The cornea is a multilayered tissue characterized by its unique optical properties with transparency, avascularity, the ability to refract light, and to filter out incoming ultraviolet (UV) radiation. Corneal disorders due to injury, disease, or genetic defects account annually for several million new cases of impaired vision and are second to cataracts as the most important cause of blindness in the world [1]. Owing to a limited knowledge of the basic molecular mechanisms involved in their pathophysiology most corneal diseases have traditionally been classified according to clinical manifestations and their morphological appearance. The consequence has been a lack of rational strategies for both the prevention and treatment of many corneal disorders. Thus, increased biochemical knowledge of normal and diseased corneas is essential for the understanding of corneal homeostasis and pathophysiology.

With the completion of the Human Genome Project, the tissue-specific and differential expression of the proteins encoded by the 20,000 to 25,000 human genes has become a main focus in research (Human Proteome Organization). Proteins exist in distinct variants due to alternative mRNA splicing, posttranslational modifications, and proteolytical processing. These modifications regulate and alter the function of the proteins and, therefore, mapping of the human proteome is an important step toward understanding protein function under normal and pathophysiologic conditions. With the advent of proteomic technology, we recently reported the identification of 141 distinct proteins in normal human corneas. This dataset represents the most comprehensive protein study of the cornea to date and provides a useful reference for further studies of normal and diseased human corneas. The list of identified proteins is available at the Cornea Protein Database. In the present paper, we review the utilized procedures for extraction and fractionation of corneal proteins and discuss the potential roles of the identified proteins in relation to homeostasis, diseases, and wound-healing of the cornea. In addition, we compare the list of identified proteins with high quality gene expression libraries (cDNA libraries) and Serial Analysis of Gene Expression (SAGE) data. Of the 141 proteins, 86 (61%) were recognized in cDNA libraries from the corneas of dogs and rabbits, or humans with keratoconus, and 98 (69.5%) were recognized in SAGE data of mouse and human corneas. However, the percentages of identified genes in each of the protein functional groups differed markedly. Thus, exceptionally few of the traditional blood/plasma proteins and immune defense proteins that were identified in the human cornea were recognized in the gene expression libraries of the cornea. This observation strongly indicates that these abundant corneal proteins are not expressed in the cornea but originate from the surrounding pericorneal tissue.

**CHARACTERIZATION OF THE HUMAN CORNEA PROTEOME**

The techniques for analyzing gene expression, such as cDNA sequencing and oligonucleotide microarray systems, fail to identify proteins and determine their abundance within a tissue. Therefore, direct protein identification methods such as Edman degradation and mass spectrometry (MS) are required to characterize the protein expression profile of a tissue. In a recent proteomic study, we reported the identification of 141...
distinct proteins from intact normal human corneas involving all cell layers: epithelium, Bowman’s layer, stroma, Descemet’s membrane, and endothelium [2]. Initially, the dissected corneal tissue (7 mm diameter buttons) were lyophilized and homogenized to generate a corneal powder. The human cornea proteome was then fractionated using four different extraction protocols and separation methods involving two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), one-dimensional polyacrylamide gel electrophoresis (1-D PAGE), and strong cation exchange (SCX; protocols 1-4; Figure 1).

The utilization of these different protocols facilitated the identification of both soluble and insoluble proteins and increased the number of identified proteins in general.

Protocol 1: To denature and release all proteins from the tissue, excluding insoluble structures such as collagen fibers, proteins from the corneal powder were extracted in a denaturing and reducing buffer (lysis buffer) containing 5 M urea, 2 M thiourea, 2% (w/v) 3-(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 2% (w/v) N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (SB3-10), 0.5% (v/v) IPG buffer, 10 mM dithiothreitol (DTT), and a protease inhibitor cocktail (2 mM EDTA, 2 mM 1,10-phenanthroline, 40 μM E-64, and 2 mM pefabloc SC). The sulphydryl reducing agent, DTT, was included in the extraction solution to release proteins linked to the extracellular matrix through disulfide-bridges as previously observed for the abundant corneal adhesion protein, transforming growth factor beta induced protein (TGFBIp) or βig-h3) [3,4]. Subsequently, the extracted proteins were separated by 2-D PAGE using five different pH gradients (3.5-4.5, 4.5-5.5, 5.5-6.7, 4.0-7.0, and 6.0-9.0).

Protocol 2: To analyze the water-soluble proteome of the human cornea, proteins were extracted from the corneal powder by a nondenaturing and nonreducing buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, and the protease inhibitor cocktail. The extracted water-soluble proteins were precipitated by adding 90% (v/v) ethanol, and the pellet was lyophilized and dissolved in lysis buffer. The proteins were separated by 2-D PAGE using a 4.0-7.0 pH gradient.

All 2-D gels were silver stained to visualize the proteins. To generate peptides suitable for analysis by mass spectrometry, spots were excised and digested with trypsin, which cleaves peptide bonds at the carboxyl side of arginine and lysine residues. Sixty-seven distinct proteins were identified from 165 spots on the 2-D gels by peptide mass fingerprinting (PMF) using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). With PMF the absolute mass of the peptides is accurately measured with mass spectrometry and the resulting mass list is then compared with the theoretical peptide masses generated by in silico trypsin digestion of all proteins in a database to search for significant matches.

The staining intensities and the number of different protein isoforms from the 2-D PAGE analysis indicated that the most abundant proteins in the corneal extracts were TGFBIp (29 isoforms), albumin (13 isoforms), immunoglobulin κ light chain (11 isoforms), α-1-antitrypsin (6 isoforms), pigment epithelium-derived factor (PEDF; 4 isoforms), with aldehyde dehydrogenase type 3 member A1 (ALDH3A1; 4 isoforms), α-enolase (2 isoforms), haptoglobin (6 isoforms), β- and γ-actin (4 isoforms), vimentin (5 isoforms), and collagen α1(VI) chain (3 isoforms). Of the 67 identified proteins, six were present in at least five different isoforms.

That several spots were found to be different fragments for certain proteins, such as TGFBIp and albumin, shows that degradation of proteins is common in the normal human cornea. Most degradation fragments of TGFBIp and albumin are not water-soluble as revealed by a comparison of the water soluble corneal proteome with the protein profile obtained using protocol 1.

Protocol 3: The corneal powder was also cleaved chemically with cyanogen bromide (CNBr), which splits proteins on the carboxyl side of methionine residues. In contrast to trypsin, CNBr is a small molecule able to access and cleave compact proteins such as those in collagen fibers. Following this treatment with CNBr the entire corneal proteome is converted into peptides, enabling the insoluble structures to be identified by liquid chromatography tandem mass spectrometry (LC-MS/MS). To generate peptides more suitable for mass spectrometry analysis, the CNBr-fragmented peptides were further digested with trypsin. The resulting peptides were then separated using SCX chromatography, and each of the pools were analyzed by LC-MS/MS. Only 31 distinct proteins were identified using this procedure, probably due to the high abundance of collagen peptides in all the fractions. As expected, the proteins identified using protocol 3 were dominated by 10 different chains of collagen types I, II, III, V, VI, and XII, proteoglycans (decorin, biglycan, lumican, and keratocan), proteins classified as corneal enzyme-crystallins (α-enolase, ALDH3A1, and transketolase [TKT]), and other abundant corneal proteins such as immunoglobulins (Ig; Ig heavy chains α-1 and γ-1, and light chains κ and λ), albumin, and TGFBIp.

Protocol 4: To separate the majority of the less abundant from the high abundant proteins prior to their identification by LC-MS/MS the corneal powder was also boiled in reducing sodium dodecylsulfate (SDS) sample buffer and proteins were separated by 1-D PAGE. Slices of the gel lane were digested with trypsin and each sample was subjected to LC-MS/MS. From this protocol, 103 distinct proteins were recognized. It is noteworthy that only seven of the ten collagen chains identified using protocol 3 were detected using protocol 4. Collagen α1(II), 1(III), and 2(V) chains were not identified using protocol 4.

By directly analyzing corneal proteins as opposed to procedures based on antibody-antigen interactions, which often cause false positives due to cross-reactivity, we have identified 141 distinct proteins in the cornea. About 70% of them have not been previously detected in any mammalian corneas using direct protein identification techniques. The categorization of the corneal proteins revealed that 60% (85 of 141) are located intracellularly, 38% (54 of 141) are extracellular matrix proteins, while only two proteins are plasma membrane bound.
COMPARISON OF THE HUMAN CORNEA PROTEOME WITH CDNA LIBRARIES AND SAGE DATA

The dataset of identified proteins in the human cornea [2] was compared with genes expressed in human, dog, and rabbit corneas using the following un-normalized cDNA libraries: (1) NbLib0073; NEI human keratoconus cornea un-normalized [5], (2) NbLib0089; NEI dog cornea un-normalized, and (3) NbLib0086; NEI rabbit cornea un-normalized, which are all available at the NEIBank [6], and the SAGE data sets of (4) the normal intact mouse cornea [7], (5) the human normal [8], and Fuchs’ dystrophy corneal endothelium [9], available at CorneaNET. The high quality NEIBank cDNA libraries were used for the alignments since a comprehensive library is presently unavailable for the normal human cornea. The 141 protein sequences in FASTA format were searched against these libraries, using the BLAST program and the BLOSUM-62 substitution matrix without filter. For the keratoconus cDNA library, alignments with at least 97% identity over at least 75 amino acids are considered a match. In the case of the dog and rabbit cornea libraries, clones generating alignments with at least 60% identity to the detected human corneal protein over at least 75 amino acids were further investigated. These investigations included an analysis of the clone description and GenBank entry. Furthermore, the GenBank entry sequence was then compared to the human Swiss-Prot database to determine

![Diagram](http://www.molvis.org/molvis/v12/a52/)

Figure 1. Schematic diagram showing the four different protocols used for the extraction, separation, and identification of proteins from the human cornea. The right side of the figure characterizes each step of the procedures.
the most homologous human protein and thereby verify that the clone sequence showed the highest identity with that specific human protein.

From the 141 proteins, 86 (61.0%) were recognized in the keratoconus, dog, and rabbit cornea cDNA libraries, while 55 (39.0%) could not be found (Appendix 1). Of the 86 identified cDNA clones, 73 of the proteins were identified in the keratoconus library, ten in the dog, and three could only be recognized in the rabbit cornea cDNA library. The relative number of identified clones in each of the protein functional groups (Appendix 1) were found to be different. Thus, 25 (64.1%) of the structural or structural-associated proteins, three (14.3%) of the classical blood/plasma proteins, 15 (75.0%) of the metabolic proteins, four (40.0%) of the immune defense proteins, 12 (100%) of the redox regulation and oxidative stress defense-proteins, and nine (100%) of the proteins assisting in protein folding and degradation, and 16 (61.5%) of the proteins with other functions were identified in the cDNA libraries. The dataset of identified cornea proteins was also compared with the gene expression profiles of the intact mouse cornea [7] and human corneal endothelium obtained with SAGE [8,9] (CorneaNET). From these comparisons, 98 (69.5%) of the proteins were recognized in the mouse and human cornea SAGE lists (Appendix 1). This analysis showed similar results to the cDNA libraries concerning the relative number of expressed genes in each of the protein functional groups. Thus, only five (23.8%) of the classical blood/plasma proteins and two (20.0%) of the immune defense proteins were recognized in the SAGE lists of corneal genes. All comparisons of the protein content and gene expression in the cornea failed to identify most of the classical blood/plasma proteins including immunoglobulins, indicating that they are not expressed in the cornea. That the lists of expressed corneal genes have several entries encoding proteins that were not detected in the “Human Cornea Proteome” is likely due to the lack of correlation between gene expression and protein expression as well as the difficulties of detecting less abundant proteins from a complex protein mixture like a tissue.

**ROLES OF IDENTIFIED CORNEAL PROTEINS**

**Blood/plasma proteins:** About 52% (28 of 54) of the corneal extracellular proteins are common plasma proteins when the identified immunoglobulin chains and complement C3 are included (Appendix 1). This group of corneal proteins also contain different serpins (α-1-antichymotrypsin, α-1-antitrypsin, and antithrombin III), α-1-microglobulin, different apolipoproteins, fibrinogen, haptoglobin, hemopexin, albumin, amyloid P-component, tetranectin, transferrin, transthyretin, and vitamin D-binding protein. Thus, these proteins are either synthesized by the corneal cells as previously reported for α-1-antichymotrypsin [10], α-1-antitrypsin [11], and apolipoprotein J [12], or originate from blood and enter the cornea with the bulk flow from the ciliary arteries located in the corneoscleral limbus area as previously suggested for albumin [13] and other proteins [14]. The identification of α-1-antichymotrypsin, α-1-antitrypsin, apolipoproteins D, E, J, complement C3, and the immunoglobulin J chain in gene expression profiles of human, dog, rabbit, and mouse corneas strongly indicates that these proteins are synthesized in the cornea. In contrast, the other common plasma proteins such as albumin, haptoglobin, hemopexin, amyloid P-component, transferrin, and transthyretin as well as the immunoglobulin chains were not identified in the gene expression libraries suggesting that these proteins are synthesized outside the cornea and imported from the blood stream of the surrounding tissue. Furthermore, apolipoprotein D, which binds phospholipids and cholesterol, has previously been identified in the tear fluid and is most likely synthesized in the lacrimal gland [15]. Thus, some of the corneal apolipoprotein D may also originate from tears.

The functions of plasma proteins in the avascular cornea are probably similar to those displayed in plasma including transport of fatty acids, heme, iron, hormones, vitamins, proteins, or participate in the blood-clotting cascade. Thus, haptoglobin binds free hemoglobin in plasma after hemolysis and transports it to the liver for degradation. In addition, this complex formation prevents oxidative damage by free hemoglobin [16,17]. Hemopexin carries released heme to the liver for breakdown and iron recovery, while transferrin binds and delivers iron to cells. Other identified transport proteins common in plasma included transthyretin, which transports the hormone, thyroxine, and is also involved in retinol (vitamin A) transport [18]. Vitamin D-binding protein has been reported to have multiple functions including delivery of vitamin D metabolites [19], binding of various fatty acids [20], and the formation of complexes with free actin in the circulation [21]. Likewise, albumin is known to bind different hydrophobic metabolites, including heme.

**Coagulation and fibrinolysis:** Previous studies have indicated the importance of fibrin/fibrinogen in corneal wound-healing [22]. Fibrinogen α and γ chains play a pivotal role in blood coagulation. Thus, cleavage of fibrinogen by thrombin leads to spontaneous fibrin polymerization and cross-linking catalyzed by factor XIIIa. However, coagulation in the normal cornea may be regulated by the presence of antithrombin III, a strong inhibitor of the blood clotting serine proteases thrombin and factors XIIa, XIa, and IXa. Furthermore, tetranectin has been shown to bind plasminogen [23] and tissue-type plasminogen activator [24], and stimulates the activation of plasminogen to plasmin, the serine protease responsible for the degradation of fibrin clots.

**Antiangiogenic factors:** In accordance with the avascularity of the normal cornea, angiogenic factors such as basic fibroblast growth factor and vascular endothelial growth factor were not identified. However, several inhibitors of angiogenesis were found including PEDF [25], maspin [26], α-1-antitrypsin [27], and antithrombin III [28]. These all belong to the serine protease inhibitor family (serpins) and possess antiangiogenic activity. PEDF shows no serine protease inhibitory activity and has previously been detected in the corneal epithelium and endothelium by immunohistochemistry [29]. Similarly, maspin is an inactive serpin with antiangiogenic activity as shown by its ability to inhibit neovascularization [26]. Studies have indicated that maspin is expressed by epi-
thelial cells, keratocytes, and endothelial cells of the cornea [30]. The active serine protease inhibitor, α-1-antitrypsin, has also been shown to inhibit neovascularization [27]. Interestingly, the expression of corneal α-1-antitrypsin is specifically induced by retinol and retinaldehyde [31], which concomitantly inhibits the expression of vascular endothelial growth factor [32] indicating that retinoids (vitamin A derivatives) may play a role in the maintenance of a normally avascular cornea. In addition to its function in fibrinolysis, antithrombin III molecules with latent-conformations have shown antiangiogenic activities [28]. Thus, this serpin may also contribute to the maintenance of avascularity in the normal cornea. In addition to the identified antiangiogenic serpins, the hemoglobin-binding protein haptoglobin has been shown to inhibit angiogenesis [33].

Adoptive and innate immune defense: Corneal infections by bacteria, fungi, or viruses are common problems that can lead to corneal opacification. From the adoptive immune system, we identified both light chains (κ and λ), the heavy chains of IgA (α chain) and IgG (γ chains), and the Ig-binding protein immunoglobulin J chain [2]. In addition, C3 from the complement system and a few proteins with antimicrobial activities were identified. As expected, lysozyme C, which is primarily secreted by the lacrimal glands and is present in tears [34], was identified in the cornea. Lysozyme is an important factor of the innate defense system of the eye as it destroys the cell wall of Gram-positive bacteria. Interestingly, the antibiotic peptide, dermicidin, originally found in sweat [35] was also identified. A previous study indicated dermicidin was not present in tears, suggesting it was produced locally by the corneal cells [36]. However, dermicidin was not recognized in the cornea cDNA libraries or the SAGE lists. Dermicidin may serve an important function as a defense factor against bacterial infections in the cornea. The cornea proteomic study [2] also revealed the presence of the cytokine, macrophage migration inhibitory factor, that is known to stimulate the host defense system by activating T-cells upon antigenic responses. This cytokine has previously been found in the basal epithelial cells and in the corneal endothelium, indicating that it may play an important role in corneal cell immunity [37].

Extracellular structural proteins: The corneal stroma (thickness about 450 µm) is a highly ordered, avascular connective tissue consisting mainly of collagen lamellae (about 200 lamellae through the thickness of the human corneal stroma). Studies using indirect methods have suggested that the corneal stroma is dominated by collagen types I, V, and VI [38,39]. The lamellae are formed by fibers of type I, III, and V collagens [39-41], having a uniform diameter of about 30 nm and creating a regular array with a spacing of about 60 nm between the centers. We identified all subunits of collagen type I (α2(I) and α1(I) chains), type II (α1(II) chain), type III (α1(III) chain), type V (α1(V) and α2(V) chains), type VI (α1(VI), α2(VI), and α3(VI) chains), and type XII (α1(XII) chain). Collagen types I, II, III, and V are fibril-forming collagens, type VI is a microfibrillar collagen, while type XII is a fibril-associated collagen with interrupted triple helix (FACIT). None of the collagens have previously been identified in the cornea using direct analyses.

The diameters and organization of the collagen fibrils in the stroma are partly controlled by small leucine-rich proteoglycans. Lumican [42] and keratan can [43], which contain keratan sulfate chains, play important functions in stromal matrix assembly, and are essential for corneal transparency [44,45]. Likewise, decorin [46] and biglycan [47], which contain chondroitin/dermatan sulfate chains, may also be involved in the regulation and structural organization of the corneal extracellular matrix [47-49]. A frameshift mutation in the DCN gene (c.967delT) that encodes for decorin causes congenital stromal dystrophy of the cornea [49].

Protection from and transport of heme and iron: Reactive oxygen species (ROS) such as the superoxide radical (O2-) and hydrogen peroxide (H2O2) are ubiquitous products in aerobic organisms. These ROS have rather low reactivities but can be converted to highly damaging radical species. Thus, the extremely reactive hydroxyl radical (OH) can be generated from hydrogen peroxide by ultraviolet photolysis or chemically by metal ion-induced reduction (Fe2+ to Fe3+) known as the Fenton or Haber-Weiss reaction which involves superoxide [50]. As the outermost tissue of the eye, the transparent cornea is highly exposed to UV-radiation and, therefore, protection against the formation of reactive oxygen species is important. The identification of extracellular superoxide dismutase (EC-SOD) is in accordance with previous studies indicating that this superoxide scavenger is abundant in the cornea where it may protect against UV-induced damage [51]. EC-SOD converts superoxide to hydrogen peroxide (2O2- + 2H+ -> H2O2 + O2), which consequently can lead to an increased formation of hydroxyl radicals. In contrast, scavengers of H2O2 such as the heme-containing protein, catalase, and selenium-containing glutathione peroxidase (GPX3), were not identified in the cornea.

The presence of haptoglobin, a hemoglobin-binding protein, in the avascular cornea is surprising when hemoglobin itself was not identified. However, trace amounts of hemoglobin and heme from plasma may diffuse from the corneoscleral limbus area into the peripheral cornea [13]. Catalase, which is a hemeoprotein, has previously been identified in the cornea and is probably damaged by UV-radiation [52] which also may lead to the release of free heme. Free hemoglobin and heme are toxic as autooxidation of ferrous (Fe2+) heme generates ROS. Thus, the presence of small amounts of free hemoglobin and heme in the transparent cornea would cause oxidative stress and tissue damage.

Previous studies have shown that haptoglobin efficiently reduces hemoglobin-stimulated lipid peroxidation [16] and protein oxidations [17]. Thus, haptoglobin may have a protective role against the formation of ROS in the cornea. Alternatively, corneal localization of haptoglobin could be related to its reported antiangiogenic activity [33]. The protection against iron-dependent oxidations is further reflected in the presence of hemopexin, a high affinity heme-binding plasma protein, which has been shown to protect against heme-de-
ependent oxidative stress [53]. In addition, α-1-microglobulin has been shown to bind and participate in the degradation of heme [54]. Furthermore, these heme-binding proteins including albumin also sequester heme from pathogens in the cornea. Transferrin binds ferric iron (Fe^{3+}) and is responsible for the solubilization, transport, and delivery of iron to the cells by receptor-mediated endocytosis. Furthermore, transferrin protects iron from participation in oxidations and from being utilized by microorganisms [55]. We also identified ferritin light and heavy chains, which are intracellular iron-binding proteins. According to previous studies, ferritin heavy chain in the cornea is mainly located in the nuclei of epithelial cells [56]. Nuclear ferritin protects against UV-induced oxidative damage of DNA in corneal epithelium probably by sequestering iron [57]. Our findings of extracellular proteins preventing the release of free iron are in accordance with the proposed role of nuclear ferritin in the corneal epithelium indicating that protection against oxidative stress is also important outside the cells in the cornea. Furthermore, lactoferrin, which was not identified in the normal cornea, but has been found in subepithelial deposits of amyloid in familial subepithelial corneal amyloidosis (gelatinous drop-like dystrophy of the cornea) [58], is an iron-binding protein in tears where it may protect the corneal epithelium from UV-induced damage [59].

**Redox regulation and oxidative stress:** Redox regulation is required to maintain an efficient oxidative stress defense in the cornea. The DJ-1 protein is a redox-dependent chaperone that may play a key role in the sensing of reactive oxygen species and protection of cells against endogenous oxidative stress [60,61]. We also identified glutathione S-transferase P, thioredoxin, and the peroxiredoxins 1, 5, and 6, which are all involved in the redox regulation process. In addition, NAD(P)H:quinone oxidoreductase 1 was identified in the proteomic study of the human cornea. This enzyme is known to be stimulated by free radicals to produce antioxidant forms of ubiquinone and α-tocopherol (vitamin E) [62]. It has previously been shown that NAD(P)H:quinone oxidoreductase 1 is abundant in the corneal epithelium, indicating it may be involved in the protection against UV-induced damage [63]. UV-radiation may damage DNA and proteins, but also generates cytotoxic aldehydes due to peroxidation of lipids. ALDH3A1 detoxifies these aldehydes by converting them into acids [64,65]. Other studies indicate that ALDH3A1 may protect the cornea directly by absorbing the UV-radiation [66]. ALDH3A1 is abundant in the human corneal epithelium and keratocytes and it has been classified as an enzyme-crystallin.

**Enzyme-crystallins and metabolism:** Several of the identified intracellular proteins have been classified as corneal enzyme-crystallins because of their abundance in epithelial cells and keratocytes, their enzymatic activity, and a suspected role in corneal transparency. Thus, in addition to ALDH3A1 [67], the metabolic proteins aldehyde dehydrogenase type 1 member A1 (ALDH1A1) [68], TKT [69], and α-enolase [70] have been observed to accumulate in mammalian corneas. Enzyme-crystallins that have been defined in other animals include gelsolin and actin (zebrafish) [71], peptidyl-prolyl cis-trans isomerase A (cyclophilin A; chicken), and glutathione S-transferase P (squid) [70]. It has been hypothesized that the enzyme-crystallins may serve important structural roles, giving the keratocytes and epithelial cells their refractive index and transparent optical properties [68,72]. This hypothesis has, however, been opposed by studies showing that ALDH3A1-deficient mice and TKT<sup>−/−</sup> mice have normal transparent corneas [73,74]. Another function of enzyme-crystallins may be protection of the corneal cells against the oxidative damage induced by UV-radiation [64].

The enzymatic activity of ALDH1A1 oxidizes retinaldehyde to retinoic acid [75,76]. TKT is part of the pentose phosphate pathway, while α-enolase, fructose-bisphosphate aldolase A, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (liver and muscle isozymes), phosphoglycerate mutase 1 or 2, neuron-specific enolase (γ-enolase), and pyruvate kinase (isozymes M1/M2) participate in glycolysis. In addition, L-lactate dehydrogenase (A and B subunits), which converts pyruvate to L-lactate under anaerobic conditions, was identified in the human cornea.

**Protein misfolding and degradation:** Identified proteins assisting in the folding of other proteins include heat shock cognate 71 kDa protein, heat shock 70 kDa protein 1, heat shock protein 27 kDa, peptidyl-prolyl cis-trans isomerase A and B, and protein disulfide isomerase A3, while proteins participating in the ubiquitin-proteasome system only included ubiquitin and ubiquitin-protein ligase Nedd-4. The rather moderate number of identified proteins involved in misfolding and degradation from the normal cornea is in accordance with our previous proposal that protein misfolding and aggregation induced by increased metabolic oxidative stress is responsible for the loss of cellular transparency in wound-healing corneal fibroblasts [77]. Thus, UV-light may be the dominating contributor to oxidative stress in the healthy cornea, while the respiratory chain and metabolic enzyme systems may be the major sources of ROS in the wound-healing cornea.

**Intracellular structural proteins:** The group of cytoplasmic structural proteins in the cornea is dominated by different cytokeratins. Keratins show tissue-specific expressions and are divided into type I (acidic) and type II (basic), forming “keratin pairs.” Specific pairs associate to form intermediate filaments partly giving strength to the epithelial cells [78,79]. All together, we identified 15 different keratins representing at least seven particular keratin pairs (pairs K10/1, K12/3, K13/4, K14/5, K16/6A or 6C, K16/6B, and K16/6F). According to the literature, it is generally accepted that pairs K12/3, K14/5, and K16/6 are present in the corneal epithelium and that K12 and K3 are cornea-specific keratins [79]. Previous studies have shown that K12/3 is responsible for the integrity of the corneal epithelium [80] and mutations in the genes that code for them cause Meesmann corneal dystrophy [81]. Keratin 7 (sarcolectin) is expressed in a wide range of simple epithelia together with K8/18 but has been previously reported to be absent in the cornea [82]. However, keratin 1, epidermal keratin 2, keratin 7, and keratin 9 are often present in the environment and, therefore, might be contaminations from the laboratory [83].
CORNEAL TRANSPARENCY AND WOUND-HEALING

Corneal transparency is thought to arise from the regular arrangement of the collagen fibers of uniform diameter and spacing within the corneal stroma that lead to destructive interference of light except in the forward direction and thereby creating optical transparency [84,85]. Thus, this lattice theory suggests that the wave amplitudes of light scattered away from the forward direction by the collagen fibrils are opposing each other (destructive interference), while the wave amplitudes of light in the forward direction emphasize each other (constructive interference). Corneal edema as seen in endothelial cell disorders [86] disrupts the collagen lattice array and therefore causes stromal opacification and visual loss. A disorder of corneal transparency may also arise from the deposition of macromolecules in the stroma as in several inherited corneal diseases. For example, numerous mutations in the TGFBI gene [87] encoding TGFBIp cause an accumulation of mutant TGFBIp in the stromal extracellular matrix. Corneal opacification may also follow common disorders of the cornea such as keratitis, ulceration, and trauma that are accompanied by the growth of blood vessels into this normally avascular tissue [88].

Recently, loss of corneal transparency has also been attributed to stromal keratocytes that become activated and transformed into highly reflective fibroblasts and myofibroblasts with increased cellular light scattering following their injury or exposure to stress [68,89]. The mechanisms underlying the development of this cellular haze during wound-healing may be linked to the loss of the abundant enzyme-crystallins. In another study, we identified 118 distinct proteins in the water-soluble fraction from cultured spindle-shaped human corneal fibroblasts representing an in vitro model of the reflective wound-healing keratocyte phenotype [77]. A comparison of the two proteomic studies reveals that only 32 of the 85 intracellular proteins found in the intact human cornea were also identified in the wound-healing corneal fibroblast. Despite that not all of the intracellular proteins identified in the intact human cornea originate from keratocytes, this observation supports previous studies showing significant changes in the gene and protein expression patterns upon transition from quiescent to wound-healing keratocyte phenotype [68,90,91]. Furthermore, that only 11% (9 of 85) of the identified intracellular proteins from the intact cornea are involved in protein folding and degradation compared to 27% (32 of 118) from the wound-healing corneal fibroblasts [77] is in accordance with our proposal that the corneal haze resulting from increased cellular light scattering from spindle-shaped corneal fibroblasts during wound-healing may be related to an unfolding and aggregation of various intracellular proteins [77].

CURRENT PERSPECTIVES

It is essential to know the biochemical composition of the healthy cornea in an effort to understand the molecular mechanisms behind corneal disorders. Thus, the dataset of human corneal proteins provides a useful background for further studies to define the roles of the proteins and to use for comparative proteomic studies. Thus, an identification of the differences in the expression profiles between normal and diseased corneas may lead to avenues for therapeutic interventions. Because the cornea is so accessible, the potential for developing effective drugs for the treatment of corneal diseases is good. In addition, the specific knowledge of the corneal proteome during normal and pathological conditions may also lead to improved molecular classifications of corneal diseases and facilitate the development of new treatments including gene therapy and the design of artificial corneas for transplantation.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the National Eye Institute (RO1-EY12712), the Danish Medical Research Council, the Danish Natural Science Research Council, and the Carlsberg Foundation.

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91:133-40.


The appendix is available in the online version of this article at http://www.molvis.org/molvis/v12/a52/.

The print version of this article was created on 9 May 2006. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.
Mature red blood cells lack all internal cell structures and consist of cytoplasm within a plasma membrane envelope. To maximize outcome, total red blood cell protein was divided into two fractions of membrane-associated proteins and cytoplasmic proteins. The proteome is the entire set of proteins that is, or can be, expressed by a genome, cell, tissue, or organism at a certain time. It is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions. Proteomics is the study of the proteome. While proteome generally refers to the proteome of an organism, multicellular organisms may have very different proteomes in different cells, hence it is important to distinguish proteomes in cells and organisms. The human cornea proteome: bioinformatic analyses indicate import of plasma proteins into the cornea. Mol Vis 2006;12:451-60. Katoh Y, Katoh M. Comparative integromics on Angiopoietin family members. Int J Mol Med 2006;17(6):1145-9. Kawamoto K, Morishige N, Yamada N, et al. Delayed corneal epithelial wound healing after penetrating keratoplasty in individuals with lattice corneal dystrophy. Am J Ophthalmol 2006;142(1):173-4. The human proteome, hence, is the complete set of proteins that can... The concentrations of various plasma components are routinely used to diagnose disease and monitor recovery. In theory, if we knew which biomarker represented which clinical condition, we could diagnose and monitor thousands of diseases through a single blood draw. Expectedly, 100s of biomarkers have been proposed over the years by researchers around the globe. The problem is, given that there are a large number of proteins in the proteome and once again the concentration range is massive, these sample preparation workflows become extremely cumbersome, non-scalable, and in some cases, bespoke. All of these have throttled throughput of proteomics discovery workflows.