Transketolase Is a Major Protein in the Mouse Cornea*

(Received for publication, June 14, 1996, and in revised form, September 30, 1996)

Christina M. Sax‡‡, Csaba Salamon‡, W. Todd Kays‡, Jing Guo‡, Fushin X. Yu‡, R. Andrew Cuthbertson‡, and Joram Piatigorsky‡

From the ‡Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, MSC 2730, Bethesda, Maryland 20892-2730 and ¶The Schepps Eye Research Institute, Harvard Medical School, Boston, Massachusetts 02114-2508

The cornea and lens are both transparent avascular tissues responsible for the refraction of light. The cornea accounts for two-thirds while the lens accounts for the remaining one-third of the refraction in the eyes of terrestrial vertebrates (1, 2). Abundant (80–90%) water-soluble proteins called crystallins are required for the transparency and refractive properties of the lens (3). The taxon-specific crystallins are either related identical to metabolic enzymes and thus are known as the enzyme crystallins (4–6). The high concentration of the enzyme-crystallins in the lens contributes to its high refractive index and focusing power. The enzyme-crystallins exhibit the property of gene sharing, once thought to be restricted to the lens, apply to other transparent ocular tissues.

Earlier experiments in this laboratory identified a highly expressed 65–68-kDa protein in both mouse and human corneas (Cuthbertson, R. A., Tomarev, S. I., and Piatigorsky J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4004–4008). Here, we demonstrate that this protein is transketolase (TKT; EC 2.2.1.1), an enzyme in the non-oxidative branch of the pentose-phosphate pathway, based on peptide and cDNA isolation and sequence analysis of mouse cornea protein and RNA samples, respectively. While expressed at low levels in a number of tissues, the 2.1-kilobase TKT mRNA was expressed at a 50-fold higher level in the adult mouse cornea. The area of most abundant expression was localized to the cornea epithelial cell layer by in situ hybridization. Western blot analysis confirmed TKT protein abundance in the cornea and indicated that TKT may comprise as much as 10% of the total soluble protein of the adult mouse cornea. Soluble cornea extracts exhibited a correspondingly high level of TKT enzymatic activity. TKT expression increased progressively through corneal maturation, as shown by Northern blot, in situ hybridization, Western blot, and enzymatic analyses. TKT mRNA and protein were expressed at low levels in the cornea prior to eye opening, while markedly increased levels were observed after eye opening. Taken together, these observations suggest that TKT may be a cornea enzyme-crystallin, and suggest that the crystallin paradigm and concept of gene sharing, once thought to be restricted to the lens, apply to other transparent ocular tissues.

The cornea is comprised of an anterior epithelial surface composed of six cell layers, an extracellular collagenous stromal matrix encasing a sparse keratocyte population, and a posterior single cell layer of endothelial cells (8). Reminiscent of enzyme-crystallin expression in the lens, the epithelial cells of the cornea also accumulate high concentrations of metabolic enzymes in a taxon-specific manner (9). Indeed, it has been estimated that aldehyde dehydrogenase class III comprises as much as 30–40% of the soluble protein of the cornea epithelium in mammals, an amount suggesting a structural role as well as a metabolic role (9–12). Other previously described abundant cornea epithelium enzymes, which may be putative enzyme-crystallins, include α-enolase (human, mouse, and chicken), glutathione S-transferase (chicken), and peptidyl prolyl cis-trans-isomerase (cyclophilin; chicken) (9, 13).

An abundant soluble 65–68-kDa protein of unknown identity was previously observed in both human and mouse corneas (9). Here, we demonstrate that this protein is transketolase (TKT; EC 2.2.1.1; 14–17), an enzyme in the pentose-phosphate (PP) pathway. The PP pathway produces pentoses and reducing agents in the form of NADPH (16). TKT has broad substrate specificity, transferring a ketol group to an aldehyde acceptor molecule, and requires Mg2+ and thiamine pyrophosphate for optimal enzymatic activity. TKT catalyzes several reactions within the nonoxidative branch of the PP pathway, and together with transaldolase serves as a reversible link between the PP and glycolytic pathways, allowing the cell to adapt to a variety of metabolic needs under changing environmental conditions. TKT is often used to assess thiamine status in man (18), and alterations in TKT chemiofisphysical or kinetic properties have been implicated in a variety of pathological conditions including Wernicke-Korsakoff’s Syndrome (19, 20), Alzheimer’s disease (21, 22), fibromyalgia (23), severe malnutrition (24, 25), and alcoholism (26).

The present studies provide evidence that TKT expression in the mouse cornea epithelium is analogous to crystallin expression in the lens in that a soluble enzyme accumulates to a sufficient concentration to play a refractive role. TKT gene expression increases progressively through cornea maturation, exhibiting a burst of expression that correlates with eye opening. Our observations support the idea that the highly preferred expression of TKT in the corneal epithelium may result from inductive processes associated with environmental conditions, in contrast to the developmentally controlled tissue-preferred transcriptional mechanisms operative in the lens (27, 28).

* These studies were supported in part by National Eye Institute Grant EY10869 (to F. X. Y.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 301-402-4342; Fax: 301-402-0781.

1 The abbreviations used are: TKT, transketolase; HCP, human cornea protein; MCP, mouse cornea protein; oligo, oligonucleotide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PP, pentose-phosphate; RT, reverse transcriptase.
MATERIALS AND METHODS—The abundant mouse 65–68-kDa protein was subjected to trypsin digestion and peptide sequencing as described previously (9), and a new peptide was identified (MCP-2). A 3′-untranslated gold cornea epithelial cell-enriched UniZap (Stratagene) cdNA expression library (oligo-T primed) was screened using an oligonucleotide (oligo); 5′-CTCCCCACACAGCAGCAGAACA- CAGCGCTGCAGGGCGCTCTGATAGTGGTGCTCCACCTG-3′ encoding MCP-2. The cdNA was subcloned (p71) into pBluescript (Stratagene), and sequenced by standard dideoxy methods. p71 and the published mouse TKT CDNA sequence (29, U05809) were used to design oligo primers for the isolation of additional cdNA clones via RT-PCR (Gene-Amp/RNA PCR kit, Perkin-Elmer) of total adult mouse cornea RNA. The PCR primers used and their positions (29) within TKT mRNA were as follows (see Fig. 1): 5′ primers spanning 482–511, 902–931, and 1502–1531; 3′ primers spanning 1610–1634, 1680–1684, 1684–1709, and 2028–2062. The cdNAs were subjected to electrophoretic separation, hybridization analyses using internal oligo probes, and cloned into pCRScript (Stratagene), and sequenced.

RNA Isolation and Northern Blot Analyses—Total RNA was prepared from the tissues of FVB/N mice using RNAzol (Tel-Test), and subjected to Northern blot analyses at 65°C using QuickHyb (Stratagene) and 2 × 10⁶ cpm/ml of one of the following radiolabeled cdNA probes: mouse TKT (11), chicken β-actin (Oncor), or a human glycerol-dehyde-3-phosphate dehydrogenase (Clontech). Cornea epithelium was prepared by the Dispase method of Gipson and Grill (30). A poly(A)⁺ RNA Northern blot was obtained from Clontech. RNA on Northern blots was visualized following autoradiography as follows: 15 min wash in 5% acetic acid, 10 min staining in 0.04% methylene blue, 0.5 M NaOAc, pH 5.2, and destaining in H₂O.

In Situ Hybridization Analysis—Mouse eyes (1 day, 14 day, and 6 weeks) were excised, embedded in OCT compound, and quickly frozen on dry ice. The frozen tissue was sectioned (6 μm), placed on slides coated with 0.25% gelatin and 0.025% chromium potassium, allowed to attach at room temperature for 2 min and then in the cryostat for at least 10 min, fixed with 4% paraformaldehyde in PBS for 10 min, rinsed three times with PBS, air-dried, and stored at −80°C until use. Mouse TKT probes were labeled by in vitro RNA transcription from linearized p71 using T7 (antisense probe) or Sp6 (sense probe) polymerase in the presence of [a-³²P]UTP. The size of the probes was adjusted by treating cRNAs with 0.2 M sodium carbonate at pH 10.2.

Prepared sections were brought to room temperature, hydrated for 2 min in PBS, treated with 1 μg/ml proteinase K in TE buffer for 10 min at 37°C, followed by incubation with 0.2% glycine in PBS. Sections were washed with PBS for 3 min, immersed in 0.1 M triethanolamine HCl, pH 7.5, containing 0.25% acetic anhydride for 10 min, rinsed with 2 × SSPE, dehydrated with ethanol, and air dried. Radiolabeled sense or antisense TKT probes (0.2 μg/ml per kilobase pairs of probe) were applied in 60 μl of hybridization mixture containing 0.5 M NaCl, 10 mM Tris·HCl, pH 7.6, 5 mM EDTA, 0.02% (w/v) Ficoll 400, 0.02% (w/v) bovine serum albumin, 50% formamide, 10% dextran sulfate, 10 μM dithiothreitol, and 0.1 mg/ml yeast tRNA. The mixture was heated at 80°C for 5 min and maintained at 50°C until use. Hybridization was carried out in a humid chamber at 42°C overnight using Parafilm coverslips. Sections were washed twice at 50°C in Solution A (2 × SSPE, 50% formamide, 0.1% β-mercaptoethanol), treated at 37°C for 30 min with 20 μg/ml boiled RNase A in 0.5 M NaCl, 0.5% Tris·HCl, pH 8.0, washed twice in Solution A, washed twice at 50°C in Solution B (0.1 × SSPE, 0.1% β-mercaptoethanol), dehydrated with ethanol, and air-dried. The slides were exposed to Kodak NTB-2 Emulsion, stored in the dark at 4°C for 3–10 days, the autoradiograms developed in Kodak 12 D-19 developer for 5 min, rinsed in H₂O for 30 s, and fixed for 8 min. The sections were then counterstained with hematoxylin and eosin. The sections were visualized, and photographed under bright- and dark-field illumination using a Nikon Optiphot microscope.

Preparation of Cellular Extracts and Enzyme Assay—Soluble cell extracts were prepared from the tissues of perfused 6-week-old FVB/N mice: the tissues were homogenized in H₂O containing proteinase inhibitors (130 μM bestatin, 50 μM chymostatin, 1 μM leupeptin, and 1 μM pepstatin) in the cell debris pelleted and discarded, the supernatant subjected to one freeze-thaw cycle, precipitated material discarded following centrifugation, and the resulting supernatant fraction saved. Total protein content was determined using the Bio-Rad Reagent. Direct TKT enzyme assays were carried out in vitro by colorimetric determination of 7-sedoheptulose phosphate production in soluble cell extracts (31).

Antibody Preparation and Western Blot Analyses—An antibody di-rected against the mouse TKT peptide sequence NH₂-GYHKP-DQQP-WLKDANTECOOGKCS-3–26 was raised (BioSynthesis Inc.) in rabbits following an analysis of the antigenicity, hydrophobicity, and hydrophilicity profiles of the deduced mouse TKT amino acid sequence (29; GenBank U05809). A search of the protein data base did not detect significant sequence similarities with any other protein besides TKT. A positive enzyme-linked immunosorbent assay reaction was obtained with the immunogen peptide (10 μg/ml) and a 1:100 dilution of serum taken from the first bleed of immunized rabbit 435. The serum of the fifth bleed of rabbit 435, taken 14 weeks after immunization, exhibited a single strongly cross-reacting band of the expected size on Western blots and was used for all subsequent studies. Soluble cell extracts (prepared above) were subjected to chemiluminescent Western blot analysis using a 1:10,000 dilution of the rabbit and mouse TKT antisera and a 1:500 dilution of peroxidase-labeled anti-rabbit antibody (Amersham). Total proteins were stained for 5 min in 0.1% Amido Black, 10% acetic acid, 45% methanol, and destained in 2% acetic acid, 90% methanol following electrophoresis and transfer onto Duralon membrane (Stratagene). Both x-ray films and stained membranes were scanned by densitometry.

RESULTS

The 65–68-kDa Protein Is Transketolase—Previous peptide analysis of the abundant mouse and human cornea 65–68-kDa protein identified an identical peptide sequence (ILATP-QEADPSVDAIR; see Fig. 1) in the two species denoted as MCP (mouse) and HCP (human) (9). Here, we report the sequence of an additional mouse peptide termed MCP-2: ILTVEDHYYGGIEAEVASAAYGEPGTVTQ(R) (see Fig. 1). A search of the SwissProt sequence data base for similarities with MCP/HCP detected a perfect match with both mouse (P40142) and human (P29401) transketolase (TKT, EC 2.2.1.1). The MCP-2 sequence was 100% identical with a region in mouse TKT (R at the last position), and matched the analogous region of human TKT at 29 of 31 positions. No other significant sequence similarities were detected, and thus these peptide sequences appeared to be specific for TKT.

A 66-base pair oligo encoding an internal 22 amino acid stretch of MCP-2 was used to screen an adult mouse cornea epithelial cell-enriched cdNA library. One positive subclone (p71) was isolated and sequenced. p71 exhibited a perfect match (positions 1618–2062) with the mouse TKT cdNA sequence previously obtained from the Y1 adenocortical tumor cell line (29; GenBank U05809). The only other significant sequence similarities detected were those with TKT cdNAs and genes isolated from a variety of other species (data not shown). p71 did contain the MCP-2-encoding region, but did not contain the MCP-encoding region. To verify that p71 indeed represent expression of the TKT gene encoding MCP, a series of TKT oligo primers were used to generate additional cdNAs (PCR-PCR in Fig. 1) from adult mouse cornea RNA via RT-PCR. In every case, the PCR-generated cdNAs matched the expected size upon electrophoresis (see Fig. 1 for schematic representation of results), exhibited the appropriate hybridization pattern using internal oligo probes (data not shown), and displayed a perfect match with the reported mouse Y1 cell line TKT cdNA sequence (data not shown). We thus concluded that the abundant 65-kDa protein observed in mouse and human corneas is TKT.

Northern Blot Analysis of TKT Expression—Northern blot analyses were performed to determine the level of TKT gene expression in the cornea, relative to other tissues. As expected from TKT's role in a basic metabolic pathway the TKT gene was ubiquitously expressed (32–34), as determined by Northern blot analysis of 2 μg of poly(A)⁺ RNA isolated from adult mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (Fig. 2A). The expected (29, 35, 35) 2.1-kilobase mRNA was observed in all tissues examined, although at differing levels. Low levels of TKT mRNA were observed in heart and testis following an extended exposure of autoradiograms.
This represents the first reported survey of TKT gene expression in multiple tissues of the mouse. Northern blot analysis performed concurrently on 5 μg of total RNA (Fig. 2B) indicated that TKT mRNA was expressed at a 50-fold higher level in total adult mouse cornea than in lens, liver, and kidney, as determined by densitometric scanning (data not shown). The TKT gene has also been shown to be abundantly expressed in Y1 mouse adrenocortical tumor cell line mutants as a result of TKT gene amplification (29). Hybridization analysis of genomic DNA isolated from adult mouse cornea and brain indicated that the TKT gene was present in the two tissues at equal copy number (data not shown). Thus, our observations indicate that the TKT gene is preferentially expressed in the adult mouse cornea as a result of transcriptional or post-transcriptional regulation, rather than an increase in gene copy number.

TKT expression in the maturing eye was examined in RNA samples isolated before and after eye-opening, which typically occurs at 12–13 days of age in the mouse. Early time points in development were studied using RNA isolated from 17-day embryonic (E17), newborn, and 15-day-old whole mouse eyes. Low and nearly equal TKT mRNA levels were observed in E17 and newborn whole eyes, with a 2-fold increase observed at 15-days old. These observations represent TKT gene expression occurring in the several different cell types present in the whole eye, so we next analyzed RNA isolated from corneas at different stages of maturation. Northern blot analysis of RNA isolated from 7-day, 15-day, and 8-week-old mouse corneas exhibited a progressive increase in steady state TKT mRNA levels (Fig. 3A). Low levels of TKT mRNA were observed in 7-day (before eye opening) samples and a prominent 15-fold increase was observed in 15-day (after eye opening) samples, as determined by densitometric scanning. At 8 weeks, 2-fold higher levels of TKT mRNA were observed as compared to 15-day levels. In 8-week RNA samples additional higher molecular weight hybridizing bands were occasionally observed. The possible origin of these bands, such as incomplete hnRNA processing or the use of additional initiation (36, 37) or poly(A) sites, is not yet known. Staining of this Northern blot with methylene blue confirmed that an equal amount of RNA was loaded in each

**Fig. 1. Schematic diagram of TKT mRNA and cDNA clones.** The mouse TKT mRNA (2062 base pairs in length; GenBank U05809; 29) is shown with the position of the ATG start codon, the MCP- (9) and MCP-2 peptide-encoding regions, stop codon, and poly(A) site denoted. The p71 cDNA was isolated by hybridization screening of an adult mouse cornea epithelial cell-enriched cDNA library with an oligo encoding MCP-2. cDNAs PCR1-PCR8 were generated by RT-PCR of adult mouse cornea RNA using oligo primers generated from the nucleotide sequence of p71 and a mouse Y1 adrenocortical tumor cell line TKT cDNA (29).

**Fig. 2. Northern blot analysis of TKT gene expression.** Either 2 μg of poly(A)+ RNA (panel A) or 5 μg of total RNA (panel B) isolated from adult mouse tissues was subjected to Northern blot analysis using the p71 TKT cDNA clone as a probe. Hybridization and autoradiography were performed concurrently; A was exposed to film for 2 h, while B was exposed for 18 h. The position of TKT mRNA is denoted by an arrow. TKT mRNA was observed in heart and testis in panel A after extended exposure of the autoradiogram.
lane (as determined by the intensity of the 18 S and 28 S rRNA bands; Fig. 3A). Control Northern blots were performed using β-actin and glyceraldehyde-3-phosphate dehydrogenase cDNA probes (Fig. 3B). Glyceraldehyde-3-phosphate dehydrogenase gene expression did not increase during cornea maturation. β-Actin expression increased approximately 5-fold between 7 and 15 days, as might be expected given the proliferative burst of the stratifying epithelium during this time. This is much less than the 15-fold increase in TKT mRNA during the same time period. Also in contrast to TKT, a significant rise in β-actin mRNA was not observed between 15 days and 8 weeks. These observations indicate that a prominent specific increase in TKT expression correlates with eye opening.

**In Situ Hybridization Analysis of TKT Expression—**In situ hybridization analysis of adult mouse corneas was performed using the antisense p71 TKT radiolabeled probe to determine the relative levels of TKT expression in the epithelial, stromal, and endothelial layers of the cornea. TKT was expressed at low levels in all three layers of the newborn mouse cornea, as revealed by the weak and homogeneous in situ hybridization signal observed in a 7-day exposure to film (Fig. 4A). Low levels have also been observed in the adjacent limbus and conjunctiva (34). TKT mRNA levels were markedly and progressively increased in the epithelium of 14-day (Fig. 4B) and 6-week-old (Fig. 4C) mouse corneas, as revealed by the increased in situ hybridization signals observed in 3-day exposures to film. The endothelial layer also exhibited TKT expression, and significantly lower basal levels of TKT expression were observed in the stroma. Use of the sense p71 probe produced no detectable signal in any layer of the cornea (data not shown).

A noticeable dark “band” is observed within the label over the epithelial side of the 14-day cornea section (dark field; Fig. 4B). The dark band is an artifact, and should not be interpreted as Bowman’s membrane. The bright signal on the apical side of the dark band represents radiolabeled probe trapped outside the cornea epithelium. This is supported by the appearance of a broad layer of dark grains outside the epithelium in bright field photos (Fig. 4B). In addition, the bright signal (dark field; Fig. 4B) on the apical side of the dark band is below the plane of focus (the sectioned tissue), and thus does not represent hybridizing mRNA within cornea cells. In contrast, the bright signal (dark field; Fig. 4B) on the epithelial-side of the dark band is in the plane of focus (the sectioned tissue), and represents hybridizing mRNA existing with cornea epithelial cells, just as the signal observed in stromal fibroblasts and endothelial cells is also in the plane of focus. In order to confirm that a strong area of TKT hybridization does not occur in the stroma, Northern blot analysis was performed on RNA prepared from both whole cornea and the isolated cornea epithelium of 14-day-old mice and subjected to Northern blot analysis (Fig. 5). The level of TKT-hybridizing signal was quantitated by densitometry and expressed relative to the level of rRNA present in each sample (see figure legend). These results indicate that 75% of the hybridizing signal in whole cornea is accounted for by the epithelium, suggesting that an area of significant TKT expression does not occur in the stroma just beneath Bowman’s membrane. Taken together, these findings indicate that TKT is
Transketolase

Fig. 5. Northern blot analysis of TKT gene expression in cornea epithelium. 5 µg of total RNA isolated from either total corneas or the cornea epithelium of 14-day-old mice was subjected to Northern blot analysis using the radiolabeled p71 TKT cDNA as a probe (top panel). Methylene blue staining (bottom panel) was carried out as in Fig. 3. Densitometric scanning of the autoradiograph (whole cornea, 1.0; epithelium, 0.5) and the methylene blue-stained blot (whole cornea, 1.5; epithelium, 1.0) was performed to quantify the relative levels of TKT mRNA and rRNA in the samples. TKT mRNA levels were then standardized per rRNA levels: whole cornea, 0.66; epithelium, 0.5. The position of TKT mRNA in the autoradiograph, and 28 S and 18 S rRNA in the stained blot are denoted.

Abundantly expressed in the mature mouse cornea epithelium.

Western Blot Analysis of TKT Expression—Chemiluminescent Western blot analyses were performed to study TKT expression in the cornea at the protein level. Soluble cell extracts were prepared from the corneas of 7-day, 15-day, and 6-week-old mice, and non-corneal tissues of perfused 6-week-old mice. Perfused mice were used in order to accurately quantify the relative levels of TKT activity, given the high level of TKT protein and enzyme activity observed in erythrocytes (38–40). The use of perfused non-corneal tissues was also important, given the avascular nature of the cornea. An antiserum raised against deduced TKT amino acids 3–20 (29) produced a single strongly cross-reacting band of the expected size in cornea samples (Fig. 6A, lanes 1–3), whose intensity increased during development. Densitometric scanning indicated that the cross-reacting band observed in 15-day extracts was 8-fold more intense than that in 7-day extracts (data not shown). In contrast, only a weakly immunoreactive band was observed in lung and brain cell extracts (Fig. 6A, lanes 4 and 5, respectively). These findings are consistent with previous studies that demonstrated low levels and variable amounts of TKT protein in most mouse tissues (32, 41).

To determine whether TKT was expressed in the cornea at levels comparable to the crystallin proteins of the lens, Western blot analysis and Amido Black staining of electrophoretically separated soluble cornea proteins were performed in parallel (Fig. 6B). The TKT-immunoreactive band was aligned with one of the major soluble protein bands observed in total adult cornea cell extracts. Densitometric scanning of the Amido Black-stained proteins indicated that the presumptive TKT band constituted roughly 10% of the total stained proteins (data not shown). Given that sequencing of this band produced only peptides corresponding to TKT (our findings here and Ref. 9) it is reasonable to presume that this band is composed primarily, although probably not solely, of TKT. Thus, TKT may represent up to 10% of the total soluble protein of the cornea. Our observations suggest that TKT accumulates in the cornea to levels resembling that for individual crystallin proteins.

Assay of TKT Enzymatic Activity—We next assayed whether the abundantly expressed TKT protein retained its enzymatic activity. The same soluble cell extracts prepared for Western blot analysis were assayed for TKT enzymatic activity by measuring the production of 7-sedoheptulose phosphate (Fig. 7; Ref. 31), a direct by-product of TKT-catalyzed reactions. TKT enzymatic activity increased progressively during development with a 4.5-fold increase observed between 7- and 15-day extracts. Cornea extracts produced 10–25-fold higher levels of 7-sedoheptulose phosphate in vitro than non-corneal extracts prepared from 6-week-old mice. These findings are consistent with previous studies suggesting that while TKT protein is constitutively expressed, the level of TKT activity varies among different tissues of the same species (14). The elevated level of TKT protein in the adult cornea was thus paralleled by an increased level of TKT enzymatic activity.

DISCUSSION

We have demonstrated that the abundant 65–68-kDa protein found in mouse and human corneas is TKT, an enzyme in the nonoxidative branch of the PP pathway. The TKT gene is most highly expressed in the epithelial cell layer of the mature mouse cornea. Our findings are consistent with early studies which demonstrated high levels of PP pathway activity in cornea epithelium (42–45). Moreover, Kinoshita and Masurat (46) demonstrated the thiamine pyrophosphate-dependent production of high levels of sedoheptulose in bovine cornea epithelial cell extracts, a hallmark of TKT enzymatic activity. Most recently, Nishida et al. (47) demonstrated significant TKT expression in the adult human cornea epithelium. Abundant 62–68-kDa cornea proteins have been previously observed (13, 48, 49). Since our sequencing studies produced only peptides corresponding to TKT, we presume that the abundant 65–68-kDa protein band of the adult mouse cornea is primarily composed of TKT.

Although not as abundant as aldehyde dehydrogenase type III (30–40% of the soluble protein; Refs. 9–12), the relatively high concentration of soluble TKT (10%) in the mouse cornea
epithelium suggests strongly that it may serve a structural as well as a metabolic role, as do the lens enzyme-crystallins (4–6). Even in most vertebrate lenses individual crystallins may constitute only 5–10% of the water-soluble protein while the composition of the total crystallins amounts to 80–90% of the protein. The low expression of TKT in essentially all of the non-corneal tissues we have examined and its strikingly higher expression in the corneal epithelium, higher than expected for purely metabolic needs, are precisely the scenario observed for the enzyme-crystallins of the lens, which play both structural and metabolic roles. The ability of a single protein, encoded by a single gene, to perform multiple functions in the same and different tissues is known as gene sharing (7).

By accumulation to high concentrations in the corneal epithelium, TKT may play structural roles in light refraction and tissue transparency, or in light absorption. A uniformly high concentration of soluble proteins throughout the corneal epithelium could minimize concentration fluctuations that would scatter light (50, 51). TKT may also protect the internal structures of the eye from the damaging effects of UV radiation (52) by directly absorbing UV light. The cornea is responsible for absorbing 80% of incident UV light (53). The water-soluble fraction (>12 kDa) of the bovine cornea is capable of absorbing 40–45% of 290–300 nm UV radiation, a property which may be conferred by the primary structure of the proteins in this fraction (54). A major component (19%) of this fraction is a 62-kDa protein of unknown identity. It is tempting to speculate that this protein is TKT, given that we have observed significant levels of TKT mRNA in the bovine cornea.

TKT exhibits a progressive increase in expression throughout cornea maturation that correlates with eye opening. This finding is consistent with previous observations on developing corneas which demonstrate an increase in PP pathway activity during cornea maturation (44, 55).

is in contrast to induced TKT expression in Craterostigma plantagineum in which translational controls have been implicated (56). Mouse aldehyde dehydrogenase type III2 and a rat ocular surface glyocalyx (57) are also up-regulated during cornea maturation and correlated with eye opening.

The abundant expression of TKT in the mature mouse cornea epithelium, and an up-regulation coincident with eye opening, may reflect an increased requirement for its protective enzymatic activity in these cells. Exposure of the cornea to UV light results in the production of free radicals and H2O2, agents potentially damaging through oxidative mechanisms (52, 58, 59). The removal of such toxic agents is of critical importance in avascular tissues such as the cornea. The PP pathway is the major supplier of NADPH reducing agents (16) and is tightly coupled to cellular processes which require NADPH and other reductase systems. TKT, therefore, may play a critical role in maintaining a reducing environment. The bovine and rabbit cornea epithelium exhibit high levels of PP activity (42, 43, 45), and PP activity can be stimulated in rabbit and chicken corneas upon exposure to oxidizing agents (42, 60). Also consistent with this idea is the abundant expression of similarly protective enzymes in the cornea epithelium of various species (9, 11, 12, 47, 61–66).

Despite their common property of enzyme-crystallin expression, the mechanisms for recruitment of tissue-preferred gene expression in the lens and cornea may differ. In the lens, the crystallin genes are regulated by tissue- and developmental-specific cis-acting transcriptional regulatory elements (67–69). In contrast, high level gene expression in the cornea may occur as a result of inductive environmental events which coincide with eye opening, including exposure of the cornea to incident light, the proliferative burst and subsequent stratification of the epithelial cell layer just prior to eye opening (44), the establishment of an avascular environment as corneal epithelial cells move from the limbal region into the cornea proper (8), and the change of relative CO2 and O2 concentrations between the closed and open eye situation (70). In fact, the maintenance of high levels of aldehyde dehydrogenase type III expression in cultured corneal epithelial cells requires exposure to light (71), indicating the importance of light exposure for specific gene expression in these cells. It is interesting to note in this regard that the TKT genes of C. plantagineum (56) and Amycolatopsis methanolica (72) exhibit transcriptional responsiveness to environmental stimuli. These observations support the contention that crystallin expression in the cornea may have been selected for by environmental and physiological conditions and stimuli which act at the level of transcriptional regulation (27, 28). The abundant expression of TKT in the cornea is not necessarily restricted to one of these possibilities, and may in fact be explained by a combination of these mechanisms, or an as yet unidentified selective mechanism.

Acknowledgments—Sequence analyses were carried out on the supercomputing facilities of the NCI/FCRF computers. We thank P. Zelenka and S. Tomarev for a critical reading of the manuscript and M. Chervenak for technical assistance.

REFERENCES

1. Land, M. F. (1988) Contemp. Phys. 29, 435–455
2. Land, M. F., and Fernald, R. D. (1992) Annu. Rev. Neurosci. 15, 1–29
3. Wistow, G. J., and Piatigorsky, J. (1988) Annu. Rev. Biochem. 57, 479–504
4. Piatigorsky, J., and Wistow, G. J. (1989) Cell 77, 197–199
5. de Jong, W. W., Hendriks, W., Mulders, J. W. M., and Bloemendal, H. (1989) Trends Biochem. Sci. 14, 365–368
6. Wistow, G. J. (1990) Trends Biochem. Sci. 18, 301–306
7. Piatigorsky, J., O'Brien, W. E., Normán, B. L., Kalumuck, K., Wistow, G. J., Borrás, T., Nickerson, J. M., and Wawrousek, E. F. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5479–5483
8. Klyce, S. D., and Beuerman, R. W. (1988) in The Cornea (Kaufman, H. E., 2 C. M. Sax and J. Piatigorsky, unpublished data.
9. W. T. Kays and J. Piatigorsky, unpublished data.
