Evidence for Gelsolin as a Corneal Crystallin in Zebrafish*

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We have shown that gelsolin is one of the most prevalent water-soluble proteins in the transparent cornea of zebrafish. There are also significant amounts of actin. In contrast to actin, gelsolin is barely detectable in other eye tissues (iris, lens, and remaining eye) of the zebrafish. Gelsolin cDNA hybridized intensely in Northern blots to RNA from the cornea but not from the lens, brain, or headless body. The deduced zebrafish gelsolin is ~60% identical to mammalian cytosolic gelsolin and has the characteristic six segmental repeats as well as the binding sites for actin, calcium, and phosphatidylinositides. In situ hybridization tests showed that gelsolin mRNA is concentrated in the zebrafish corneal epithelium. The zebrafish corneal epithelium stains very weakly with rhodamine-phalloidin, indicating little F-actin in the cytoplasm. In contrast, the mouse corneal epithelium contains relatively little gelsolin and stains intensely with rhodamine-phalloidin, as does the zebrafish extraocular muscle. We propose, by analogy with the diverse crystallins of the eye lens and with the putative enzyme-crystallins (aldehyde dehydrogenase class 3 and other enzymes) of the mammalian cornea, that gelsolin and actin-gelsolin complexes act as water-soluble crystallins in the zebrafish cornea and contribute to its optical properties.

Focused vision in the vertebrate eye depends upon light transmission through the transparent lens and cornea. The lens is an encapsulated tissue containing a layer of anterior, cuboidal epithelial cells and posterior, elongated fiber cells (1). By contrast, the transparent cornea has an anterior squamous epithelium comprising 5–7 cell layers overlying a relatively thick extracellular stroma containing ordered collagen fibers, proteoglycans, glycosaminoglycans, and keratocytes, and finally a posterior single layer of endothelial cells (2–5).

The transparent and refractive properties of the eye lens depend upon the crystallins, which often differ among species in a taxon-specific fashion (6). The diverse crystallins comprise approximately 90% of the water-soluble proteins of the lens. Many lens crystallins are either closely related or identical to metabolic enzymes (the enzyme-crystallins) or stress proteins (small heat shock proteins) that are used outside of the lens for non-refractive purposes (7–9). The dual use of crystallins for metabolism and refraction has been called gene sharing (10).

Because transparency of the cellular lens involves the intracellular crystallins (11–13), studies on corneal transparency have concentrated on the highly structured extracellular stroma (14, 15). However, corneal epithelial cells, like lens cells, contain unexpectedly high proportions of selected proteins (16–19), raising the possibility that they may have structural roles related to transparency as do lens crystallins. Furthermore, these abundant intracellular corneal proteins are often enzymes, reminiscent of the enzyme-crystallins in the lens, suggesting that they are not serving strictly metabolic roles. For example, a major protein in most mammalian corneal epithelial cells is aldehyde dehydrogenase class 3 (ALDH3),1 which represents 20–40% of the water-soluble protein of the bovine (20, 21), rodent, and marsupial (22, 23) corneal epithelial cells. Transketolase (TKT) comprises at least 10% of the water-soluble protein of the mouse corneal epithelial cells (24) and NADP+-dependent isocitrate dehydrogenase comprises approximately 13% of the water-soluble proteins of the bovine corneal epithelial cells (25). An additional similarity with the enzyme-crystallins of the lens is that the abundant proteins in the corneal epithelial cells are taxon-specific. For example, although ALDH3 is the major protein in corneal epithelial cells of most mammals, it does not accumulate in the corneas of chicken, toad, or fish (18, 23, 26). Stromal keratocytes of the cornea also have putative enzyme-crystallins (27). For example, ALDH1 and TKT comprise ~30% of the water-soluble protein of the transparent keratocytes of the rabbit cornea, whereas this value is markedly reduced in the reflective keratocytes after freeze injury. It thus appears that corneal cells, like lens cells, accumulate certain multifunctional, water-soluble proteins that contribute structurally to their optical properties as well as having other non-refractive roles (28).

In the present report we show that gelsolin and non-filamentous actin are major intracellular water-soluble proteins of the zebrafish cornea. Gelsolin binds to and severs filamentous actin in a calcium-dependent manner by non-enzymatically dissociating neighboring subunits (29). Gelsolin can also nucleate F-actin formation depending upon the calcium concentration and the presence of phosphoinositides (30–33). By analogy with the lens crystallins and putative enzyme-crystallins of the cornea (28), the overexpression of gelsolin and the abundance of non-filamentous actin in the zebrafish cornea suggest a crystallin-like role for these cytoskeletal proteins related to vision and tissue transparency.

MATERIALS AND METHODS

Preparation of Proteins—The cornea and other tissues from the zebrafish (Danio rerio), rose barb (Puntius conoconius), and tricolor shark (Puntius conoconius). The abbreviations used are: ALDH, aldehyde dehydrogenase; TKT, transketolase; BSA, bovine serum albumin; TBS, Tris-buffered saline; PCR, polymerase chain reaction; kb, kilobase pair; PBS, phosphate-buffered saline; bp, base pair.

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1 The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession number AF175294.
(Balantiocheirus melanopterus) were surgically isolated. The tissues were homogenized with a Pellet Pestle motor (Kontes) for 20 s in 63 mM Tris-HCl, pH 7.4, 5% β-mercaptoethanol and 10% glycerol. After centrifugation at 10,000 × g at 4 °C in an Eppendorf centrifuge, the supernatant fraction was removed and considered as the water-soluble protein. The pellet was resuspended in the original volume of the homogenate in 125 mM Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, and 5% bromophenol blue, boiled for 5 min, and centrifuged at 10,000 × g. The resulting supernatant fraction was considered as the insoluble proteins.

The protein samples were stored at −20 °C.

**Fractionation and Sequencing of Proteins**—Proteins were fractionated by 10% SDS-polyacrylamide gel electrophoresis. After Coomassie Blue staining the separated proteins were blotted onto nitrocellulose membranes (NOVEX), blocked with 3% (w/v) BSA in TBS, and incubated with the primary antibody (1:1000 in TBS) for 1 h at room temperature. After washing 3 times in TBS for 5 min each time, the membranes were incubated with the alkaline phosphatase-coupled secondary antibody (1:10,000 in TBS) at room temperature and developed using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as substrates according to the manufacturer’s protocol (Roche Molecular Biochemicals).

**cDNA Cloning**—Total corneal RNA was isolated with the Stratagene isolation kit. The first cDNA strand was synthesized using the modified oligo(dT) primer and CapSwitch TM oligonucleotide provided by the CLONTECH SMART kit and digested with RNase A (2 units/20 μl). The CLONTECH Advantage cDNA PCR kit was used for PCR cloning of the 5'-half of the zebrafish gelsolin-like cDNA. The 3'-primer contained a degenerate sequence (5'-ACRAANACRTCTRTNGT-3') encoding a peptide sequence (TNDVFV) of the major gelsolin-like corneal protein of the zebrafish; the 5'-primer was from the manufacturer. PCR was for 30 cycles at 94 °C for 45 s, 44 °C for 1.5 min, and 72 °C for 3 min. PCR bands were selected by Southern blot hybridization at 50 °C for 1 h in QuikHyb hybridization solution (Stratagene) using a 1.5-kb Xenopus gelsolin cDNA probe (kindly provided by Dr. Werner Franke, Heidelberg, Germany). After hybridization the blot was washed once at 45 °C in 0.1% SSC followed by 0.1% SDS for 10 min. The positive PCR products were cloned using the PCR-Script™ AMP Cloning kit (Stratagene). Twelve independent clones selected for initial screening were found to be gelsolin-related. Both strands of one of the clones (pN4) were sequenced; pN4 was found to encode the 5'-half of the cDNA. The 3'-half of this cDNA was cloned by PCR using a 5'-primer (5'-AGAGTCGAGTGGGCACATTAC-3') derived from pN4 and the 5' cDNA synthesis primer from the manufacturer. PCR was for 30 cycles at 94 °C for 45 s and 52 °C for 4 min. The resulting 1.2-kb band was cloned into the PCR-Script™ AMP vector (Stratagene). Sequencing established that this clone (pK24) encodes the 3'-half of the gelsolin-related protein. Double-stranded DNA sequencing was performed in ABI 377 and 373 Stretch sequencers, and sequence analysis was carried out with the GCG program.

**Northern Blot Hybridization**—Total RNAs from the cornea, lens, brain, or headless body were fractionated by electrophoresis through formaldehyde-agarose gels and blotted onto Nylon membranes (DuPont). The CLONTECH Advantage cDNA PCR kit was used for PCR cloning of the 3'-half of the gelsolin-related protein. Double-stranded DNA sequencing was performed in ABI 377 and 373 Stretch sequencers, and sequence analysis was carried out with the GCG program.

**Western Immunoblotting**—An antibody was raised against a synthetic peptide comprising the N-terminal 17 amino acids of the zebrafish-derived gelsolin-related protein (Research Genetics, Inc., Huntsville, AL) and affinity purified by standard methods. The rabbit alkaline phosphatase-labeled second antibody was obtained from The Jackson Laboratory, and the mouse second antibody was from Roche Molecular Biochemicals. After SDS-polyacrylamide gel electrophoresis, the separated proteins were blotted onto nitrocellulose membranes (NOVEX), blocked with 3% (w/v) BSA in TBS, and incubated with the primary antibody (1:1000 in TBS) for 1 h at room temperature. After washing 3 times in TBS for 5 min each time, the membranes were incubated with the alkaline phosphatase-coupled secondary antibody (1:10,000 in TBS) at room temperature and developed using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as substrates according to the manufacturer’s protocol (Roche Molecular Biochemicals).

**dye (Sigma) in 0.1 M phosphate-buffered saline for 5 min, acetylated with 100 mM triethanolamine, pH 8.0, and hybridized overnight with digoxigenin-labeled RNA (0.2 μg/ml) in a humidified chamber at 42 °C with hybridization buffer (10 mM Tris-HCl, pH 7.6, 900 mM NaCl, 5 mM EDTA, 1% Denhardt’s solution, 50% deionized formamide, 10% dextran sulfate, 10 mM dithiothreitol, and 0.1 mg/ml yeast tRNA). The mixture was heated initially at 80 °C for 5 min and maintained at 50 °C. Posthybridization conditions and immunological detection were according to the application manual supplied by Roche Molecular Biochemicals.

**Phalloidin Staining**—Frozen 10-μm sections of the zebrafish eye were fixed in 3.7% formaldehyde in PBS for 10 min at room temperature.
Following two washes in PBS, the sections were treated with acetone at 22 °C for 3 min and rinsed again in PBS. The sections were preincubated in 1% BSA for 30 min and then stained with rhodamine phalloidin (Molecular Probes, Eugene, OR) at 5 units/ml in 1% BSA for 20 min at room temperature. The sections were washed in PBS, coverslipped using Aqua Poly/Mount (Polysciences, Warrington, PA), and immediately subjected to fluorescence microscopy with a Zeiss Endow GFP filter.

**RESULTS**

**Corneal Proteins**—We first compared the water-soluble proteins of the cornea from the mouse and zebrafish by SDS-polyacrylamide gel electrophoresis (Fig. 1). As expected, the 54-kDa ALDH3 was the major water-soluble protein of the mouse cornea (18–20, 23) (Fig. 1, lane 2, arrowhead). By contrast, there was little if any 54-kDa protein in the zebrafish corneal extract. Instead, one of the most prevalent bands of water-soluble protein in the zebrafish cornea migrated with an approximate molecular mass of 80 kDa (arrows in Fig. 1, lane 3, and in Fig. 2a, lane 2). There was little if any stained 80-kDa protein detectable in the zebrafish extracts from the lens (Fig. 2a, lane 1), iris (Fig. 2a, lane 3), and combined remaining eye tissues (Fig. 2a, lane 4). Another abundant water-soluble protein in the zebrafish cornea was approximately 43 kDa (open arrowheads in Fig. 1, lane 3, and in Fig. 2a, lane 2). In contrast to the 80-kDa protein, the 43-kDa protein was also abundant in the iris (Fig. 2a, lane 3) and the rest of the eye (Fig. 2a, lane 4).

We next compared the water-soluble (supernatant) and -insoluble (pellet) proteins of the zebrafish cornea. After washing in buffer, the 10,000 × g pellet was boiled in containing 4% SDS in the same volume as the original homogenate, and equal volumes of the water-soluble and insoluble protein extracts were compared by SDS-polyacrylamide gel electrophoresis (Fig. 2b). The insoluble proteins contained the high molecular weight collagen and proteoglycans normally present in the corneal stroma (4). Most of the 80-kDa protein, however, was in the water-soluble, supernatant fraction (Fig. 2b, lane 2, arrow). Although it is likely that the 80-kDa bands in the water-soluble and pellet fractions are the same protein, this requires confirmation. It is also noteworthy that the 43-kDa protein was considerably more prevalent in the water-soluble supernatant fraction than the insoluble pellet (open arrowhead, Fig. 2b, lanes 2 and 3, respectively). It was not visible in the lens (Fig. 2a, lane 1), probably because of the high proportion of lower molecular weight crystallins in this tissue.

**Peptide Sequences**—The corneal, water-soluble 80-kDa protein band of the zebrafish was purified from the SDS-polyacrylamide gel, and its tryptic peptides were fractionated by high pressure liquid chromatography. Four tryptic peptides were sequenced. One (NTIQVMPGCQ) of the tryptic peptides showed 88% identity to mouse adseverin (D5) (34), a protein related to gelsolin. The sequences of the other three tryptic peptides did not show significant homology with any protein in the GenBank™ data base.
tryptic peptides derived from the 43-kDa protein band purified from the SDS-polyacrylamide gel were identical to actin, whereas the sequences of the other 6 tryptic peptides were identical to human creatine kinase (data not shown). Thus, actin is a major component of the water-soluble protein of the zebrafish cornea.

**cDNA Cloning**—A cDNA encoding the 80-kDa corneal protein of adult zebrafish was cloned in two parts using degenerate oligonucleotide primers derived from the tryptic peptide sequences (see “Materials and Methods”). The compiled cDNA is 2528 bp long and has an open reading frame of 2160 bp (Fig. 3). It has 5′ and 3′ non-coding regions of 96 and 272 bp, respectively. The open reading frame encodes a protein of 720 amino acids with a calculated molecular mass of 80,077 Da. The four tryptic peptides derived from the 80-kDa protein that were sequenced are present in the deduced protein and are shown in **bold letters** below the protein sequence in Fig. 3. The cDNA appears to be full-length since 12 independent clones were identical in the 5′-untranslated region. The four tryptic peptides that were obtained from the 80-kDa protein are perfect or near-perfect matches to the predicted protein sequences. Whereas a putative polyadenylation site is underlined in the cDNA sequence in Fig. 3 (AATAA), it is imperfect, and a complete poly(A)+ sequence seems to be missing from the 3′ end. Thus, it is possible that the 3′-untranslated sequence is not complete in our cDNA.

**Sequence Analysis of the Gelsolin-like Protein**—Computer analysis of the deduced gelsolin-like protein showed significant sequence identity to gelsolin and gelsolin-related proteins of other species. The percent identity between the zebrafish gelsolin-related protein and different gelsolins is 60% (pig), 59% (human), 57% (Xenopus), 44% (ascidian), and 39% (lobster and Drosophila). Within this protein family gelsolin and villin (35) have a 6-fold repeated structure, and fragmin (36), severin (37), and Mbhl (myc basic motif homolog-1) (38) have a 3-fold repeated structure, and fragmin (36), severin (37), and Mbhl (myc basic motif homolog-1) (38) have a 3-fold repeated structure. Each repeated domain is considered a segment (S), with S1–S3 and S4–S6 clustered in the three-dimensional crystal structure of horse plasma gelsolin (39). The six segments each contain three motifs arranged in the order B, A, and C (40). The alignment presented in Fig. 4 shows that motifs B, A, and C are conserved in each of the six segments of the zebrafish gelsolin-related protein.

In general, the highest degree of similarity in the gelsolin family of proteins is found within the first 200 amino acids in the N-terminal half of the protein comprising the S1 and part of the S2 segments. These protein segments are functionally important and contain an actin-binding site and two Ca2+-binding sites in human gelsolin (41). The critical amino acids for these functions are conserved in the zebrafish gelsolin-like protein (Fig. 5). Moreover, phosphatidylinositol biphosphate binding sites in human gelsolin (41) are highly conserved in the amino acids with a calculated molecular mass of 80,077 Da. The four tryptic peptides derived from the 80-kDa protein that were sequenced are present in the deduced protein and are shown in **bold letters** below the protein sequence in Fig. 3. The cDNA appears to be full-length since 12 independent clones were identical in the 5′-untranslated region. The four tryptic peptides that were obtained from the 80-kDa protein are perfect or near-perfect matches to the predicted protein sequences. Whereas a putative polyadenylation site is underlined in the cDNA sequence in Fig. 3 (AATAA), it is imperfect, and a complete poly(A)+ sequence seems to be missing from the 3′ end. Thus, it is possible that the 3′-untranslated sequence is not complete in our cDNA.

**Expression of the mRNA Encoding the Zebrafish Gelsolin-like Protein**—Northern blot hybridization tests were performed to compare the relative amount of mRNA for the gelsolin-like protein in the zebrafish cornea and other tissues (Fig. 7, **upper panel**). The intense hybridization signal derived from total RNA extracts from cornea indicated that this gene is highly expressed in this tissue. The mRNA size for the gelsolin-like protein is approximately 3 kb long, consistent with the length of the cDNA. The hybridization signal was approximately 300-fold higher in the RNA extracts from the cornea than from the lens and rest of eye of these zebrafish (Fig. 6B). We conclude that the cloned cDNA encodes the 80-kDa protein and that this protein is highly enriched although not specifically expressed in the zebrafish cornea.
adenylation signals.

Spatial Distribution of the mRNA Encoding the Gelsolin-like Protein in the Zebrafish Cornea—We next determined the location of the mRNA for the gelsolin-like protein within the zebrafish cornea by in situ hybridization. A 1.2-kb antisense digoxigenin-labeled RNA derived from the 3' half of the cDNA encoding the gelsolin-like protein was obtained by in vitro transcription and used as a probe. The hybridized section showed that the mRNA encoding the gelsolin-like protein accumulates in the corneal epithelium of the zebrafish (Fig. 8a).

It is uncertain if the slight staining in the stroma and endothelium of the cornea represents a low level of RNA or background coloration. The corresponding sense RNA was used as a negative control and showed negligible staining to the corneal section (Fig. 8b).

Rhodamine-Phalloidin Staining of the Zebrafish and Mouse Corneal Epithelium—Gelsolin is known to bind and sever F-actin into water-soluble G-actin subunits (see Ref. 45 and below). We thus stained frozen sections of the zebrafish eye with rhodamine-phalloidin, which selectively stains F-actin in the cytoplasm (46, 47), in order to test for the presence of F-actin in the zebrafish corneal epithelial cells. For comparison, we also examined sections of the mouse cornea, which contains relatively little gelsolin, as judged by the paucity of 80-kDa protein in SDS-polyacrylamide gels (see Fig. 1, lane 2). The mouse corneal epithelial cells fluoresced intensely with rhodamine-phalloidin, indicating the presence of F-actin in the cytoplasm (Fig. 9b). A similar result was obtained with the extraocular muscle of the zebrafish (Fig. 9f), which is expected to contain high concentrations of F-actin. By contrast, no fluorescence signal was obtained in the zebrafish cornea after an equal exposure (3.5 s) (Fig. 9d). A 10-fold longer exposure (35 s) of the sections stained with rhodamine-phalloidin revealed the presence of some assembled F-actin in the most anterior corneal epithelial cells of the zebrafish (Fig. 9e). These results indicate that actin exists almost entirely as dissociated G-actin in the zebrafish cornea, in striking contrast to its associated F-actin state in the mouse cornea.

DISCUSSION

Previous investigations have shown that the corneal cells of vertebrates accumulate unexpectedly high concentrations of enzymes among their water-soluble proteins that differ among species (28). For example, ALDH3 (22, 23), TKT (24), and isocitrate dehydrogenase (25) are present at considerably higher levels in the epithelial cells of mammalian corneas than would be ex-

![Fig. 5. Comparison of the amino acid sequences of the functional regions in segments 1 and 2 of gelsolins from zebrafish (zgs), human (hgs), porcine (pqs), and Drosophila (dgs). The amino acid residues involved in G-actin-binding are represented by shaded boxes. The residues responsible for calcium-binding are marked by open boxes. The phosphatidylinositol biphosphate-binding region is underlined.](image1)

![Fig. 6. Coomassie Blue-stained SDS-polyacrylamide gel (a) and Western immunoblot (b) of approximately 15 μg of 10,000 × g water-soluble eye proteins of the zebrafish (lanes 1–3), rose barb (P. concomitans) (lanes 4–6), and tricolor shark (B. melanopterus) (lanes 7–9); 14 μg of proteins were used as markers (M). Lanes 1, 4, and 7, cornea; lanes 2, 5, and 8, lens; lanes 3, 6, and 9, the rest of the eye (eye minus lens and cornea). The Western blot in b was performed from a duplicate of the blot shown in a. The rabbit antiserum raised against the synthetic peptide comprising amino acids 129–145 derived from the deduced protein shown in Fig. 3 was used as the primary antibody.](image2)

![Fig. 7. Northern blot hybridization of zebrafish gelsolin RNA. Upper panel, 10 μg of total RNA from the cornea (lane 1), lens (lane 2), brain (lane 3), and headless body (lane 4) was hybridized with the radioactively labeled 1.2-kb 3' zebrafish gelsolin cDNA (p1K24, see "Materials and Methods"). Lower panel, same blot as upper panel, but hybridized with the zebrafish actin cDNA probe (see text). Corneal Epithelium—Gelsolin is known to bind and sever F-actin into water-soluble G-actin subunits (see Ref. 45 and below). We thus stained frozen sections of the zebrafish eye with rhodamine-phalloidin, which selectively stains F-actin in the cytoplasm (46, 47), in order to test for the presence of F-actin in the zebrafish corneal epithelial cells. For comparison, we also examined sections of the mouse cornea, which contains relatively little gelsolin, as judged by the paucity of 80-kDa protein in SDS-polyacrylamide gels (see Fig. 1, lane 2). The mouse corneal epithelial cells fluoresced intensely with rhodamine-phalloidin, indicating the presence of F-actin in the cytoplasm (Fig. 9b). A similar result was obtained with the extraocular muscle of the zebrafish (Fig. 9f). A 10-fold longer exposure (35 s) of the sections stained with rhodamine-phalloidin revealed the presence of some assembled F-actin in the most anterior corneal epithelial cells of the zebrafish (Fig. 9e). These results indicate that actin exists almost entirely as dissociated G-actin in the zebrafish cornea, in striking contrast to its associated F-actin state in the mouse cornea.](image3)
Gelsolin in Zebrafish Cornea

The protein encoded in the zebrafish cDNA cloned in the present study is a homologue of gelsolin of other species. It does not have a headpiece characteristic of villin and advillin. The six repeated domains and the sequences involved in the binding and calcium-dependent severing of actin and in the phosphoinositide regulation of F-actin formation are generally conserved in the zebrafish protein. The residues responsible for a high affinity calcium-binding site that modulates gelsolin-actin interaction are identical between human gelsolin and the deduced protein from the cloned zebrafish cDNA. These sequence similarities suggest that the deduced zebrafish gelsolin binds and severs actin in the same manner as human gelsolin. The absence of rhodamine-phalloidin staining of the zebrafish cornea in the present study is consistent with the abundant gelsolin severing F-actin and capping the G-actin in the cytoplasm of the epithelial cells.

Other vertebrate (59–61) and Drosophila (55) gelsolins are encoded in single copy genes that produce at least three different proteins by alternative RNA splicing. One of the gelsolin isoforms is secreted into the plasma where it dissociates and contributes to the clearing of F-actin released by dying cells (62, 63). Plasma gelsolin has an N-terminal signal sequence that is required for its secretion. Two intracellular gelsolin isoforms are known. The more common cytoplasmic gelsolin lacking the signal sequence shows a wide tissue distribution. An isoform called gelsolin 3 contains an additional peptide in the N-terminal region and is preferentially expressed in oligodendrocytes of the central nervous system, testis, and lung (61). Our sequence and in situ hybridization data are consistent with the abundant gelsolin in the zebrafish corneal epithelial cells being the commonly expressed cytoplasmic gelsolin. Southern blot hybridization tests on genomic DNA using p1K24 (the 3’ 1.2-kb of the present cDNA) indicated that there is but a single gene for gelsolin in the zebrafish (data not shown), as in other species. It remains possible, however, that zebrafish have an additional gelsolin gene that has specialized for corneal expression, especially since a chromosome doubling event occurred in this species (64).

Due to its actin-modulating properties, cytoplasmic gelsolin has been implicated in a number of biological functions in non-muscle cells. These include the control of cell shape and various differentiative changes, phagocytosis, exocytosis, contraction, mitosis, and cytokinesis (see Refs. 65–67 for examples and further references). Gelsolin and other actin-binding proteins have been given special attention with respect to cellular locomotion (68). Overexpression of cytoplasmic gelsolin in NIH 3T3 fibroblasts enhances migration and wound healing in tissue culture (69). The high content of gelsolin in developing oligodendrocytes (70) and its localization in neuronal growth cones (71) are consistent with a role in motility. Gelsolin null mice have shown that, although not essential for motility during early embryogenesis, gelsolin is required for rapid motile responses in stressed cells involved in hemostasis, inflammation, and wound healing (72). Platelet shape changes were noted in gelsolin-negative mice as well as decreased migration of neutrophils and fibroblasts (72) and delayed retraction of filopodia in neurites (73). Gelsolin plays a role in the ruffling response and motility of dermal fibroblasts and appears to be a downstream effector of Rac-mediated actin dynamics (74). Recently, gelsolin has been shown to be substrate for caspase-3 and has been implicated in the apoptotic pathway (75, 76). Thus, gelsolin performs many critical cellular functions usually related to cellular shape, motility, and contraction by virtue of its ability to influence F-actin formation and the cytoskeleton.

**FIG. 8. Gelsolin gene expression in the adult zebrafish cornea.**
a, frozen corneal section was subjected to in situ hybridization using the antisense 3’ 1.2-kb gelsolin cDNA probe (p1K24) labeled with digoxigenin. The black arrow points to the stained corneal epithelium overlaying the extracellular stroma. The white arrow points to the single-layered corneal endothelium. b, in situ hybridization using the sense cDNA probe.
In view of the role of gelsolin in cell motility and the significance of actin for corneal wound healing (77), it will be interesting to investigate if the differences that have been reported in the rate of corneal wound healing among fish (78) are related to differences in the relative concentrations of gelsolin or of other cytoskeletal regulatory proteins (79). Preliminary tests have shown that both the trout and sculpin corneal epithelial cells stain as weakly with phalloidin as do the zebrafish corneal epithelial cells,2 despite the fact that the trout cornea heals considerably more slowly than the sculpin cornea (78). Western immunoblots using the zebrafish gelsolin peptide antibody made in the present study indicated that the trout cornea has gelsolin, although apparently relatively less than that in the zebrafish cornea as judged by staining of the SDS-polyacrylamide gel.3 Gelsolin was not detected in the sculpin cornea in Western immunoblots using the same antibody; however, this may be due to species differences in the gelsolin epitope. Thus, further experiments are necessary to establish whether regulation of the cytoskeleton by gelsolin and/or other proteins plays a critical role in the rate of corneal wound healing in fish.

Here we propose a new function for gelsolin and actin related to vision. We estimate from the present data that gelsolin and actin represent at least half of the water-soluble proteins of the epithelial cells of the zebrafish cornea. The absence of significant staining with rhodamine-phalloidin in the zebrafish corneal epithelial cells suggests that the abundant actin is in the form of water-soluble, gelsolin-capped subunits filling the cytoplasm. By analogy with the transparent lens, which relies on the accumulation of diverse crystallins for its optical properties (11–13), we propose that gelsolin and its complexes with actin may contribute to the optical properties of the transparent zebrafish cornea. The abundance of these water-soluble proteins in the cytoplasm might minimize the concentration fluctuations responsible for light scattering and maintain the cellular refractive index compatible with corneal transparency. In mammals, ALDH3 and other enzymes may play this role (28). The high proportion (~30%) of ALDH1 and TKT in the transparent keratocytes and the specific reduction in these putative enzyme-crystallins in the reflective keratocytes of the freeze-injured, hazy rabbit cornea also support the notion that the abundance of particular proteins is structurally important for cellular transparency in the cornea (27). It would seem especially important to have water-soluble crystallins in the corneal epithelium of fish since the epithelium comprises ~40% of the transparent cornea in these organisms (78, 80). This contrasts with humans and other vertebrates where the epithelium comprises a smaller percentage of the cornea (2, 5).

Although different proteins are used as taxon-specific crystallins in the lens and cornea of individual vertebrate species, including zebrafish as shown in the present study, similar proteins are used as crystallins in the lens and cornea in some cephalopods. For example, the glutathione S-transferase related S-crystallins are the major proteins of both the lens and the cornea in

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2 J. Davis and J. Piatigorsky, unpublished observations.
3 A. Kim, J. Davis, and J. Piatigorsky, unpublished observations.
