA) and were visualized using the Vectastain Elite Kit as specified by the manufacturer. Sections were counterstained with hematoxylin. Identical procedures were carried out in the absence of primary antibody as control.

Results

Identification of Specific MT Transcripts

Expression of 11 different MT isoforms was examined by RT-PCR analysis of whole lens RNA. The data are shown in Figure 1A. High levels of transcripts specific for MT isoforms IE[31] (211 bp, lanes 5 and 6), IF[31] (226 bp, lanes 7 and 8), IG[32] (234 bp + 270 bp top band, lanes 9 and 10), IH[33] (213 bp, lanes 11 and 12), and IIA[34] (236 bp, lanes 17 and 18) were detected by this analysis. By contrast, lower amounts of product were detected using primers designed to recognize isoforms IA[35] (237 bp expected + 260 bp top band, lanes 1 and 2) and IL[36] (226 bp + 250 bp top band, lanes 15 and 16). No product was detected for isoforms IB[37] (lanes 3 and 4), III[29] (lanes 19 and 20), or IV[30] (lanes 21 and 22). As control, high levels of GAPDH were detected in control reactions (840 bp, lanes 13 and 14). Single products were detected in all reactions except for IA, IL, and IG that also contained a (270 bp) top band of unexpected size. All reactions yielding high levels of product were specific for the presence of reverse transcriptase (Fig. 1B, lanes 1–14).

To confirm the identities of the resulting transcripts, products of the expected size that were specific for isoforms IA, IE, IF, IG, IH, IL, and IIA were cloned and sequenced. Analysis of three separately isolated clones confirmed these transcripts to be authentic, except for IA, which contained a mixture of transcripts encoding IG and IH. The identities of the larger bands detected in the IA, IG, and IL reactions were not determined.

To further characterize the specific MT isoforms expressed by the lens, RT-PCR was performed using primers (universal primers) designed to recognize and amplify all class I and class II MT transcripts. The forward primer is specific for exon 1, and the reverse primer overlaps exons 2 and 3 of the MT class I and II coding sequences. The forward primer sequence is identical with the corresponding sequences of isoforms IA, IF, IG, IH, and IL and differs by only 1 bp with the corresponding sequences of isoforms IB, IE, IR, and IIA. The reverse primer sequences is identical with the corresponding sequences of isoforms IA, IF, IG, IH, IL, and IR and differs by only 1 bp with the corresponding sequences of IB, IE, and IIA.

The universal primers were designed to produce a 102-bp RT-PCR product. Purification and cloning of this product, followed by sequencing and identification of individual clones, should identify the spectrum of class I and II transcripts present in the lens. The number of individual clones divided by the total number of clones should approximate the relative frequency of each transcript expressed by the lens.

RT-PCR of pooled whole lens RNA using the universal primers yielded a single band of the expected size (Fig. 1A [102 bp]; lanes 23 and 24) that was dependent on the presence of reverse-transcriptase (Fig. 1B; lanes 9 and 10). The 102-bp band stained less intensely than the isoform-specific bands (cf. Fig. 1, lanes 23 and 24 with lanes 5–12, 17, and 18). This most likely is the

result of reduced amplification efficiency of the 102-bp product because the universal primers are likely to have reduced binding efficiency relative to the isoform-specific primers. The 102-bp product was excised from the gel, purified, and cloned. Seventy-five separately isolated clones were identified by sequencing.

The results are shown in Table 1. Consistent with the identities of transcripts detected using the isoform-specific primers (Fig. 1), six MT isoforms were identified by this analysis including MTs IE, IF, IG, IH, IL, and IIA. The number of clones of each isoform identified and its relative proportion in the total population of clones are as follows: IL: 51 clones, 68%; IG: 8 clones, 11%; IIA: 7 clones, 9%; IF: 5 clones, 7%; IH: 3 clones, 4%; and IE: 1 clone,1%.

Analysis of MT Transcripts in Microdissected Lens Epithelia and Fibers

To examine the spatial expression patterns of the identified transcripts, the levels of five MT transcripts (IE, IF, IG, IH, and IIA) were compared by RT-PCR analysis using pooled RNA obtained from microdissected lens epithelium and lens fibers. To control for cross-contamination between the lens epithelia and fiber RNA preparations, the epithelium-specific transcript SPARC[38] and the fiber-specific transcript MIP 26[39] were also examined.

The data are shown in Figure 2A. Transcripts encoding MTs IE, IF, IG, and IH (lanes 1–8) were detected in both lens epithelium and lens fibers. By contrast, MT IIA transcripts (lanes 11 and 12) were detected only in the lens epithelium. Using higher amounts of RNA and increased numbers of PCR cycles, low amounts of MT IIA were detected in the fibers (data not shown). As found with whole lens RNA (Fig. 1, lanes 9 and 10), a second higher-molecular-weight band than the authentic MT IG band was also observed (Fig. 2, lane 5). Although the identity of this band was not determined, it is interesting to note that it is confined to lens epithelial RNA preparation (cf. Fig. 2, lanes 5 and 6).

As expected, the SPARC control was detected only in the lens epithelial RNA preparation (Fig. 2B, lanes 3 and 4). Correspondingly, the MIP 26 control was almost entirely restricted to the lens fiber RNA preparation (Fig. 2B, lanes 5 and 6), although trace amounts of MIP 26 were detected in the epithelial preparation, indicating possible contamination of lens epithelial RNA with fiber RNA. Because greater levels of almost all transcripts were found in the lens epithelium relative to the fibers and no fiber-specific gene expression was detected, it is unlikely that trace contamination of epithelial RNA with fiber RNA would significantly alter the present results.

Analysis of MT Transcripts in Individual Lenses

It is possible that MT expression might vary between individual lenses. Because the previous experiments (Figs. 1 and 2) examined MT levels in pooled lenses, the expression of two MT isoforms (MT IG and MT IIA) was examined using equal amounts of RNA isolated from individual lens epithelia or fibers. The data are shown in Figure 3. The levels of both MT isoforms were variable in individual lens epithelia and fibers. This variability does not appear to be related to age or sex. Interestingly, the levels of MT IIA and MT IG were not proportional in the same lens epithelia (cf. Fig. 3A to Fig. 3B, lanes 2, 4, and 5). The levels of MT IG were lower in lens fibers than in epithelia (Fig. 3A, lanes 6 and 7), and trace amounts of MT IIA were detected in lens fibers (Fig. 3B, lanes 6 and 7). MT IIA exhibited greater variation between individual lenses than did IG (cf. Figs. 3A and 3B), indicating different levels of variation between specific MT isoforms in individual lenses.

Spatial Analysis of MT Protein

To evaluate the corresponding levels of MT protein in the lens, Western blot analysis was performed using a MT-specific antibody and equal amounts of extracts prepared from pooled

whole lens, lens epithelia, and lens fibers. Because antibodies specific for individual MT isoforms are not available as a result of the very high degree of amino acid sequence identity between MT classes I and II (at most a one–amino acid difference), an antibody recognizing both MT classes[40] was chosen for this analysis.

The data are shown in Figure 4. Consistent with the reported molecular weight for MT, a 6- to 8-kDa immunoreactive band comigrating with the corresponding 6- to 8-kDa purified MT band (lane 4) was detected with epithelial (lane 3) and whole lens extracts (lane 1). A second 16- to 20-kDa band is also visible in the purified MT (lane 4), lens epithelia (lane 3), and weakly in whole lens (lane 1). Although the identity of this band is not known, it is likely that it contains cross-linked MTs,[40] which may also represent the higher immunoreactive bands (35–50 kDa) restricted to the purified MT control (lane 4). In contrast to the whole lens or lens epithelial protein extracts, no MT was detected using equal amounts of lens fiber extracts (lane 2) indicating that MT protein is confined to the lens epithelium.

To further explore the levels of MT protein in specific lens subregions, immunostaining was performed with a second antibody recognizing MT class I and II isoforms.[41] The data are shown in Figure 5A. Represented is a portion of the lens between the anterior and peripheral regions. Consistent with the immunoblotting results (Fig. 4), MT protein was detected at significantly higher levels in the lens epithelium than in the lens fibers. No staining was detected in the lens capsule (Fig. 5A). It is interesting to note that the weak staining in the lens fibers appears to be restricted to undifferentiated fibers. As control, no staining was detected in the absence of primary antibody (Fig. 5B).

Discussion

The present results demonstrate that high levels of both MT mRNA and protein are present in the adult human lens, and they establish the identities of the individual isoforms of MT expressed by the lens. Transcripts encoding MTs IE, IF, IG, IH, IL, and IIA were confirmed to be expressed by the lens (Fig. 1). By contrast, transcripts encoding isoforms IA, IB, III, and IV were not detected (Fig. 1). The lack of isoform III or IV expression is not surprising because isoform III has been previously reported to be specific for brain,[29] and isoform IV has only been detected in squamous epithelium.[30] The identification of isoform IH (also known as isoform MT 0) in the adult lens is surprising because this isoform has been reported to be confined to fetal tissues.[42]

Consistent with the results obtained with the isoform-specific primers, analysis of MT expression using primers recognizing all class I and II MTs (universal primers) identified the same transcripts obtained with the isoform-specific primers (Table 1). Based on the number of specific transcripts to total transcripts detected using the universal primers, the predominance of MTs in the lens is IL > IG > IIA > IF > IH > IE. It is surprising that IL is the predominate isoform detected using the universal primers because relatively low levels of IL were detected in using IL-specific primers. The reason for this inconsistency is not known, but it is likely to result from differences in priming efficiency between the isoform-specific and the universal recognition primers.

The identified transcripts exhibited different spatial expression patterns between lens epithelium and lens fibers. MT I isoforms IE, IF, IG, and IH (Fig. 2, lanes 1–8) were detected in both lens subcomponents. Isoforms IE and IF (Fig. 2, lanes 1–4) were expressed at slightly higher levels in the lens epithelium than in the lens fibers. By contrast, MT IIA was only detected in lens epithelium (Fig. 2, lanes 11 and 12), although low amounts of MT IIA were detected in fibers from individual lenses (Fig. 3) and with very high amounts of RNA. Because of low amplification specificity (Fig. 1), spatial analysis was not performed on isoform IL. The

differences in spatial expression patterns between class I and II MTs may indicate distinct roles for these isoforms.

Consistent with the spatial expression patterns of MT transcripts detected by RT-PCR, both immunoblotting (Fig. 4) and immunostaining (Fig. 5) revealed that MT protein is mostly confined to the lens epithelium. The predominance of MT protein in the lens epithelium relative to the fibers implicates the epithelium as a primary site for possible MT function in the lens. It is interesting to note that expression of MT appears to be restricted to the outermost layers of the fibers (Fig. 5), indicating that MT expression may be restricted to those fibers that are not fully differentiated.

Significant differences in the levels and proportions of two MTs (MT IG and MT IIA) were detected in epithelia and fibers isolated from individual lenses (Fig. 3). Although further studies with large numbers of lenses will be required to establish relationships between characteristics of individual lenses and MT expression levels, the differences in MT levels detected between individual lenses in the present study appear to be independent of sex or age. Further studies will be required to establish whether these differences result from different environmental exposures, physiological conditions, and/or genetic backgrounds and if these differences may be related to possible functional differences of the individual MT isoforms.

At present, the specific function of MTs in the lens is not known. Future studies will involve identifying those factors regulating MT expression in the lens and the potential role of MTs in lens protection and cataract. The present report provides the groundwork required to carryout these studies by establishing those MTs expressed by the lens and their spatial expression patterns.

Acknowledgments

The authors thank Paula Ousley and Rory Dunaway of the Lions Eye Bank of Oregon; John Hawse, Ashley Halstead, Julie McWhorter, and Kija Cauffman of the Kantorow laboratory; Jorge Flores of the West Virginia University Department of Biology; and Marcos Chacone of Paragon Bioservices, Baltimore, Maryland.

Supported by National Institutes of Health, National Eye Institute Grant EY13022 and conducted in partial fulfillment of the MS degree requirements for BO.

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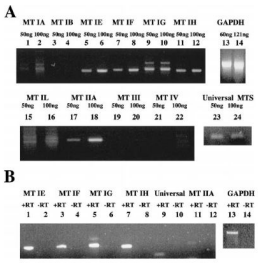

Figure 1.

Expression of MT isoforms in the adult human lens. (**A**) Ethidium bromide–stained gel showing indicated MT transcripts (*lanes 1* to *12* and *15* to *22*) amplified by RT-PCR (32 cycles) using isoform-specific primers and indicated amounts of whole lens RNA. Transcript sizes are MT IA (237 bp), MT IE (211 bp), MT IF (226 bp), MT IG (234 bp), MT IH (213 bp), MT IL (226 bp), and MT IIA (236 bp). Also shown are RT-PCR products obtained with primers (universal MTs) designed to recognize all class I and II MT isoforms (102 bp; *lanes 23* and *24*) and, as control, GAPDH (840 bp; *lanes 13* and *14*). (**B**) Ethidium bromide–stained gel showing indicated transcripts (*lanes 1* to *14*) amplified in the presence (+RT) or absence (−RT) of reverse-transcriptase using 50 ng of whole lens RNA and 32 PCR cycles.

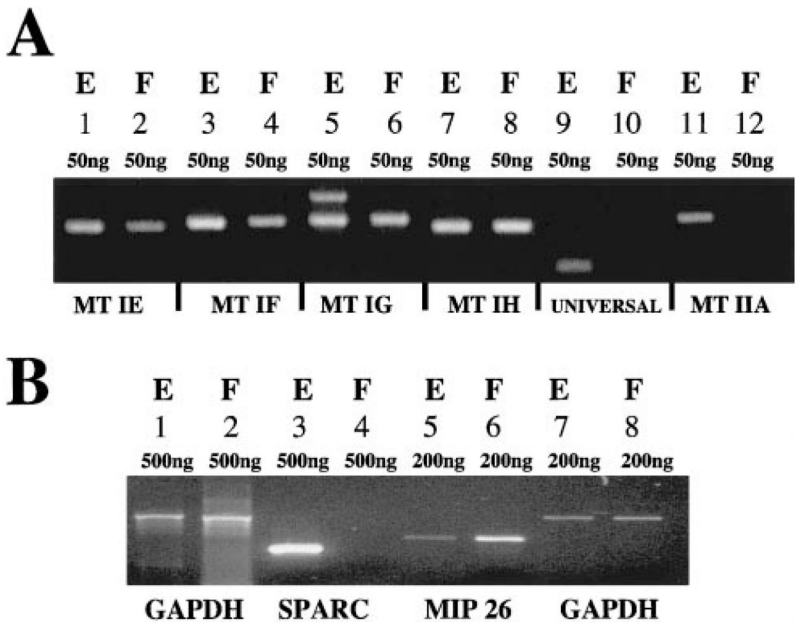

Figure 2.

Expression of MT isoforms in pooled lens epithelia and lens fibers. (**A**) Ethidium bromide–stained gel showing indicated MT transcripts (*lanes 1* to *12*) amplified by RT-PCR (32 cycles) using isoform-specific primers and 50 ng of RNA isolated from lens epithelia (E) or fibers (F). (**B**) Ethidium bromide–stained gel showing indicated control transcripts (*lanes 1* to *8*) amplified by RT-PCR (32 cycles) using SPARC-or MIP 26-specific primers and indicated amounts of (E) epithelial or fiber (F) RNA.

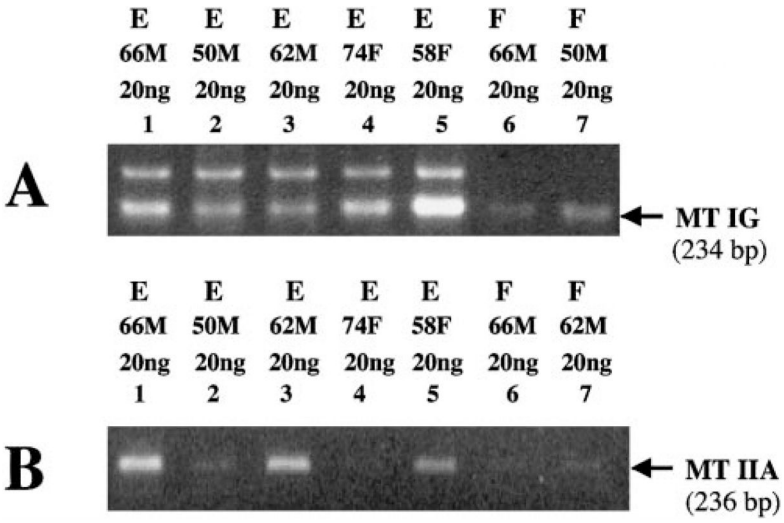

Figure 3.

Expression of MT IG and MT IIA in individual lens epithelia and fibers. (**A**) Ethidium bromide–stained gel showing MT IG transcripts amplified by RT-PCR (25 cycles) using 20 ng of epithelia (E) or fiber (F) RNA. Donor ages and sexes (M, male; F, female) are indicated. (**B**) Ethidium bromide–stained gel showing MT IIA transcripts amplified by RT-PCR (25 cycles) using 20 ng of epithelia (E) or fiber (F) RNA. Donor ages and sexes (M, male and F, female) are indicated.

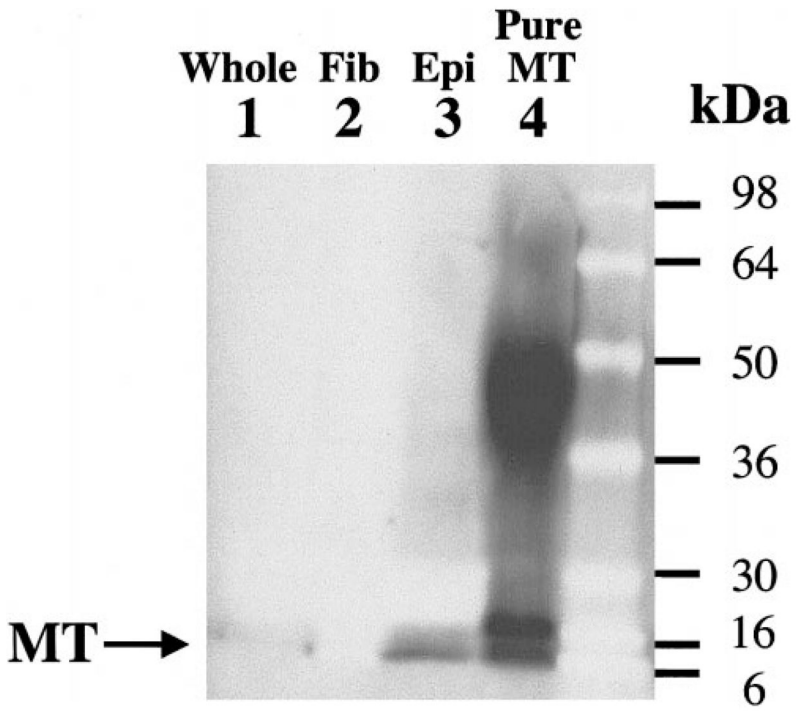

Figure 4.

Immunoblotting of lens extracts with MT-specific antibody. Shown is the autoradiogram of the corresponding blot. *Lane 1* contains 1.25 μg of whole; *lane 2*, 1.25 μg of fiber (Fib); *lane 3*, 1.25 μg of epithelial (Epi) lens extracts. *Lane 4* contains 50 ng of purified rabbit liver MT protein. Indicated is the 6- to 8-kDa immunoreactive MT band and the positions of molecular weight standards.

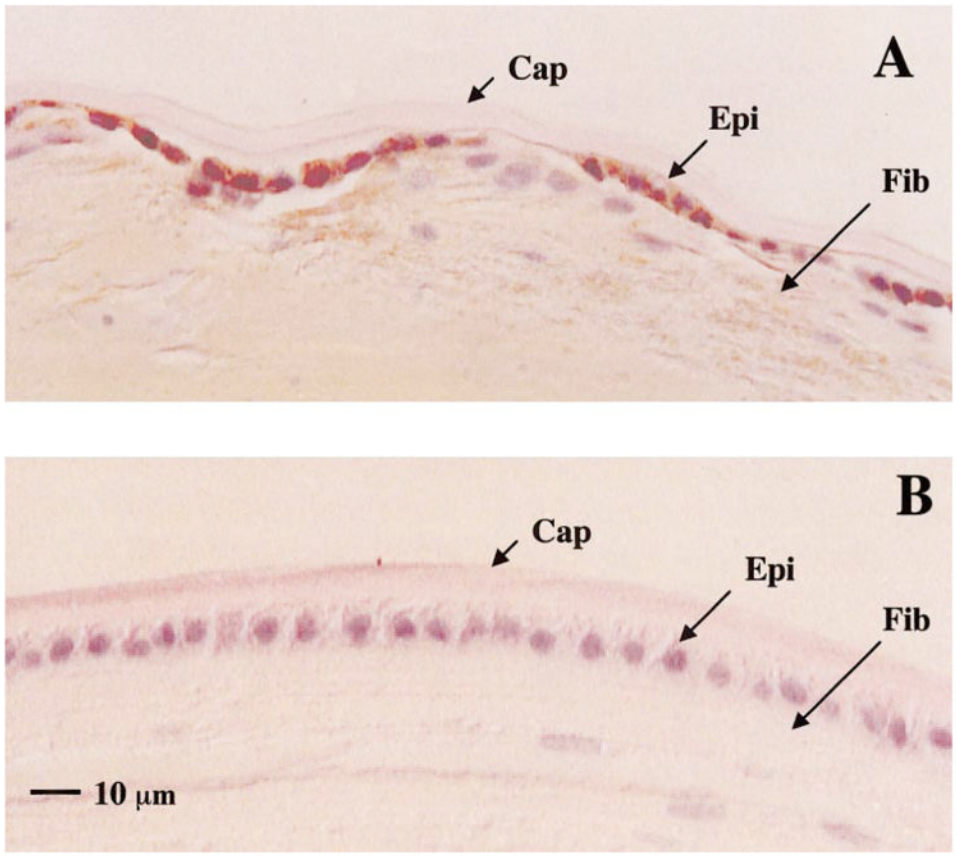

Figure 5.
Immunostaining of adult human lens with MT-specific antibody. An 18-year-old female human lens was immunostained with (**A**) anti-MT antibody or (**B**) secondary antibody alone. Indicated are the lens capsule (Cap), lens epithelium (Epi), and lens fibers (Fib).

Table 1

Identities and Relative Proportions of MT Transcripts Amplified from Whole Lens RNA with Universal Recognition RT-PCR Primers

MT Isoforms Detected	No. of Clones Identified	Proportion of Total Clones (%)
MT IE	1	1
MT IF	5	7
MT IG	8	11
MT IH	3	4
MT IL	51	68
MT IIA	7	9