GRIFIN, a Novel Lens-specific Protein Related to the Galectin Family*

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The vertebrate lens is a relatively simple cellular structure that has evolved to refract light. The ability of the lens to focus light on the retina derives from a number of properties including the expression at high levels of a selection of soluble proteins referred to as the crystallins. In the present study, we have used differential cDNA display techniques to identify a novel, highly abundant and soluble lens protein. Though related to the family of soluble lectins called galectins, it does not bind β-galactosides sugars and has atypical sequences at normally conserved regions of the carbohydrate-binding domain. Like some galectin family members, it can carry a single carbohydrate binding domain (CBD), whereas the galectins 4, 6, and 8 carry two CBDs in tandem (4). Furthermore, of the galectins with a single CBD, galectins 1, 2, and 7 exist as noncovalent homodimers under physiological conditions (8, 9), whereas galectin 5 exists as a monomer (10). Galectin-3 (also known as Mac-2 (11)) exhibits disulfide-mediated multimerization (12) that can increase its affinity for glycoconjugates. Crystallographic studies have shown that galectins with a single CBD have β-sandwich topology that may be related to the structure of the legume lectins such as concanavalin A (3).

Galectins play diverse biological roles. Indeed, it appears that even a given galectin may have multiple functions. For example, both galectins 1 and 3 have been implicated in pre-mRNA splicing through the immunodepletion of in vitro splicing extracts (13, 14). In contrast, but not surprising given their lectin activity, some galectins appear to be involved in regulating adhesion. In particular, galectin-1 was shown in vitro to promote binding between olfactory neurons and a laminin glycoconjugate that lines the axonal migration path in vivo (15). More recently, it has been shown that mice missing the galectin-1 gene have disrupted axonal architecture for a subset of olfactory neurons (16), thus supporting the idea that galectin-1 mediates fasciculation. Different again is the demonstration from culture experiments that galectin-1 can induce programmed cell death in T-cells in a CD45-dependent manner (17). More recently, a urate transporter identified through expression screening was shown to have a high degree of identity to the galectins (18). It is designated galectin-9, has tandem carbohydrate binding domains with affinity for lactose (19), and represents one of the few galectins for which a function is defined.

In the current report, we describe the characterization of a novel protein related to the galectins, designated GRIFIN (galectin-related inter-fiber protein), that is restricted in its expression to the lens. In the adult, GRIFIN is located at the interface between adjacent fiber cells. Although GRIFIN is clearly a galectin family member based on amino acid sequence similarity, it differs in sequence at two locations that are absolutely conserved among the galectins and that are involved in carbohydrate binding; GRIFIN does not display β-galactoside-binding activity.

EXPERIMENTAL PROCEDURES

Animal Breeding—Timed pregnant Sprague-Dawley rats were obtained from Taconic Farms (German Town, NY) and maintained according to institutional guidelines. Ages of rats are noted as days postconception (e.g. PC 31.5).

Arbitrarily Primed PCR—The arbitrarily primed PCR method was used in these experiments.
adapted from previously established protocols (20). RNA samples for arbitrarily primed PCR were obtained from eyes taken from Sprague-Dawley rats at 29, 33, and 50 days postconception. Eye tissue used for RNA preparations included lens, ciliary body, and the ocular vasculature with the remaining eye tissues excluded through dissection.

The DNA was extracted using the RNAzol B kit (Biozet) and poly(A) enriched using oligo dT cellulose (Boehringer Mannheim) according to manufacturer instructions. RNA samples were treated with 0.5 units of RNase free DNase (Promega) in 40 μl Tris-HCL, 10 μl NaCl, and 6 μl MgCl2, in the presence of 0.5 units of RNasin (Promega) for 10 min at 37 °C. After adding EDTA to 10 μl to halt the reaction, samples were incubated with equal volumes of phenol, then chloroform, and then precipitated with 0.3 M NaOAc (pH 5.5) and ethanol.

cDNA was produced from 20 ng of RNA samples using 50 units Moloney murine leukemia virus reverse transcriptase (Promega) in a 20-μl reaction mixture containing 50 μl Tris-HCL, pH 8.3, 75 μl KCl, 10 μl dithiothreitol, 3 μl MgCl2, 200 μl dNTPs, 4 pmol of each of two random 10-mer primers (Operon), and 20 units of RNasin. Reactions were incubated at 37 °C for 1 h. To this, 20 μl of a PCR reaction mixture containing 10 μl Tris-HCL (pH 9.0), 50 μl KCl, 1.5 μl MgCl2, 4 pmol of each primer, 5 μCi [α-32P]dCTP, and 2.5 units of Taq polymerase was added and subjected to one low stringency round of amplification (94 °C for 3 min, 27.5 °C for 3 min followed by a 5-min temperature ramp to 72 °C holding for 3 min). Following this, 40 rounds of high stringency amplification were performed (94 °C for 1 min, 39 °C for 1 min, and 72 °C for 2 min) with a final extension incubation of 72 °C for 15 min.

Products were visualized on a 5% polyacrylamide, 7 μM urea 1x Tris borate-EDTA gel after denaturation (5 μl of PCR reaction in 10 ml of 80% formamide with dye heated to 65 °C for 10 min). The gels were dried according to standard protocols and exposed to film overnight. Film and gel were aligned with radioactive ink dots, and bands were cut out with a scalpel and placed in a microfuge tube containing 50 μl of TE (10 μl Tris-CI, 1x Tris EDTA). DNA was allowed to elute overnight. 5 μl was then removed and subjected to the high stringency amplification protocol previously mentioned. PCR products were then visualized on a 1.2% agarose gel stained with ethidium bromide. PCR products were cloned using the pCR II T-A cloning kit (Invitrogen), and positive clones were identified using blue/white selection. Miniscreens were performed using the Qiaex II kit (Qiagen). Automated sequencing was performed bidirectionally using SP6 and T7 primers. Consensus sequences were elucidated using Geneworks software (IntelliGenetics) and matched against the GenBankTM data bank using the FASTA search engine.

cDNA Library Screen—10 μg of poly(A) RNA was extracted from whole eyes of PC 33.5 Sprague-Dawley rats, and cDNA was synthesized and then unidirectionally sub-cloned into λ-ZAP II Express vector according to the manufacturer recommendations (Stratagene). A cDNA library of 2.1 × 109 isolates resulted. Library screens and phagemid amplification were also performed according to the manufacturer instructions. cDNA clones hybridizing with the GRIFIN probe were sequenced from the pBK-CMV phagemid bidirectionally using T3 and T7 primers. The GenBankTM accession number for the rat cDNA sequence for GRIFIN is AP082160.

PCR Amplification of GRIFIN 5’ End—Experience had shown that cDNAs identified by arbitrarily primed PCR always matched (at their 5’ end) the most 3’ nucleotides of the random 10-mer primer. Given this, we were able to design a 20-mer primer to complement the seventeen most 5’ nucleotides of the GRIFIN cDNA subclone and the three 3’ terminal nucleotides of the OPA-4 10-mer used in the arbitrarily primed PCR amplification (Operon Technologies, Inc.). When this primer was used in conjunction with a T3 primer (complementary to a region of plasmid DNA 5’ to the cloning site), we were able to amplify the relevant 5’ sequences from GRIFIN cDNAs when library DNA was used as template.

Specifically, 750 μl of the 2 × 107 plaque forming unit/ml amplified eye cDNA library was digested for 1 h at 55 °C in 20 μl EDTA, 0.5% SDS with 50 μg/ml proteinase K. Phenol, phenol/chloroform, and chloroform extractions followed with DNA precipitation using 0.3 M NaOAc and ethanol. DNA was resuspended in 50 μl of TE. One μl of this DNA and 100 ng of both a T3 primer and the 5’ primer (5’-TGTAATGCCATTACCCTCAG-3’) were used in a 50 μl reaction containing 10 μl Tris-HCL (pH 9.0), 50 μl KCl, 1.5 μl MgCl2, 100 ng of each primer, and 2.5 units of Taq polymerase and cycled for 40 rounds (94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min) followed by a 15-min extension at 72 °C. The products were visualized on a 1.5% ethidium/agarose gel, subcloned into the pCR II vector, and sequenced.

Transcription Analysis—Total RNA or poly(A)RNA purified as above was run out on a 1% agarose, 18% formaldehyde gel and transferred overnight onto Hybond-N (Amersham Pharmacia Biotech). Randomly primed probes labeled with [α-32P]dCTP (ICN) were generated according to RAPD priming protocol (Amersham Pharmacia Biotech). Results were viewed and quantitated for intensity using a PhosphorImager (Molecular Dynamics).

Isolation of the GRIFIN Gene—GRIFIN genomic DNA was isolated from a 129 sv genomic library in the AFIX11 vector (Stratagene). Hybridization screening of the library was performed with the cDNA to GRIFIN according to the manufacturer instructions. Subcloning and sequencing of GRIFIN genomic DNA was carried out using conventional methods. The accession number for the mouse genomic sequence for GRIFIN is AF092155.

Preparation of Recombinant Galectin-1 and GRIFIN—The cDNA encoding Galectin-1 was cloned from a rat liver cDNA library using the pRSET plasmids essentially as recommended by the manufacturer (Invitrogen). Recombinant, untagged galectin-1 was generated from a PCR-amplified cDNA subcloned into the pRSETB plasmid with the region encoding the His tag removed. All proteins were overexpressed in E. coli strain BL21(DE3). Galectin-1 was purified from a bacterial extract using lactose-Sepharose (Amersham Pharmacia Biotech). Recombinant His-tagged GRIFIN was purified using a nickel resin column. Untagged GRIFIN was overexpressed in the E. coli strain BL21(DE3) using the vector pRSETB. Cells were lysed in 150 mM NaCl, 50 mM Tris-HCL pH 8.5 (± 10 mM β-mercaptoethanol) by French Press. The cell lysate was centrifuged at 1 °C at 15,000 rpm. Polyethylene glycol precipitation was added to the supernatant to a final concentration of 0.5% and the solution was stirred at 4 °C for 30 min. The suspension was then centrifuged at 15,000 rpm for 30 min and the supernatant loaded on a Q-Sepharose (Pharmacia) column that was pre-equilibrated with 50 mM Tris-HCL (pH 8.5). The column was washed with 300 mM NaCl, 50 mM Tris-HCL (pH 8.5), and the protein was step eluted with 400 mM NaCl, 50 mM Tris-HCL (pH 8.5) (± 10 mM β-mercaptoethanol). The protein obtained from anion exchange chromatography was concentrated and loaded on a Superdex-75 (Amersham Pharmacia Biotech) gel-filtration column, using 20 mM Tris (pH 7.5), 150 mM NaCl as the elution buffer at 1 ml/min. The peak at 68–72 min corresponded to GRIFIN (or its mutant forms). SDS-PAGE conducted on the protein containing fractions from gel filtration chromatography resulted in a single band of appropriate molecular weight when visualized by silver staining. Electrospary ionization mass spectrometry was used to confirm precise subunit molecular masses. Using either of these two techniques, recombinant material was purified to homogeneity at a yield of 6–7 mg/liter of bacterial culture.

Polycystic Kidney Disease—Rabbit anti-GRIFIN polyclonal sera were prepared against either the His-tagged or unmodified forms of GRIFIN using standard immunization protocols (21). Immunization and harvesting procedures were performed by Covance (Denver, PA). Detection of recombinant GRIFIN by Western blotting was used as a means of testing antisera titers.

Immunoblotting—Immunoblotting was performed according to standard procedures (21) using Renaissance chemiluminescence visualization reagents (NEN Life Science Products). Protein A-horseradish peroxidase (Kirkegaard and Perry Laboratories Inc.) was used at a 1:5000 dilution. The secondary antibody used was a goat anti-rabbit IgG peroxidase (Kirkegaard and Perry Laboratories Inc.) was used at a 1:5000 dilution and the polyclonal antisera to GRIFIN at a 1:2500–1:5000 dilution.

Histological Procedures—Tissues for histological analysis included developmentally staged embryos or whole eyes from postnatal animals. Tissue samples were processed using conventional methods (22). The figures in this paper were prepared digitally using a ProGress 3012 digital camera, a Nikon slide scanner, and Adobe® Photoshop® and Quark Express® production software.

Immunofluorescence—Axial sections (4 μm) of paraffin-embedded, phosphate-buffered saline-formalin (4%) fixed eyes were immunofluorescently labeled according to conventional methods (20). Polyclonal rabbit antisera to GRIFIN was used at a range of dilutions between 1:50 and 1:250. The secondary antibody used was a goat anti-rabbit IgG conjugated with rhodamine (Boehringer Mannheim).

RESULTS

Isolation and Characterization of the GRIFIN cDNA—The cDNA encoding GRIFIN was identified using an arbitrarily primed PCR assay (20) designed to detect mRNAs differentially expressed during the postnatal stages of lens development. A PCR-amplified cDNA for GRIFIN arose in two separate reactions and appeared as an abundant product expressed at higher levels at PC day 50 than at PC 33.5 or PC 29.5 (Fig. 1,
A and B). Both PCR products were bracketed with sequences complementary to the primers used. Subcloning and partial sequencing of the amplified cDNA revealed that the conceptual translation of one open reading frame showed a high degree of sequence identity to members of the galectin class of lectins.

A rat eye cDNA library in LZIP II Express (Stratagene) was screened by hybridization for GRIFIN representatives using the amplified cDNA subclone fragment as a probe. GRIFIN hybridizing plaques represented 1 in 5–10,000 clones. Six were plaque purified, and the pBK-CMV phagemid was excised from ZAP II Express cDNA vector and the inserts sequenced.

Conceptual translation (Fig. 1C) of the GRIFIN cDNA revealed a single open reading frame matching the amino acid sequence predicted from the amplified cDNA from arbitrarily primed PCR. The library screen failed to provide enough 5' sequence to include the candidate start codon present in the original PCR fragment, so further PCR of phage DNA was undertaken (see "Experimental Procedures"). This strategy yielded another 23 nucleotides, and the initiator methionine codon was tentatively assigned to position 33 in the cDNA based on a reasonable match with the Kozak consensus sequence (23).

A search of the nonredundant protein data base (at NCBI; http://www.ncbi.nlm.nih.gov/) using the BLAST algorithm revealed that the GRIFIN open reading frame encoded a protein of the galectin class. The GRIFIN amino acid sequence was aligned with many of the known galectin family members (Fig. 1D) using the alignment program Clustawl (http://www.publi c.iastate.edu/~pedro/rt_1.html) and was shown to be highly related. The alignment program was directed to choose the alignment order based on relatedness. Thus, being positioned last in the alignment series, GRIFIN is the most distant family member. Of the eight Gal-2 residues identified as forming direct contacts with carbohydrate (24) and the three residues whose mutation abolished lactose binding in Gal-1 (25), GRIFIN has two that diverge from the consensus galectin sequence: Lys-48 instead of Asn and Val-72 instead of Arg/Lys (Fig. 1D, asterisks). GRIFIN did not show any characteristics of a secretory leader sequence (26) nor of a GPI-linked protein (27). A hydrophobicity plot (generated by the IntelliGenetics Geneworks software) did not provide any indication of the presence of a transmembrane domain.

GRIFIN mRNA and Protein Are Lens-specific and Present at High levels—To assess the expression pattern of GRIFIN, mRNA was isolated from a variety of rat tissues and subject to Northern blotting analysis. Stringent wash conditions were employed in an experiment using the rat GRIFIN cDNA as a hybridization probe. GRIFIN mRNA was detected at high levels in the eye but not elsewhere (Fig. 2A). The band representing GRIFIN was clearly not a single species when compared
Northern blotting analysis using poly(A)-cloning regions (Fig. 3A) stream of exon 1, and a canonical polyadenylation sequence DNA revealed that the GRIFIN gene was made up of five exons subcloned into pGEM7z and restriction mapped. The exonic conditions revealed three GRIFIN mRNA species closely related in size and are differentially modulated over developmental time. a-c gene indicates that the GRIFIN mRNA consists of three species (Fig. 3, B). Species b increased in intensity only at PC 46 and PC 50 (Fig. 2). As with the internal control (the mRNA representing glyceraldehyde-3-phosphate dehydrogenase (GAPDH)). A further Northern hybridization to RNA prepared from dissected lenses as well as the remaining eye tissues revealed that GRIFIN expression was restricted to the lens (data not shown).

Because GRIFIN had been identified based on differential representation after PCR amplification, we determined whether its mRNA was modulated through development. Northern blotting of total RNA under stringent washing conditions revealed three GRIFIN mRNA species closely related in size but with different patterns of modulation (Fig. 2B). The largest of the species (species a) was expressed at high levels at PC days 18 and 20 but showed diminished intensity from PC 22 onward. Species b increased in intensity only at PC 46 and PC 50 (Fig. 2B, tracks labeled 46 and 50). Species c is represented at higher levels from PC 22–30, reaching a peak at PC 34. Hybridization of the same Northern filter used in panel B with a probe to GAPDH indicates that approximately equal levels of RNA were loaded.

Structure of the GRIFIN Genomic Locus—We isolated and characterized λ-clones encompassing the GRIFIN gene from a 129 sv strain DNA library in AFIXII (Stratagene). Three hybridizing λ-clones were plaque purified, and the inserts were subcloned into pGEM7z and restriction mapped. The exonic region of the gene was identified through Southern hybridization with a rat GRIFIN cDNA probe, and a restriction fragment carrying the gene was subcloned and sequenced. Comparison of the sequences of the cDNAs and the genomic DNA revealed that the GRIFIN gene was made up of five exons and was a little less than 2 kilobases from the 5′- to 3′-untranslated regions (Fig. 3A). A TATA box sequence is present upstream of exon 1, and a canonical polyadenylation sequence corresponding to that identified in the rat cDNA was located at the 3′ end of the gene (Fig. 3C).

Three sets of imperfect direct repeats were detected in the region of the gene using a dot matrix comparison method (http://alces.med.umcn.edu/raowdot.html). Two of these repeats were located in the region upstream of the promoter and consisted of two 58-bp repeats (Fig. 3B, long arrows) and three 10-bp repeats (Fig. 3B, small arrows). Two of the 10-bp repeats appear at the 5′ end of the 58-bp repeats. Within the 58-bp repeats, sequences that match the consensus binding sites for the transcription factors c-myb (28) and δEF1 (29, 30) are found (Fig. 3B, gray bars). Two additional potential binding sites for each of these transcription factors are located between the direct repeats and the TATA box.

The second set of imperfect direct repeats appear in the exon 4-intron 4 region (Fig. 3C). These repeats are each 65 bp in length and contain two consensus binding sites for the transcription factor δEF1. The first repeat overlaps the splice donor for exon 4, and this places one potential δEF1 binding site within the coding region of exon 4 (Fig. 3C).

The GRIFIN Protein Forms a Stable Dimer and Does Not Bind Lactose—An examination of GRIFIN protein by immunoblotting revealed that, during the developmental period corresponding to fiber cell differentiation, the level of GRIFIN increased markedly (Fig. 4A). According to an estimation based on the use of recombinant GRIFIN as a control, endogenous GRIFIN represents about 0.5% of total lens protein in the adult (data not shown). Despite the appearance of multiple mRNA species (Fig. 3, A and B), only a single GRIFIN protein species is observed on a native gel (Fig. 4A). Furthermore, this single species runs at approximately 28 kDa, suggesting that it might be a dimer of the 15 kDa monomer.

Given this observation, we sought additional evidence for the dimerization of GRIFIN. On SDS-PAGE gels, both recombinant and endogenous GRIFIN were observed at the expected monomeric size of about 15 kDa (Fig. 4B). In contrast, recombinant untagged GRIFIN eluted from a size-exclusion column between the 25- and 43-kDa size standards (Fig. 4C) suggesting that both this measure too, GRIFIN might exist in dimeric form.

To test directly whether GRIFIN would dimerize, we determined whether His-tagged recombinant GRIFIN could form a heterodimer with either the untagged recombinant form or endogenous material in a lens extract (Fig. 4D). When His-tagged GRIFIN was combined with either untagged GRIFIN (Fig. 4D, track 4) or lens extract containing endogenous GRIFIN (Fig. 4D, track 5), three distinct species were observed on a native gel. Included as controls were His-tagged recombinant GRIFIN (Fig. 4D, track 1), untagged recombinant GRIFIN (Fig. 4D, track 2), and endogenous GRIFIN in lens extract (Fig. 4D, track 3). In order of reducing molecular weight, the species apparent in tracks 4 and 5 correspond to the His-tagged GRIFIN homodimer, a heterodimer between untagged and his-tagged GRIFIN, and a homodimer of untagged protein. No monomeric GRIFIN was observed. Combined, these experiments provide strong evidence that GRIFIN can form a stable dimer and that it probably exists as a dimer in the lens.

Anticipating that GRIFIN would display the lactose-binding characteristics of the galectin family members, we tested its ability to bind lactosyl-Sepharose (Amersham Pharmacia Biotech). In initial experiments, GRIFIN did not display any lactose-binding activity. GRIFIN differs in sequence from the galectin family of proteins at three key positions involved in sugar-binding. To test the possibility that lack of sequence conservation at these locations was responsible for the inability of GRIFIN to bind lactose, we modified residues at positions 47, 48, and 71 (Fig. 1D, residues indicated in red) so that the
GRIFIN sequence conformed closely to the conserved CBD consensus. The resulting altered protein was referred to as the GRIFIN triple mutant (TM) and included the changes Val-47 to Phe, Lys-48 to Asn and Val-71 to Arg. Recombinant GRIFIN-TM was overexpressed in *E. coli* and chromatographically purified as described previously for wild-type GRIFIN. The pure protein was tested for its ability to bind lactosyl-Sepharose beads under conditions that effectively immobilized recombinant Gal-1. GRIFIN and GRIFIN-TM did not bind lactosyl-Sepharose (Fig. 4E). In contrast, in parallel experiments approximately 90% of recombinant galectin-1 bound to lactosyl-Sepharose and was eluted with lactose (Fig. 4E). Recombinant GRIFIN proteins with either one or two of the changes in the triple mutant also displayed no discernible lactose-binding activity (data not shown).

**GRIFIN Is Detected in the Lens Fiber Cells by Immunofluorescence**—To allow a detailed examination of the location of GRIFIN in the lens, we generated rabbit polyclonal antiserum
using recombinant material and performed immunofluorescence labeling of histological sections from the eye (Fig. 5). Immunoreactivity for anti-GRIFIN antiserum was detected in the fiber cells of the lens at the day of birth (PC 22) (Fig. 3A).

Immunoreactivity was highest in the central region of the lens, whereas the lens epithelium and the anterior tip of the fiber cells immediately underlying the epithelial layer have minimal immunoreactivity (Fig. 5, A and B). Lens sections incubated with nonimmune rabbit serum in the place of anti-GRIFIN antiserum showed minimal background fluorescence (Fig. 5C).

Interestingly, adult lens sections show a distinctly different pattern of immunoreactivity to GRIFIN antiserum in adult rat lens section; D, pattern of immunoreactivity to GRIFIN antiserum in adult rat lens section; E, nonimmune control of adult lens section; F and G, higher magnification of the sub-epithelial region of fiber cells seen in panel D showing intense immunoreactivity between rows of fiber cells. The strongly labeled circular regions appear to correspond to small areas where the tissue has not adhered to the glass slide. The intense labeling within these regions is specific for GRIFIN and may be a consequence of the antibodies having access to both surfaces of the section.
pattern of GRIFIN immunoreactivity (Fig. 5, D-G). At low magnification, discrete areas of immunoreactivity are observed in a peripheral region underlying the lens epithelium (Fig. 5D) and were not observed in control sections incubated with pre-immune serum (Fig. 5E). This provides evidence that there may be a relocalization of the GRIFIN protein dependent on developmental stage.

In the adult lens, GRIFIN immunoreactivity is observed at the angular interface between fiber cells over an extended region of the fiber cell stacks; labeling is present but much less intense at the flat interface between adjacent fiber cells (Fig. 5, F and G). The changing pattern of GRIFIN labeling seen in Fig. 5D is probably a reflection of the changing direction of the fiber cell axes at a lens suture (white arrowhead).

**DISCUSSION**

We have identified a lens-specific protein that is related to the family of small, soluble lectins called galectins (1–4). The protein, designated GRIFIN (galectin-related inter-fiber protein) appears to be expressed exclusively in the lens and is restricted to the differentiated fiber cells of this organ. GRIFIN forms a stable dimer in vitro and, by immunofluorescence, is located at the interface between adjacent lens fiber cells. The location of expression, cellular distribution, and biochemical characteristics of GRIFIN raise a number of interesting questions concerning its likely function.

We currently have no satisfactory explanation for the appearance of three GRIFIN mRNA species. The structure of the genomic locus did not reveal potential splicing signals besides those that would give the intron-exon structure indicated. Furthermore, the many GRIFIN cDNAs shown provided no unequivocal evidence of multiple splice variants. The existence of a single protein product probably means that the existence of three mRNA species is of limited biological significance.

The GRIFIN gene has a set of large direct repeats that encompass the 3′ splice site of exon 4. Within each repeat are two potential binding sites for the transcription factor δEF1. This is a zinc finger and homeodomain transcription factor that has previously been implicated in conferring lens-specific expression of the chicken δ1-crystallin gene (29). The enhancer at which δEF1 acts is located toward the 3′ end of the δ1-crystallin gene in the third intron (30). The similar location of the potential δEF1 binding sites in the GRIFIN gene and its lens-specific expression may indicate some similarities in the mechanism of transcriptional regulation.

GRIFIN does not bind β-galactoside sugars under conditions where both galectin-1 and galectin-3 will. All current members of the galectin family show β-galactoside binding activity even though many have been identified using techniques that did not rely on lectin activity (2–4). The lack of two residues in the CBD that are conserved in other family members (Lys-48 and Val-71) might have explained the lack of lectin activity except for the fact that a mutant form of GRIFIN, conforming to the conserved motifs, did not show β-galactoside binding activity either. While sequence homology suggests that the polypeptide fold of GRIFIN is likely to be similar to that of the galectins, the lack of demonstrable lectin activity indicates stereochemical differences in the putative carbohydrate binding pocket. It is also possible that GRIFIN will bind particular sugars under conditions not tested here.

GRIFIN forms very stable dimers in vitro according to multiple criteria. Striking is the observation that no monomeric form is apparent under native conditions. Many of the galectins will form dimers or multimers (3) or have two tandemly repeated CBDs (3, 4), and this argues that their biological function requires the cross-linking of glycoconjugates. In the absence of a lectin activity for GRIFIN, it is not clear whether the same significance can be attached to its dimerization.

One interesting possibility for the function of GRIFIN is that it may be a new lens crystallin. The class of soluble, abundant proteins collectively referred to as the crystallins are expressed exclusively or primarily in the lens (31). They have evolved from proteins with high solubility and provide the high protein concentration necessary for light refraction. With the characterization of multiple crystallins, it has become clear that any protein with high solubility can evolve for function as a crystallin (32). Crystallin proteins include an α-enolase (33), an argininosuccinate lyase (34), and lactate dehydrogenase (35).

Like many of the crystallins, GRIFIN is expressed exclusively in the lens, and it is both abundant and highly soluble. Although we cannot exclude the possibility that GRIFIN has a function in binding a saccharide, it does not have the characteristic lactose-binding activity of the class of molecules to which it is most closely related. Combined, these characteristics might support the idea that GRIFIN represents a novel crystallin that has evolved from a galectin but has not retained its capacity to bind lactose.

The location of GRIFIN at the interface between lens fiber cells tends to counter the argument that it is a crystallin because those that have been characterized show a uniform cytoplasmic distribution. Because many of the galectins are secreted from cells despite the lack of a classical signal sequence, there is a possibility that the observed distribution of GRIFIN reflects a position outside the plasma membrane. Whether intra- or extracellular, the pattern of immunofluorescence for GRIFIN suggests that its primary function is executed at the membrane. Further studies on molecules that interact with GRIFIN may prove revealing.

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**Addendum**—While this paper was in review, human genomic sequence encoding the gene for GRIFIN was added to the high-throughput genomic sequence data base (accession number AC004840, nucleotide segment 11172 to 17312). Analysis of the intron-exon structure and the deduced mRNA sequence revealed an encoded protein showing a high degree of identity to rat GRIFIN. Importantly, the amino acids in rat GRIFIN within the carbohydrate-binding domain that deviate from the galectin consensus are also variant in human GRIFIN.

**REFERENCES**

1. Drickamer, K., and Taylor, M. E. (1993) *Annu. Rev. Cell Biol.* 9, 237–264
2. Barondes, S. H., Cooper, D. N. W., Gitt, M. A., and Leffler, H. (1994) *J. Biol. Chem.* 269, 20867–20879
3. Kasai, K., and Hirabayashi, J. (1996) *Biochem. J.* 319, 1–8
4. Colnot, C., Ripoche, M. A., Sceaur, F., Fouilis, D., and Poirier, F. (1996) *Biochem. Soc. Trans.* 24, 141–146
5. Hirabayashi, J., Satoh, M., and Kasai, K. (1992) *J. Biol. Chem.* 267, 15485–15490
6. de Waard, A., Hickman, S., and Kornfeld, S. (1976) *J. Biol. Chem.* 251, 7581–7587
7. Nowak, T. P., Kobler, D., Roel, L. E., and Barondes, S. H. (1977) *J. Biol. Chem.* 252, 6026–6030
8. Gitt, M. A., Massa, S. M., Leffler, H., and Barondes, S. H. (1992) *J. Biol. Chem.* 267, 10601–10606
9. Magnaldo, T., Bernard, F., and Darmon, M. (1995) *Dev. Biol.* 168, 259–271
10. Gitt, M. A., Wiser, M. F., Leffler, H., Herrmann, J., Xia, Y. R., Massa, S. M., Cooper, D., Luis, A., and Barondes, S. H. (1995) *J. Biol. Chem.* 270, 5032–5038
11. Hughes, R. C. (1994) *Glycobiology* 4, 5–12
12. Woo, H. J., Jeon, M. H., Jung, J. U., and Mercurio, A. M. (1994) *J. Biol. Chem.* 269, 18141–18142
13. Dagher, S. F., Wang, J. L., and Patterson, R. J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 1213–1217
14. Vyakarnam, A., Dagher, S. F., Wang, J. L., and Patterson, R. J. (1997) *Mol. Cell. Biol.* 17, 4730–4737
15. Mahantappa, N. K., Cooper, D. N., Barondes, S. H., and Schwartz, G. A. (1994) *Development* 120, 1573–1584
16. Puche, A. C., Poirier, F., Hair, M., Bartlett, P. F., and Key, B. (1996) *Dev. Biol.* 179, 274–287
17. Perillo, N. L., Pace, K. E., Seilhamer, J. J., and Baum, L. G. (1995) *Nature* 378,
A Galectin-related Lens-specific Protein

Eur. J. Biochem. **234**, 107–114

18. Leal-Pinto, E., Tan, W., Rappaport, J., Richardson, M., Knorr, B. A., and Abramson, R. G. (1997) *J. Biol. Chem.* **272**, 617–625
19. Wada, J., and Kanwar, Y. S. (1997) *J. Biol. Chem.* **272**, 6078–6086
20. Ralph, D., McClelland, M., and Welsh, J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 10710–10714
21. Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Culling, C. F. A., Allison, R. T., and Barr, W. T. (1985) *Cellular Pathology Technique*, Butterworth & Co. Ltd, London
23. Kozak, M. (1989) *J. Cell Biol.* **108**, 229–241
24. Hirabayashi, J., and Kasai, K. (1991) *J. Biol. Chem.* **266**, 23648–23653
25. Solis, D., Jimenez-Barbero, J., Matin-Lomas, M., and Diaz-Maurino, T. (1994) *Eur. J. Biochem.* **234**, 107–114
26. von Heijne, G. (1990) *J. Membr. Biol.* **115**, 195–201
27. Udenfriend, S., and Kodukula, K. (1995) *Annu. Rev. Biochem.* **64**, 563–591
28. Thompson, M. A., and Ramsay, R. G. (1995) *Bioessays* **17**, 341–350
29. Funahashi, J., Sekido, R., Murai, K., Kamachi, Y., and Kondoh, H. (1993) *Development* **119**, 433–446
30. Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
31. Piatigorsky, J. (1984) *Cell* **38**, 629–621
32. Wistow, G. J., and Piatigorsky, J. (1988) *Annu. Rev. Biochem.* **57**, 479–504
33. Wistow, G. J., Lietman, T., Williams, L. A., Stapel, S. O., de Jong, W. W., Horwitz, J., and Piatigorsky, J. (1988) *J. Cell Biol.* **107**, 2729–2736
34. Wistow, G. J., and Piatigorsky, J. (1990) *Gene (Amst.)* **96**, 263–270
35. Wistow, G. J., Mulders, J. W., and de Jong, W. W. (1987) *Nature* **326**, 622–624