Cataractogenesis in Transgenic Mice Containing the HIV-1 Protease Linked to the Lens αA-Crystallin Promoter

Santa J. Tumminia†§, Gerald J. Jonak†, Richard J. Focht†, Y-S. Edmond Cheng‡, and Paul Russell‡

From the †Laboratory of Mechanisms of Ocular Diseases, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892, ‡Dupont Merck Pharmaceutical Company, Wilmington, Delaware 19880, and ¶Fuji ImmunoPharmaceuticals, Lexington, Massachusetts 02173

Several lines of transgenic mice were generated with either active or inactive forms of the human immunodeficiency virus type 1 (HIV-1) protease gene under the control of the mouse lens αA-crystallin promoter. Mice bearing the inactive protease coding sequence displayed no gross abnormalities in the lens, while mice with the active protease developed time-dependent bilateral cataracts. One line, TG61, developed cataracts in utero while the second line, TG72, developed cataracts postnatally. TG61 mice, homozygous for the transgene, developed severe microphthalmia and were significantly smaller than the control mice at postnatal day 30. Two-dimensional-polyacrylamide gel electrophoresis analysis of the protein profiles of TG72 and TG61 lenses revealed extensive modifications in the lens crystallins. Protein leakage and the gradual breakdown of cytoskeletal elements also occurred. In contrast, the opacification of the homozygous TG61 lenses appeared to have been influenced by differentiation and developmental processes. It appears that HIV-1 protease expression activates other proteases, and these enzymes, in concert with the HIV-1 protease, are responsible for the protein modifications that eventually result in the opacification of the lens.

The vertebrate lens is primarily composed of proteins known as crystallins, which account for 90% of its total soluble protein. In the mammalian lens, these proteins are divided into three major families: α-, β-, and γ-crystallins (5). α-Crystallin is an oligomer of approximately 800 kDa and is composed primarily of two subunits, αA and αB. The β-crystallins are also oligomers, and they elute in two or more size classes during gel filtration and range from 50 to 200 kDa. They are composed of seven different types of subunits, β1A, β1B, β2, β3, βA1, βA2, βA3, and βA4. γ-Crystallins share sequence homology with the β-crystallins; however, the γ-crystallins are exclusively monomeric. It is the organization of these crystallins that maintains the transparency of the lens. Denaturation and degradation of the crystallins have been shown to occur during the formation of several experimental cataracts.

Constructs were made linking only the HIV-1 protease to the lens αA-crystallin promoter. The transgenic animals that were produced developed cataracts. By examining the progression of lens opacities in these transgenic mice, the relationship between HIV, specifically the HIV-1 protease, and the pathways leading to cataractogenesis were explored.

MATERIALS AND METHODS

Transgenic mice were constructed by linking the HIV-1 protease to the lens αA-crystallin promoter. This was accomplished using the pMSG mammalian expression vector (Pharmacia Biotech Inc.), which was modified by replacing the murine mammary tumor virus long terminal repeat promoter region with the 412-bp BglII-BamHI mouse lens αA-crystallin promoter fragment (6) kindly provided by Dr. Joram Piatigorsky (National Eye Institute, NIH). A single chain, tethered dimeric form of the active HIV-1 protease gene, termed baa* (7, 8), was modified for mammalian cell expression by replacing nucleotides 7–90 within the region amplified by the two oligonucleotide primers, the PCR

---

* 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: National Eye Institute, National Institutes of Health, Bldg. 6, Rm. 228, 6 Center Dr., MSC 2735, Bethesda, MD 20892-2735.

§ The abbreviations used are: HIV-1, human immunodeficiency virus type 1; RT-PCR, reverse transcriptase polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s).
HIV-1 Protease Induces Cataract Formation

fragment derived from genomic DNA containing the total RNA preparations (or unspliced RNA) is 625 bp long, while the fragment derived from cDNA (and thus spliced mRNA) is 559 bp long.

Strictly adhering to the procedures set forth in the NIH Guidelines for the Care and Use of Laboratory Animals, all transgenic mice were bred for hemizygous and homozygous expression of the HIV-1 protease transgene and compared with sibling control FVB/N mice. Comparison was made on the basis of gross physical characteristics, such as weight and length, and visual examination of the eyes for lens clarity. Pups were euthanized postnatally up to day 30, and the lenses were carefully dissected and stored at −70 °C. The date of birth was considered to be postnatal day 1.

Slot blots were carried out as described previously (12) for the semi-quantitative analysis of HIV-1 protease protein content in the lens homogenates. Affinity-purified recombinant HIV-1 protease (Bachem Bioscience, Inc.) was used as a standard. In all experiments, the sample volume was 25 μl. Immunoreaction was with rabbit IgG prepared against a synthetic peptide, GQKELLADTGADDTVL, from HIV-1 protease (underlined and bold is the active site). Immunodetection was carried out on 12.5% Phastgels. The samples were loaded into each well. The pI gradient was set up using Pharmalyte 3–10 (Pharmacia). Transfer of the proteins to the second dimension was carried out on 12.5% Phastgels.

HIV-1 protease digests were carried out with affinity-purified recombinant HIV-1 protease (Bachem Bioscience). Column-purified bovine or mouse α-, high (β4+) or low (βB) molecular weight β-crystallins, or γ-crystallins (4 μg each) were incubated with HIV-1 protease (0.5 μg) at 37 °C overnight. Crystallins were also incubated in the presence or absence of 20 mM EDTA and/or 7 mM CaCl2. After the incubation, an aliquot (5 μl) of the HIV-1 protease-digested material was mixed with an equal volume of SDS gel sample buffer. The samples were boiled for 3 min and then loaded onto 12.5% homogeneous Phastgels (Pharmacia).

Following the same procedure, control mouse lens homogenates were also incubated with purified recombinant HIV-1 protease. Homogenates obtained from 10- and 22-day-old control mice were incubated with purified HIV-1 protease in ratios of 10:1 or 30:1 (sample to protease). After incubation, this material was run on both single and two-dimensional-gels.

Vimentin and actin antibodies were obtained from Sigma and BioDesign, Inc. (Kennemburkport, ME), respectively. βB-crystallin antibodies were a gift from Dr. Joseph Horwitz (U. Leu Stein Institute, UCLA), and antibodies to βA3-crystallin were obtained from Drs. J. ohn N. Hope and J. Fielding Hejtmancik (National Eye Institute, NIH).

RESULTS

Cataractogenesis was observed in several transgenic lines, albeit, at different times (Table I). Those mice containing the transgene with the mutation in the active site of the protease exhibited no lens opacities. Two of the lines that developed cataracts will be reported here. TG61 mice developed cataracts in utero. Hemizygous expression of the transgene in this line resulted in severe microphthalmia and cataract formation (Fig. 1a), while hemizygous expression was characterized only by the formation of a cataract in utero. Severe microphthalmia was not observed in the lenses from the hemizygous TG62 mice. In the second line, TG72, hemizygous expression of the transgene resulted in the development of lens opacities 23–24 days postnatally, while in mice hemizygous for this transgene, cataract formation was delayed to postnatal days 25–30. In the homozygous TG72 mice, cataract formation was observed to begin in the perinuclear region of the lens (Fig. 1b). After liquefication of the cortical region, the cataract appeared to migrate to the back of the lens. The cataractous lenses from both lines of hemizygous mice appeared similar in appearance to the lenses of the homozygous TG72 mice, although the lenses of the TG61 mice were slightly smaller (not shown).

For ease of comparison, only the results obtained from the homozygous mice from the two lines will be discussed unless otherwise noted. RT-PCR analysis was used to confirm the presence of HIV-1 protease mRNA in the TG61 and TG72 lines. As shown in Figs. 2A and 3, this mRNA was detected in the lenses of TG72 and TG61 hemizygous mice. In addition, TG61 hemizygous mice also expressed the mRNA ectopically in other organs tested, such as the spleen, liver, and kidney (Fig. 3). However, in contrast to the lens cataract phenotype, there were no gross abnormalities observed in these organs as a consequence of HIV-1 protease mRNA expression.

Slot blot analysis of the lens protein homogenates revealed extremely low quantities of the protease. It did appear, however, that in the homozygous mice the HIV-1 protease was expressed early (Fig. 2B). The level of protease found in 10-day-old TG61 mice was 0.695 ng of protease/μg total protein. This level appeared to decrease over the course of the next 20 days. Protease levels were also detected in 5-day-old TG61 mice. Accurate quantitation of the decrease in the level of protease over time could not be determined because the amount of protease was only slightly above background on the blot.

When comparing the size and weight of the mice, it was observed that the homozygous TG61 mice were significantly smaller than their control counterparts (Fig. 4). At postnatal day 5, the TG61 animals (n = 6) weighed 2.79 ± 0.175 g, while 5-day-old control mice (n = 6) were 3.21 ± 0.209 g (p < 0.01). At postnatal day 20, the TG61 mice weighed 8.49 ± 0.208 g in comparison to the 11.53 ± 0.282 g (p < 0.001) of the controls. The pups were weaned approximately at day 23, yet by postnatal day 30, the TG61 pups weighed only 15.30 ± 0.38 g in comparison to the 17.99 ± 0.695 g weighed by the controls (p < 0.001). In addition to weight, size was also checked. Mouse length was measured from snout to rump. At 5 days, the body of the TG61 mice was 3.61 ± 0.174 cm in length. In contrast, the length of control mice was 4.07 ± 0.249 cm. By day 20, the TG61 mice were 5.06 ± 0.450 cm in length, and the controls were 7.12 ± 0.512 cm. The weight and length of the hemizygous TG61 mice appeared to be only slightly less than the control mice (data not shown). In contrast to the findings obtained with the TG61 mice, the size and weight of both the homozygous and hemizygous TG72 mice were comparable to the control mice (Fig. 4).

![Table I](http://www.jbc.org/)

| Mouse line | Gene | Cataract formation |
|-----------|------|--------------------|
| TG61      | baa  | In utero (embryonic day 18) |
| TG62      | baa  | Postnatal days 24–29 |
| TG72      | baa  | Postnatal days 23–24 |
| TG72      | (homozygous) | Postnatal days 25–30 |
| TG65      | baa* | None |
| TG66      | baa* | None |
| TG67      | baa* | None |

* The gene designated baa contained the active form of the HIV-1 protease, whereas the gene termed baa* contained a mutation. The active aspartic residue was changed to a glycine residue.
Preparation of the lens homogenates for gel analysis revealed differences in the protein concentration of the lenses. Lenses from four mice were harvested and homogenized separately. The protein concentrations were determined at each timepoint and statistically analyzed. Control lenses harvested at postnatal day 5 contained 0.256 ± 0.142 mg of protein/lens in the water-soluble fraction and 0.0069 ± 0.018 mg/lens in the urea-soluble fraction. As expected, the protein concentration of the water-soluble fractions increased with age to 1.21 ± 0.056 mg of protein/lens at postnatal day 24/25. The concentration of the urea-soluble fractions increased in the same manner (Table II). Lenses obtained from TG72 mice exhibited a similar trend of protein increase with age until postnatal day 22, when the amount of protein in the water-soluble fraction began to decrease. By day 25, the amount of protein in the water-soluble fraction of the lens was 0.619 ± 0.089 mg/lens. However, the trend of protein concentration in the urea-soluble fraction followed that observed with the control lenses (Table II). Concentrations for the TG61 lenses were difficult to quantitate because of the small size of the lenses. At postnatal day 5, the water-soluble fraction contained approximately 0.080 mg of protein/lens, and the urea-soluble fraction had 0.037 mg of protein/lens. The amount of protein present in both the water- and urea-soluble fractions decreased with age, making it difficult to calculate accurate protein concentrations. In addition, by postnatal day 30, the lens was becoming liquified.

Since single dimension SDS-PAGE analysis of the two lines revealed that there were strong differences between their protein profiles, two-dimensional SDS-PAGE was used to closely monitor these modifications. The homozygous TG72 mice were observed from birth to postnatal day 30 so that the protein modifications leading up to cataract formation could be followed. The progression of protein changes seen in the TG72 mice is shown in Fig. 5. Protein modifications developed in the same manner for all of the TG72 lenses examined. Although no visually obvious changes were observed in the lenses prior to the manifestation of the cataract on day 24, modifications in the proteins began at postnatal day 20 (Fig. 5C, arrow). By day 24, when the cataract is present, several new protein spots appeared (Fig. 5D).

It was important to determine whether these protein modi-

![Image](HIV-Protease-Induces-Cataract-Formation.png)
HIV-1 Protease Induces Cataract Formation

Modifications were mainly in the water-soluble or -insoluble fractions of the homogenates. Two-dimensional analysis comparing the water-soluble profiles of protein extracts from control and homozygous TG72 mice revealed that the trend of protein changes was similar to that observed in the patterns of the lenses solubilized in 8 M urea (data not shown). Urea-soluble (water-insoluble) fractions were analyzed, and the patterns were also found to be similar to the water-soluble profiles, albeit less protein was present. In addition, at postnatal day 24, the urea-soluble fractions showed that the α-crystallins underwent further processing, and the amount of γ-crystallins seemed to decrease (data not shown). Comparison with the urea-insoluble protein profiles revealed a marked increase in the amount of γ-crystallin (Fig. 6). Profiles of the hemizygous TG72 mice were similar, except the rate of proteolytic processing was slightly delayed (data not shown).

Since there appeared to be a loss of several of the crystallins from the lenses of the TG72 mice, Western blot analyses were used to determine which proteins were specifically being degraded (Fig. 7). Lens protein homogenates were extracted from control mice on postnatal days 5 and 24 and from TG72 mice at postnatal day 24. After staining with peptide-specific crystallin antibodies, it was found that βB1- and βA3-crystallins were degraded from the TG72 lenses (Fig. 7, B and C). The γ-crystallins also appeared to be degraded (data not shown), and although the α-crystallins did not disappear, it seemed as though they too were being modified in some manner (Fig. 7A). In contrast, βB2-crystallin did not appear to be affected (Fig. 7D).

The next issue to be addressed was whether or not the protease itself could cleave the crystallins. Purified HIV-1 protease was incubated with column-purified mouse or bovine crystallins. Gel profiles clearly showed that the protease was indeed capable of cleaving mouse and bovine β- and γ-crystallins (data not shown). The protease can also cleave mouse, but not bovine, α-crystallins. The protease-digested protein pattern was the same even in the presence of EDTA, which was used to chelate endogenous Ca²⁺ (data not shown). The EDTA was used to prevent activation of Ca²⁺-dependent proteases known to be present in the rodent lens. Purified HIV-1 protease, when incubated with control mouse lens homogenates, yielded a modified protein pattern (Fig. 8A); however, the two-dimensional profile exhibited a slightly different pattern than the ones obtained from the lenses of the homozygous TG61 and TG72 mice (Fig. 8B). Since it has been shown that some cytoskeletal proteins can be cleaved by HIV-1 protease (15, 16), the fate of several of these proteins was examined by Western analysis of whole lens homogenates of the TG72 line. Vimentin is present in the lens in the elongating but not mature fiber cells. It is a 56-kDa protein that was observed to exhibit some lower molecular weight bands in the lens homogenates under normal conditions. By postnatal day 22, the lower molecular weight components (26–30 kDa) disappeared, and by day 24, the 56-kDa band was lost leaving only a band of approximately 22 kDa (Fig. 9). Actin followed a similar trend of degradation with time (data not shown).

Although the protein profiles indicated that there were some differences in the pattern of modifications between the two mouse lines, two-dimensional analyses of the TG61 mice were less informative because the progression of the protein changes could not be easily monitored since cataract formation occurred in utero. Protein profiles of the lenses obtained from TG61 mice at postnatal day 5 revealed changes in the number of αA-crystallin fragments when compared to control mouse profiles. In addition, the spot observed at day 20 in the TG72 lenses homogenates was also present in the TG61 homogenates obtained at day 5 (Fig. 10). Very little γ-crystallin was present.

Table II

| Postnatal day | Control mice |
|---------------|-------------|
| Water-soluble fractions | |
| 5 | 0.256 ± 0.142 |
| 10 | 0.500 ± 0.161 |
| 15 | 0.889 ± 0.179 |
| 20 | 1.139 ± 0.186 |
| 25 | 1.205 ± 0.056 |
| 30 | 1.254 ± 0.098 |

| Urea-soluble fractions |
|------------------------|
| 5 | 0.0069 ± 0.018 |
| 10 | 0.0127 ± 0.011 |
| 15 | 0.0364 ± 0.018 |
| 20 | 0.0660 ± 0.014 |
| 25 | 0.0817 ± 0.024 |
| 30 | 0.2827 ± 0.038 |

Fig. 5. Two-dimensional SDS-PAGE analysis of the progression of lens protein modifications in TG72 mice homozygous for the expression of the HIV-1 protease transgene. Lenses were harvested postnatally on days 5 (A), 10 (B), 20 (C), and 24 (D) and homogenized in 8 M urea. Control mouse lenses were harvested postnatally on day 24 (E). The arrows represent the appearance of new protein spots, while the circle depicts the disappearance of several proteins. The major crystallins are identified in panel E, 1, αB-crystallin; 2, αA-crystallin; 3, βB2-crystallin; 4, βB1-; βB3-crystallins; 5, γ-crystallin; 6, βA3-crystallin; 7, βA4-crystallin; and 8, βA1-crystallin.

Table II

| Postnatal day | Control mice |
|---------------|-------------|
| Water-soluble fractions | |
| 5 | 0.256 ± 0.142 |
| 10 | 0.500 ± 0.161 |
| 15 | 0.889 ± 0.179 |
| 20 | 1.139 ± 0.186 |
| 25 | 1.205 ± 0.056 |
| 30 | 1.254 ± 0.098 |

| Urea-soluble fractions |
|------------------------|
| 5 | 0.0069 ± 0.018 |
| 10 | 0.0127 ± 0.011 |
| 15 | 0.0364 ± 0.018 |
| 20 | 0.0660 ± 0.014 |
| 25 | 0.0817 ± 0.024 |
| 30 | 0.2827 ± 0.038 |
and most of it appeared to be present in the urea-soluble fraction. In contrast to what was observed with the TG72 mice, βB1- and βA3-crystallins were only minimally degraded. In addition, these proteins were present even 30 days after birth, albeit at lower amounts (data not shown).

**DISCUSSION**

Several genomic products of retroviruses, such as HIV, are initially expressed as large polypeptide precursors. In the life cycle of HIV-1, one of these products, the HIV-1 protease, can cleave the polypeptide precursors into individual functional proteins. This 11.5-kDa enzyme is an aspartyl protease and is required for both polyprotein processing and virus infectivity (17–19). It is believed that the protease first cleaves itself out of the large Gag-Pol fusion precursor resulting in Gag and Pol precursors, which the protease then processes to their final forms.

The functions of the protease appear to make it an ideal target for therapeutic intervention. In studying various models for this purpose, transgenic mice containing only the HIV-1 protease were developed. An earlier transgenic mouse model showed that cataracts developed in mice containing the entire HIV genome defective only in reverse transcriptase activity. Because of the large accumulation of the Gag p24 capsid protein in the lenses, it was proposed that this protein played a role in the formation of cataracts at 3–6 months of age (4). However, the data presented in this study show that HIV-1 protease alone can cause cataractogenesis. RT-PCR and slot blot analysis revealed the presence of HIV-1 protease mRNA and protein in the lenses of both the TG61 and TG72 mice. The protein levels appeared to decrease with time probably as a consequence of autolysis or protein leakage. The HIV-1 protease is believed to be the initiating factor of the cataract, since mice bred with a mutation in the HIV-1 protease active site did not develop cataracts (Table I) even though HIV-1 protease...
mRNA was present (data not shown). In addition, the cataract phenotype was either reduced or prevented in TG61 and TG72 hemizygous mice by treating them with HIV-1 protease-specific inhibitors, thus supporting a primary role of the protease in cataract formation. The mechanisms of cataract formation are not well understood. However, it is hypothesized that once started, the pathway to opacification proceeds in certain defined steps regardless of the initiating factor. During the progression of lens opacification in the TG72 mice, protein modifications were observed approximately at postnatal day 20. The disappearance or partial loss of βA1-, βB3-, and βA3-crystallins seems to be concurrent. In addition, the appearance of a protein band at this same time point seems to correspond to a modified βA3/βA1 fragment identified by David et al. (20). By day 24, a large band appeared above the αB-crystallin spot, and based upon its location, it is suggested that it is comprised of aggregates of β-crystallin fragments. In addition, between days 20 and 24, the gradual breakdown of the cytoskeletal elements vimentin and actin occurs. This is in contrast to the data observed from the control lens homogenates, where no protein modifications were observed in the water-soluble fractions up to postnatal day 30 and only the processing of β-crystallin was observed in the urea-soluble fractions. We examined the progression of protein modifications during postnatal cataract formation in another mouse model. The Philly mouse, a strain with an inherited cataract, has an abnormal βB2-crystallin resulting from an in-frame deletion, which leads to a loss of 4 amino acids (21). The protein profile obtained prior to the formation of the cataract (postnatal day 20) was similar to that observed for control mice. At day 35, when a cataract was present, modifications to lens crystallins were similar to those observed for the TG72 mice, i.e., the loss of the βA3-, βB1-, and βB3-crystallins. The data appear to support the hypothesis that the progression of opacification is similar in postnatal cataract development.

The opacification of the TG61 lenses appeared to proceed in a slightly different manner. The βA3/βA1 spot present at day 20 in the TG72 lens homogenates was also present in the TG61 mice at day 5. However, very little γ-crystallin was present at this time even in the urea-soluble fraction, which indicates that some of the crystallins were either not expressed or were leaking out of the lens. In addition, the βB1-, βB3-, βA3-crystallins all appear to be present in the TG61 cataract. This suggests that since cataract formation occurs in utero, the mechanism of opacification proceeds in a slightly different manner and may be influenced by effects of the concurrent differentiation and early developmental processes. In addition, the smaller size of the TG61 mice compared to the TG72 or control animals might be attributed to the ectopic expression of the HIV-1 protease gene and/or a difference in its site of integration, which would result in an insertional inactivation of an essential cellular gene.

Analysis of the protein concentration of the TG72 lens homogenates revealed that the amount of protein decreased in the water-soluble fraction with time. However, the protein concentration of the urea-soluble fraction did not increase concurrently. Although there seems to be an increase in the protein concentration of the urea-insoluble fraction, it is likely that protein leakage from these lenses is also occurring. Protein leakage has been shown to occur during cataract formation in humans and in various cataractous animal models (22–27), and leakage could account for the loss of the γ-crystallins.

Although it was shown that the HIV-1 protease alone could cleave purified crystallins, the two-dimensional gel profile of the protease-digested control lens homogenates did not yield the same protein pattern as that observed for the TG72 lenses. This suggests that the protease may signal the activation of another enzyme(s) in vivo. One possible candidate for this role is calpain. The calpains are intracellular, calcium-dependent cysteine proteases (28) that have been shown to factor in the formation of nuclear cataracts (29). There are two forms of calpain. Calpain I requires micromolar amounts of Ca2+, while calpain II requires millimolar amounts of Ca2+ and is the predominant form in the lens (30). Activated calpain has been shown to proteolyze a number of lens crystallins (31–33). Calpain II appears to proteolyze βB1-, βB3-, βA3/βA1-, and βA4-crystallins. βB2-crystallin and the γ-crystallins resist calpain proteolysis. Calpains are major proteases in rodent lenses, and recent work on the selenite cataract suggests that the degradation of the crystallins by calpain II contributes to light scattering and lens opacification (34–36).

These results indicate that the entire genome of HIV is not necessary to cause cataract formation in mice. Expression of the HIV-1 protease is sufficient, and the level of expression of this protease can be quite low. The data appear to implicate other proteases in the lens for some of the protein modifications occurring during opacification. The specific initiating event in cataractogenesis caused by the HIV-1 protease has not been determined, but lens opacification caused by this protease in concert with other factors in the lens is certain.

Acknowledgments—We thank B. Krikelis, R. S. Senick, and M. J. Turner for excellent technical assistance.

REFERENCES
1. Huff, J. R. (1991) J. Med. Chem. 34, 2305–2314
2. Fitzgerald, P. M. D., and Springer, J. P. (1991) Annu. Rev. Biophys. Biophys. Sci. 20, 351–376
3. S. J. Tumminia and P. Russell, unpublished results.
3. Iwakura, Y., Shioda, T., Tosu, M., Yoshida, E., Hayashi, M., Nagata, T., and Shibuta, H. (1992) AIDS 6, 1069–1075
4. Schnaudigel, O.-E., Gumbel, H., Richter, R., Subklew, R., and Garweg, T. (1994) Ophthalmologica 91, 668–670
5. Harding, J. J., and Crabbe, M. J. C. (1984) in The Eye: Vegetative Physiology and Biochemistry (Davson, H., ed) Vol. 1b., 3rd Ed., pp. 207–492, Academic Press, New York
6. Chepelinsky, A. B., King, C. R., Lelenka, P. S., and Piatigorsky, J. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 2334–2338
7. Cheng, Y.-S. E., McGowan, M. H., Kettner, C. A., Schloss, J. V., Erickson-Vitanen, S., and Yin, F. H. (1990) Gene (Amst.) 87, 243–248
8. Cheng, Y.-S. E., Yin, F. H., Foundling, S., Blomstrom, D., and Kettner, C. A. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 960–964
9. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
10. Hogan, B., Constantini, F., and Lacy, E. (1986) Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
11. Taketo, M., Schroeder, A. C., Mobraaten, L. E., Gunning, K. B., Hanten, G., Fox, R. R., Roderick, T. H., Stewart, C. L., Lilly, C. T., and Overbeek, P. A. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 2065–2069
12. Yamada, T., Nakamura, T., Westphal, H., and Russell, P. (1990) Curr. Eye Res. 9, 31–37
13. Turnmna, S. J., Qin, C., Zigler, J. Jr., and Russell, P. (1994) Exp. Eye Res. 58, 367–374
14. Russell, P., and Yamada, T. (1990) BioTechniques 9, 422–424
15. Honer, B., Shoeman, R. L., and Traub, P. (1991) J. Cell Sci. 100, 799–807
16. Adams, L. D., Tomasselli, A. G., Robbins, P., Moss, B., and Heinrikson, R. L. (1992) AIDS Res. Hum. Retroviruses 8, 291–295
17. Kohl, N. E., Emin, E. A., Schleef, W. A., Davis, L. J., Helmbach, J. C., Dixon, R. A. F., Scolnick, E. M., and Sigal, I. S. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 4686–4690
18. Krausslich, H.-G., and Wimmer, E. (1988) Annu. Rev. Biochem. 57, 701–754
19. Peng, C., Ho, B. K., Chang, T. W., and Chang, N. (1990) J. Viral. 63, 2550–2556
20. David, L. L., Calvin, H. I., and Fuji, S. C. (1994) Exp. Eye Res. 59, 901–904
21. Chambers, C., and Russell, P. (1991) J. Biol. Chem. 266, 6742–6746
22. Sandberg, H. O. (1976) Exp. Eye Res. 22, 75–84
23. Sandberg, H. O., and Closs, O. (1979) Exp. Eye Res. 28, 601–610
24. Watanabe, H., and Shearer, T. R. (1989) Curr. Eye Res. 8, 479–486
25. Piatigorsky, J., Fukui, H. N., and Kinoshita, J. H. (1978) Nature 274, 558–562
26. Piatigorsky, J., Kador, P. F., and Kinoshita, J. H. (1980) Exp. Eye Res. 30, 69–78
27. Linklater, H. A., Dzialosznski, T., McLeod, H. L., Sanford, S. E., and Trevithick, J. R. (1990) Exp. Eye Res. 43, 305–313
28. Murachi, T., Tanaka, K., Hatanaka, M., and Murakami, T. (1981) Adv. Enzyme Regul. 19, 407–424
29. Azuma, M., David, L. L., and Shearer, T. R. (1992) Ophthalmic Res. 24, 8–14
30. Murachi, T. (1983) in Calcium and Cell Function (Cheung W. Y., ed) Vol. 4, pp. 377–410, Academic Press, New York
31. Yoshida, H., Murachi, T., and Tsukahara, I. (1984) Biochim. Biophys. Acta 798, 252–259
32. Yoshida, H., Murachi, T., and Tsukahara, I. (1985) Curr. Eye Res. 4, 983–988
33. David, L. L., and Shearer, T. R. (1986) Exp. Eye Res. 42, 227–2238
34. David, L. L., and Shearer, T. R. (1984) Invest. Ophthal. & Visual Sci. 25, 1275–1283
35. David, L. L., Wright, J. W., and Shearer, T. R. (1992) Biochim. Biophys. Acta 1139, 210–226
36. David, L. L., Shearer, T. R., and Shih, M. (1993) J. Biol. Chem. 268, 1937–1940
Cataractogenesis in Transgenic Mice Containing the HIV-1 Protease Linked to the Lens A-Crystallin Promoter
Santa J. Tumminia, Gerald J. Jonak, Richard J. Focht, Y.-S. Edmond Cheng and Paul Russell

J. Biol. Chem. 1996, 271:425-431.
doi: 10.1074/jbc.271.1.425

Access the most updated version of this article at http://www.jbc.org/content/271/1/425

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 9 of which can be accessed free at http://www.jbc.org/content/271/1/425.full.html#ref-list-1