Differential Expression of maf1 and maf2 Genes in the Developing Rat Lens

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**Purpose.** To examine the expression of maf1 and maf2, two protooncogenes in the developing rat lens.

**Methods.** Maf1 and maf2 transcripts were assayed in rat lenses on embryonic days 13 and 16 (E13 and E16) by in situ hybridization using single-stranded RNA probes. Proteins encoded by the maf2 gene were assayed immunocytochemically in embryonic (E12, 13, 16, 19) and postnatal day 14 and 90 (P14 and P90) lenses.

**Results.** In embryonic lenses, we detected maf1 messenger RNA (mRNA) in the lens epithelium and maf2 mRNA diffusely distributed in the lens fiber cells. By immunocytochemistry, Maf-2 was detected on E12 in the nuclei of almost all lens pit cells. On days E13, E16, and E19, however, lens epithelial cells showed no immunoreactivity, but nuclei of fiber cells reacted strongly. On P14, nuclei containing Maf-2 protein were confined to the equator of the lens, but at 3 months of age, no Maf-2 could be detected in the rat lens. Western blotting showed that the anti-Maf-2 antisem reacted with a single protein, of molecular weight ~39 kDa, in rat lens.

**Conclusions.** Results showed the spatial and temporal regulation of maf1 and maf2 gene expression and suggest that these genes participate in transcriptional regulation during the development of the lens in the rat. Invest Ophthalmol Vis Sci. 1997;38:2679–2683.

The normal development of the vertebrate lens involves the differentiation of epithelial cells into fiber cells. The development of such cells in the rat is characterized by the appearance and subsequent accumulation of the cytoplasmic crystallins1 and of the integral membrane protein known as the main intrinsic polypeptide.2 Synthesis of these proteins is regulated temporally and spatially within the developing lens. α-Crystallin is found in both epithelial and fiber cells, but β- and γ-crystallins, as well as main intrinsic polypeptide, are present only in fiber cells. The mechanism that regulates protein synthesis during the development of the lens, however, has yet to be determined.

Recent work has shown that DNA-binding proteins regulate the transcription of many peptides and proteins and are therefore important in development.3,4 Maf proteins are members of a DNA-binding protein family, each of which has a bZip DNA-binding domain.5,6 The binding consensus sequence of the maf gene products overlaps with those of the TPA responsive element and the cyclic adenosine monophosphate responsive element.7 In addition to forming homodimers, the Maf proteins can form heterodimers with transcription factors having bZip structures, such as Jun and Fos.7,8 Recently it was found that the Maf protein family regulates erythroid differentiation by transcriptional regulation.9

In a previous study, we isolated two maf-related clones, maf1 and maf2, from a rat liver complementary DNA library and determined that these genes are expressed in the embryonal rat lens.10 In the present study, we assayed the expression of maf1 and maf2 during development of the rat lens.

**MATERIALS AND METHODS.** Animals and Tissues. All animal experiments conformed to the ARVO Resolution on the Use of Animals in Research. Timed-pregnant Sprague-Dawley rats were purchased from Saitama Jikken Dobutu Laboratory (Saitama, Japan). The morning that the vaginal plug was detected was defined as embryonic day (E) 0. On embryonic days 12, 13, 16, and 19 (E12, E13, E16, and E19), the rats were anesthetized with an intraperitoneal injection of 6% sodium pentobarbital (0.1 ml/100 g), and the embryos were surgically removed. Eyes were surgically removed from rat 14 days and 3 months after birth (days P14 and P90, respectively). Tissue samples were washed in saline and fixed in ice-cold 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5) for 2 hours (for immunocytochemistry) and for several days (for in situ hybridization).

One day before sectioning, tissue samples were immersed in phosphate buffer (pH 7.4) containing 10% sucrose. Cryostat sections, 10 to 20 μM thick, were cut in a plane parallel to the optic axis. They were mounted onto slides coated with poly-L-lysine and used immediately for immunocytochemistry or
kept at -70°C for in situ hybridization. For each developmental stage, we examined four series of sections from two animals.

In Situ Hybridization. In brief, sections were dried for 0.5 to 1 hour and immersed in 1% Triton X-100 in 50 mM Tris-HCl buffer (pH 8.0) containing 25 mM ethylenediamine tetraacetic acid for 30 minutes and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine. The sections were dehydrated in an ascending ethanol series and air-dried. The probe (1 × 10^6 disintegrations per minute [dpm]/ml) was dissolved in a buffer containing 50% formamide, 10% dextran, 1 × Denhardt's solution, 12 mM ethylenediaminetetraacetic acid (pH 8.0), 10 mM Tris-HCl (pH 8.0), 30 mM NaCl, 0.5 mg/ml yeast tRNA, and 10 mM dithiothreitol; 100 μl of probe solution was applied to each slide. Slides were coverslipped and incubated at 55° to 60°C overnight. Coverslips were then removed and the slides were rinsed in 4 × SSC, digested with RNase A (20 μg/ml) for 30 minutes at 37°C, and then for 30 minutes in 0.1 × SSC at 60°C, before finally being dehydrated again. The sections were exposed to X-ray film (Kodak X-omat; Eastman Kodak, Rochester, NY) for 3 to 4 days, dipped in NTB2 nuclear emulsion (1:1 with water, Kodak), and exposed for 1 to 3 weeks before being developed. Counterstaining of cells was done with 0.001% bisbenzimidane.

Radioactive RNA probes of maf1 and maf2 (nucleotides 195 to 410 and 1248 to 14170, respectively) were synthesized with (α-^35S) uridine triphosphate by T7 RNA polymerase. In control experiments, adjacent sections were treated with RNase before hybridization or sense-strand cRNA probes were used for hybridization.

Immunocytochemistry. Antibody directed against a maf2 sequence was prepared with a fusion protein (with glutathione S-transferase) produced in Escherichia coli. A maf2 complementary DNA sequence encoding amino acids 64 to 158 was inserted into pGEX-T2 vector (Pharmacia LKB, Uppsala, Sweden). The fusion protein was purified according to the supplier's instructions and subsequently used to immunize rabbits. The resultant antibodies were affinity-purified.

Slides of tissue sections were dried for 1 hour, rinsed twice in phosphate-buffered saline, and incubated with a 1:1000 diluted solution of anti-Maf2 antibody. The antibody was localized using an avidin-biotin immunoperoxidase method (Vector Laboratories, Burlingame, CA), and the reaction product was developed using a nickel-enhanced glucose oxidase method. As a negative control for immunostaining, adjacent sections were treated with normal rabbit serum.

Western Blotting. Lens proteins were solubilized in sodium dodecyl sulfate sample buffer (1%, 10 mM Tris-HCl, pH 6.8) and electrophoresed on 12.5% polyacrylamide sodium dodecyl sulfate gel. Proteins were blotted onto polyvinylidene fluoride membranes (Millipore, Bedford, MA). The membranes were incubated with anti-Maf2 antibody or normal rabbit serum. Binding was detected by the peroxidase antiperoxidase method using a chemiluminescence kit (ELC kit, Amersham, Buckinghamshire, UK). The Maf2-glutathione S-transferase fusion protein was used as a positive control.

RESULTS. We assayed the expression of maf1 and maf2 messenger RNA (mRNA) in the developing rat lens by in situ hybridization. In embryos studied 13 and 16 days after conception, message specific for maf1 was detected in the lens epithelium (Figs. IA, IC), whereas maf2 mRNA was diffusely distributed throughout the lens fiber cells (Figs. IB, ID). The control probes specific for antisense maf1 and maf2 sequences did not hybridize to the E16 lens (Figs. IE, IF).
FIGURE 2. Details of maf1 and maf2 expression in the developing rat lens. Embryonic day 15 rat lens stained with (A) bisbenzimide (arrow, cornea; *, lens epithelium) or hybridized with probes for (B) maf1 messenger RNA (mRNA) or (C) maf2 mRNA (arrow, lens equator). Magnification ×500.

At a higher magnification, the expression of maf1 mRNA was observed only in the epithelial cells, not in the fiber cells of the lens (Fig. 2). In contrast, maf2 mRNA was expressed in the lens fiber cells, with the strongest expression being in the lens equator cells. It was not detected in the epithelium of the lens.

When we evaluated by immunocytochemistry the expression of Maf2 protein in embryonic rat lenses, we observed that 12 days after conception, nearly all the lens cells exhibited immunoreactive nuclei (Fig. 3). The lens epithelial cells of older embryos (13, 16, and 19 days after conception) showed no immunoreactivity, but the nuclei of the fiber cells reacted strongly. Two weeks after birth, Maf2 expression was confined to the nuclei in the lens equator. No expression of Maf2 protein was apparent in the lenses at 3 months.

Western blots were used to characterize the molecular weight of the lens cell polypeptide bound by anti-Maf2 antibody (Fig. 4). This antiserum specifically bound to a ~39-kD protein in the rat lens (Fig. 4, lane 1). This band was identical in size to that of the Maf2 protein (theoretical Mr, 38,493).

DISCUSSION. The developing lens provides an opportunity to observe the differentiation of epithelial cells into fiber cells. The ability of maf gene products to bind to DNA and to regulate gene transcription suggests that these genes may be involved in the process of cell differentiation in the developing lens. We
FIGURE 4. Western blot analysis of Maf-2 protein expression in the rat lens. (Lanes 1 and 2) Proteins extracted from postnatal day 1 rat lens and (lane 3) proteins from E. coli expressing the Maf-2-glutathione S-transferase fusion protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted to polyvinylidene fluoride membranes. Blots were incubated with (lanes 1 and 3) anti-Maf-2 antibody or (lane 2) normal rabbit serum. Molecular size markers are indicated at the left.

Therefore assayed the expression of mRNA and protein encoded by the maf1 and maf2 mRNA genes in the developing rat lens. Our findings—that maf1 and maf2 mRNA are differentially expressed in the embryonal rat lens, both temporally and spatially—suggest that these genes play important roles in lens cell differentiation and in the development of the rat lens.

We found that maf2 gene transcripts are expressed as early as E12, at the time the lens pit forms. There is convincing evidence that lens polarity is influenced by the optic cup and the retina, which differentiates from it. Thus, our data suggest that the maf-2 gene product may mediate the stimulation of lens fiber-specific genes by the optic cup. Our data show that Maf-2 protein ceases to be expressed by 3 months of age. However, the possibility remains that the Maf-2 antigenic site is not merely masked in the 3-month-old lens.

Other transcription factors have been reported to be expressed in the developing lens. For example, c-fos mRNA is expressed at high levels in the central epithelium and annular pad, and c-jun mRNA is expressed in the lens fiber cells. In addition, Nrl, a member of the maf family and a transcription factor, is expressed in both the epithelial and fiber cells of the mouse lens. Thus, our results, together with the finding that Maf1 and Maf2 proteins can heterodimerized with c-Fos, suggest that the maf genes, by binding to transcription factors, regulate the process of differentiation and development in the embryonic lens.

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Key Words

lens, maf1, maf2, transcriptional regulation

References


Risk of Acute Angle-Closure Glaucoma After Diagnostic Mydriasis in Nonselected Subjects: The Rotterdam Study

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Purpose. To report the prevalence of narrow anterior chamber angles on slit-lamp examination and the incidence of acute angle-closure glaucoma (AACG) after diagnostic mydriasis in nonselected white subjects aged 55 years and over.

Methods. Of all subjects in the population-based Rotterdam Study (n = 7983), 6760 participated in the ophthalmologic examination and received both tropicamide 0.5% and phenylephrine 5% eye drops for diagnostic mydriasis. No exclusion criteria (e.g., level of intraocular pressure, presence of narrow anterior chamber angles, history of or treatment for glaucoma) were used. After the ophthalmologic examination, all participants received thymoxamine 0.5% drops in both eyes and were warned about the symptoms of AACG.

Results. The prevalence of narrow angles was 2.2% and was twice as high in women. In two subjects (0.03%), an attack of AACG developed in one eye after diagnostic mydriasis. After medical therapy, peripheral iridectomy was performed. In two subjects, both eyes healed without loss of visual acuity or visual field.

Conclusions. In nonselected white subjects of 55 years of age or older, the 2% prevalence of narrow anterior chamber angles is similar to that in a mixed black and white population in the United States. According to our protocol, 3 in 10,000 subjects are likely to develop AACG after diagnostic mydriasis followed by miotic drops. Invest Ophthalmol Vis Sci. 1997;38:2683–2687.

Diagnostic mydriatic drops are widely used in ophthalmologic clinical practice; in most cases they are essential for an adequate examination of the ocular media and fundus. However, the use of mydriatic agents can provoke an attack of acute angle-closure glaucoma (AACG), with an increase in intraocular pressure (IOP) of up to 80 mm Hg within a few hours and a risk of permanent damage to the optic nerve. Only early recognition of the elevated (in most cases substantially) IOP and subsequent reduction of the pressure can save the visual capacity of the eye involved.

The risk of precipitating an AACG attack might be a reason why many general practitioners, internists, and other nonophthalmologic physicians are reluctant to dilate pupils for ocular examination. Also, when an ophthalmic epidemiologic study is started, the question arises as to whether it is safe to use mydriatics in all participants. In epidemiologic studies, a selection may be made based on, for example, the width of the chamber angle by penlight or slit-lamp examination. No exact data could be obtained from the literature on the risk of AACG when, with or without prior examination, all subjects in a white, population-based study would receive mydriatic drops. Thus,