Secretoglobins: Sexually Dimorphic Expression of Androgen-Binding Protein mRNA in Mouse Lacrimal Glands

Susann G. Remington1 and J. Daniel Nelson1,2

PURPOSE. This study was conducted to seek sex differences in mRNA expression in normal mouse lacrimal glands. Gene expression differences in the lacrimal gland may contribute to susceptibility to lacrimal gland or ocular surface disease.

METHODS. A differential display analysis was performed on poly(A)+ RNA isolated from male and female Swiss Webster mouse exorbital lacrimal glands. Four potential gender-specific products were subcloned and sequenced. Full-length cDNAs of each product were obtained using RACE-PCR. 32P-labeled fragments of each clone were hybridized to a blot of male and female mouse poly(A)+ RNA isolated from hardierian, lacrimal, submandibular, sublingual, and parotid glands and the liver.

RESULTS. GenBank database alignments indicated that the four clones were members of the secretoglobin family. The most closely related sequences were the mouse salivary androgen-binding protein (ABP) subunits α, β, and γ. We named the four lacrimal clones the δ, ε, ϵ, and ζ subunits of ABP. Northern blot analysis showed that mRNAs for each of these four ABP subunits were lacrimal-gland-specific. The δ and ζ subunits of ABP were expressed primarily in male mouse lacrimal gland.

CONCLUSIONS. Sequence attributes predict that the ABP subunits expressed in lacrimal glands comprise proteins that are secreted in tears. These data imply compositional differences in ABPs secreted by mouse lacrimal and submandibular glands, and in ABPs secreted by male and female mouse lacrimal glands. (Invest Ophthalmol Vis Sci. 2005;46:31–38) DOI: 10.1167/iovs.04-04216

The tear film serves as a barrier between the ocular surface and the environment. Tears comprise an aqueous-mucin gel covering the superficial epithelial cells of the cornea and conjunctiva, bounded by a thin lipid layer at the air/fluid interface. The lacrimal gland secretes the bulk of the aqueous phase, containing dissolved salts and a variety of proteins. Corneal and conjunctival epithelial cells contribute mucins. The meibomian glands embedded in the eyelid supply the lipids. The hardierian gland of nonprimate vertebrates also secretes fluid containing proteins and lipids.

Gender differences have been reported in lacrimal glands, in overall size, morphology, physiology, and molecular composition. Differing sex steroid hormones of males and females account for many of these observations. Sex-based differential expression was documented for several individual mRNAs and proteins,2–11 one of which was rat prostatein (also known as prostatic-binding protein). Prostatein belongs to the secretoglobin family.1,2 Secretoglobins are small, multimeric proteins secreted in the mucosa of mammalian barrier epithelial tissues. Uteroglobin, a homodimer, represents the founding member of the family.1 The secretoglobins detected in saliva and tears form heterotetramers, composed of two heterodimers.7,14–16 Rat prostatein has three subunits (C1, C2, and C3), comprising two disulphide-bonded heterodimers C1-C3 and C2-C3 in equimolar ratios.17–19 Mouse salivary androgen-binding protein (ABP) is also composed of three subunits (α, β, and γ), forming α-β and α-γ heterodimers.20 A universal function for secretoglobins has not been identified; however, various functions have been described for individual members of the secretoglobin superfamily. Uteroglobin (also known as CCSP in lung) binds progesterone, inhibits phospholipase A2, and has anti-inflammatory properties.21–25 Fel dl4, secreted in cat saliva, is one of the major cat allergens in humans.24,25 Mouse salivary ABP26,27 and rat prostatein bind steroid hormones.28 In rodents, secretoglobins may serve as pheromones.29 Several secretoglobins exhibited differential expression in glands of male and female animals. Examples include cat Fel dl,20,35 hamster heteroglobin,32,16 and the C3 component of rat prostatein.7 In the following report, we characterize four mouse secretoglobin mRNA sequences, some of which are differentially expressed in male and female mouse lacrimal glands.

MATERIALS AND METHODS

Animals

Male and female Swiss Webster mice were obtained from Charles River Laboratories (Wilmington, MA). The mice were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were killed at 7 to 10 weeks of age, and tissues were dissected immediately, frozen in liquid nitrogen, and stored at −70°C until needed.

For mouse lacrimal gland RNA isolations, exorbital lacrimal glands were collected from four male and female animals on each of five consecutive days, and approximately 20 lacrimal glands from each gender were pooled.

RNA Isolations and cDNA Synthesis

Total RNA was isolated from the pooled lacrimal glands by using a guanidinium hydrochloride protocol.35 Poly(A)+ RNA was subsequently prepared (PolyATtract mRNA Isolation System; Promega, Madison, WI). Poly(A)+ RNA from lacrimal glands was reverse transcribed into cDNA, as previously reported.7
Poly(A)* RNA was isolated from the remaining tissues in a single-step protocol (PolyATract System 1000; Promega). Protocol variations for individual tissues have been described.9

### Differential Display-PCR and Gel Analysis

We compared male and female lacrimal gland cDNAs using differential display-polymerase chain reaction (PCR) with three downstream primers and 24 upstream primers (72 combinations; National Biosciences, Plymouth, MN).34 Experimental details have been published.9 Briefly, male and female mouse cDNA and water controls were amplified with each primer combination. PCR products were electrophoresed in nondenaturing polyacrylamide gels, and the DNA was silver stained (Promega). Differential fragments (Y3m3, W9m2, and X2f4) were excised and eluted from hydrated gels, and reamplified using the same primers (Table 1). The resultant PCR products were subcloned into a plasmid vector (pCR2.1; Invitrogen, Carlsbad, CA) and sequenced (Silver Sequence DNA Sequencing Systems; Promega).

### Rapid Amplification of cDNA Ends–PCR

The complete cDNAs of the differential display products (Y3m3, W9m2, and X2f4) and GN17 were obtained using the rapid amplification of cDNA ends (RACE) technique.35 (GN17 was subcloned during RACE of X2f4.) cDNA was synthesized using AMV reverse transcriptase (Promega) after priming poly(A) RNA from male and female mouse lacrimal glands with a sequence-specific oligonucleotide (Sigma Genosys, The Woodlands, TX) for 5°C. RNA size standards (Life Technologies, Rockville, MD) were used. The PCR reaction mixtures were denatured at 94°C for 2 minutes, then cycled 35 times in a thermal cycler as follows: 94°C for 45 seconds, 52°C for 1 minute, and 72°C for 2 minutes. The 72°C extension step was lengthened by 10 seconds per cycle. After 35 cycles, the products were electrophoresed through a 1% agarose gel, and each fragment was re-subcloned before isolation and labeling. The PCR products were subjected to sequencing analysis.

### DNA Sequencing

Double-stranded plasmid inserts were sequenced in both directions with one of two systems (Silver Sequence DNA Sequencing System; Promega, or the T7 Sequenase 2.0 sequencing kit; Amersham Pharmacia Biotech, Piscataway, NJ).

### Sequence Analyses

Sequence information was organized with computer programs (GeneWorks; IntelliGenetics, Campbell, CA). Translation start sites, signal peptide cleavage sites and protein structure were predicted using NetStart 1.0,36 SignalP 3.0,37-39 and CPHmodels 2.0,40 respectively. Web-based programs at the Center for Biological Sequence Analysis (provided in the public domain by the Technical University of Denmark, Lyngby, Denmark and available at http://www.cbs.dtu.dk/services/). Sequences were compared with those in the GenBank databases, using the BLAST programs.41 (GenBank and BLAST are provided in the public domain by the National Center for Biotechnology Information [NCBI], Bethesda, MD, and are available at http://www.ncbi.nlm.nih.gov/GenBank/ and http://www.ncbi.nlm.nih.gov/BLAST/.) ClustalW was used to align nucleic acid and amino acid sequences.42 (ClustalW is provided in the public domain by the European Bioinformatics Institute at EMBL, Hinxton, UK, and is available at http://www.ebi.ac.uk/clustalw/.)

### Northern Blot Analysis

RNA samples were denatured in 50% formamide and 6% formaldehyde in low-ionic-strength buffer (20 mM sodium phosphate [pH 7.7]) at 60°C for 5 minutes. The samples were electrophoresed through a 1% agarose gel containing 6% formaldehyde in 20 mM phosphate buffer (pH 7.7). RNA size standards (Life Technologies, Rockville, MD) were visualized by staining a portion of the gel with 0.5 μg/ml ethidium bromide. The RNA in the remainder of the gels was capillary transferred to nylon membrane (Zeta-Probe; Bio-Rad Laboratories, Hercules, CA) from 10× SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0).

Table 3 lists the RNA fragments that were labeled for Northern blot hybridizations. Secretoglobin clones were digested with appropriate restriction enzymes, the desired fragments were purified in agarose gels, and each fragment was re-subcloned before isolation and labeling. The actin probe was a 1.5 kb mouse c-actin fragment (Stratagene, La Jolla, CA). The DNA probes were random prime-labeled (Prime-a-Gene Labeling System; Promega) with 0.5 μg/ml ethidium bromide. The DNA in the remainder of the gels was capillary transferred to nylon membrane (Zeta- Probe; Bio-Rad Laboratories, Hercules, CA) from 10× SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0). The labeled probes were denatured in a boiling water bath for 5 minutes and added to fresh hybridization solution at 1× 10^6 to 1× 10^7 cpm/ml.

For the secretoglobin probes, prehybridizations, and overnight hybridizations were performed in 5× SSC, 1× Denhardt’s reagent, 7% SDS, 50 μg/ml salmon testes DNA, and 20 μM NaHPO4 (pH 7.2) at 55°C. The filter was washed three times for 20 minutes each in 50 mL.

### Table 1. Differential Display Products

<table>
<thead>
<tr>
<th>Clone</th>
<th>Downstream Primers</th>
<th>Upstream Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>W9m2</td>
<td>W: 5’TCAV3’</td>
<td>9: 5’-GTTTTCGAGC-3’</td>
</tr>
<tr>
<td>X2f4</td>
<td>X: 5’TGG3’</td>
<td>2: 5’-CGAGAGAGG-3’</td>
</tr>
<tr>
<td>Y3m3</td>
<td>Y: 5’TGG3’</td>
<td>3: 5’-GATGGATG-3’</td>
</tr>
</tbody>
</table>

* V is A, C, G, or T.

### Table 2. RACE-PCR Primers

<table>
<thead>
<tr>
<th>Clone/Accession No.</th>
<th>Reverse Transcription Primer*</th>
<th>Bases</th>
<th>PCR Primer*</th>
<th>Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ RACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y3m3 (abph)/AF008595</td>
<td>5’-TAGCTACAGCGAGCAGAGG-3’</td>
<td>325–344</td>
<td>5’-TTAGCCATCAACATAAGG-3’</td>
<td>289–307</td>
</tr>
<tr>
<td>W9m2 (abph)/AF272844</td>
<td>5’-GAGCTGAGCTGAGATGTTAC-3’</td>
<td>492–412</td>
<td>5’-CAAGCGTACGATAGGAGG-3’</td>
<td>292–312</td>
</tr>
<tr>
<td>X2f4 (abph)/AY370634</td>
<td>5’-ATAGAATACGCTTGAGAG-3’</td>
<td>478–496</td>
<td>5’-GTCAGGCCTGAGATGTTAC-3’</td>
<td>281–300</td>
</tr>
<tr>
<td>3’ RACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GN17 (abpz) AY370635</td>
<td>5’-GACTCAGGGATCCAGAC-3’</td>
<td></td>
<td>5’-ATGCTGGACTACTATCC-3’</td>
<td>294–313</td>
</tr>
</tbody>
</table>

* For 5’ RACE, the primer sequences for the respective clones represent the complementary strands.

### Table 3. Northern Blot Probes

<table>
<thead>
<tr>
<th>Probe</th>
<th>Bases</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ abp-η</td>
<td>1–307</td>
<td>AF008595</td>
</tr>
<tr>
<td>Internal abp-η</td>
<td>99–307</td>
<td>AF008595</td>
</tr>
<tr>
<td>3’ abp-δ</td>
<td>123–524</td>
<td>AF272844</td>
</tr>
<tr>
<td>Internal abp-δ</td>
<td>123–347</td>
<td>AF272844</td>
</tr>
<tr>
<td>Internal abp-ε</td>
<td>169–355</td>
<td>AS370634</td>
</tr>
<tr>
<td>Internal abp-ζ</td>
<td>157–350</td>
<td>AS370634</td>
</tr>
</tbody>
</table>
3× SSC, 10× Denhardt’s reagent, 5% SDS, and 25 mM Na$_2$HPO$_4$ (pH 7.2) at 55°C; once in 100 mL 1× SSC and 1% SDS at 55°C; and finally in 100 mL 0.5× SSC and 1% SDS at 55°C. For the 5′ abp-$\eta$, 3′ abp-$\delta$ and internal abp-$\delta$ probes, these washes were performed at 60°C. The blot was exposed to x-ray film (XAR; Eastman Kodak Co., Rochester, NY). After each hybridization, the blot was stripped twice in 0.1× SSC and 0.5% SDS at 95°C for 20 minutes each.

For the actin probe, prehybridization, and overnight hybridization were performed in 1 mM EDTA, 7% SDS, 50 µg/mL salmon testes DNA, and 0.5 M Na$_2$HPO$_4$ (pH 7.2) at 60°C. The filter was washed twice for 30 minutes each in 50 mL 1 mM EDTA, 5% SDS, and 40 mM Na$_2$HPO$_4$ (pH 7.2) at 60°C; twice in 50 mL 1 mM EDTA, 1% SDS, and 40 mM Na$_2$HPO$_4$ (pH 7.2) at 65°C. The blot was exposed to x-ray film.

**RESULTS**

**Lacrimal Gland Secretoglobins**

To identify lacrimal gland mRNAs that exhibited sexually dimorphic expression, we compared lacrimal gland poly(A)$^+$ RNA from male and female mice using differential display analysis. Herein, we describe four related sequences. Two of these sequences represented potential male-specific cDNA clones: Y3m3 (290 bases) and W9m2 (400 bases). One sequence represented a potential female-specific clone, X2f4 (130 bases). We cloned the fourth sequence, GN17, in RACE-PCR product GN17 encoded ABP, $\eta$ (Scgb1b1) nucleic acid sequence is encoded by proposed secretoglobin nomenclature (the Human Genome Organization, University College London, available in the public domain at http://www.gene.ucl.ac.uk/nomenclature/gene-family/scgb.html). In keeping with the mouse literature, we refer to Scgb1b1 as androgen-binding protein, (ABP, $\eta$, and the corresponding gene as Abpb (described later).

The deduced amino acid sequence of (cDNA) Abpb, bases 18-299, contains 95 residues. SignalP$^{37-39}$ predicted cleavage of a signal peptide after cysteine 23, yielding a mature secreted protein of 70 amino acids with a predicted isoelectric point of 4.6. This cleavage site for ABP, $\eta$ is analogous to the signal peptide cleavage site determined empirically for ABP, $\alpha$, the closely related mouse secretoglobin family member Scgb1b2.

**ABP, $\delta$, $\epsilon$, and $\zeta$**

The 524-base, full-length nucleic acid sequence generated from clone Y3m3 encoded mouse secretoglobin, family 1B, member 1, designated Scgb1b1 (GenBank accession no. AF008595) in proposed secretoglobin nomenclature (the Human Genome Organization, University College London, available in the public domain at http://www.gene.ucl.ac.uk/nomenclature/gene-family/scgb.html). In keeping with the mouse literature, we refer to Scgb1b1 as androgen-binding protein, (ABP, $\eta$, and the corresponding gene as Abpb (described later).

The deduced amino acid sequence of (cDNA) Abpb, bases 18-299, contains 95 residues. SignalP$^{37-39}$ predicted cleavage of a signal peptide after cysteine 23, yielding a mature secreted protein of 70 amino acids with a predicted isoelectric point of 4.6. This cleavage site for ABP, $\eta$ is analogous to the signal peptide cleavage site determined empirically for ABP, $\alpha$, the closely related mouse secretoglobin family member Scgb1b2.

BLAST alignments$^{41}$ of these three sequences suggested that they each belong to the same branch of the phylogenetic tree as cat Fel dl, chain 2$^2$ and the $\beta$ and $\gamma$ subunits of mouse salivary gland ABP.$^{44}$ Several secretoglobin phylogenetic trees have been published, in which three different designations have been used for the Fel dl, chain 2 branch (http://www.gene.ucl.ac.uk/nomenclature/genefamily/scgb_tree.pdf).$^{43-45}$ Because the Scgb subfamily designation for this branch appears ambiguous, we extended the mouse salivary ABP nomenclature to name our mouse lacrimal gland sequences. The ABP designations were based on sequence homologies with the mouse salivary gland proteins. Note that androgen binding has not been demonstrated for these lacrimal gland secretoglobins. The 5′ end of (cDNA) Abpb contains two in-frame AUG codons as potential translation starts. NetStart 1.0$^{46}$ predicted use of the second initiation codon, yielding a deduced amino acid sequence of 112 residues, bases 24-362. For (cDNA) Abpb and (cDNA) Abpz, the single initiation codons predicted 114 and 112 residue proteins, respectively.

A Clustal W alignment$^{42}$ of the predicted amino acid sequences depicted the relationships among the five Fel dl, chain 2 branch subunits of mouse secretoglobins: $\delta$, $\epsilon$, and $\zeta$ of lacrimal ABP and $\beta$ and $\gamma$ of salivary ABP (Fig. 1). More amino acid identities are apparent in the N-terminal signal peptides than in the mature proteins.

SignalP predicted cleavage of signal peptides after alanine 23, yielding mature secreted proteins of 89 or 91 amino acids. The corresponding isolectric points calculated for each lacrimal gland ABP subunit were 4.3 for $\delta$, 5.6 for $\epsilon$ and 6.1 for $\zeta$.

**ABP Structure**

On the basis of sequence similarities between mouse ABP and cat Fel dl, each ABP subunit was predicted to form a fold comprising four $\alpha$ helices.$^{40}$ The crystal structure has been solved for a recombinant Fel dl dimer.$^{46}$

In the mouse salivary gland, the $\alpha$ subunit of ABP formed covalent associations with either the $\beta$ or $\gamma$ subunits.$^{20}$ In the mouse lacrimal gland, one would predict that the $\eta$ subunit of ABP, which is homologous to the salivary $\alpha$ subunit, would form heterodimers with the $\delta$, $\epsilon$, or $\zeta$ subunits. Two heterodimers would associate to form a heterotetramer, secreted in the tears.

**Nucleic Acid Sequence Alignments**

The Abpb (Scgb1b1) nucleic acid sequence is encoded by locus ID 57426 at position 7A3 on chromosome 7 of the C57BL/6J mouse (http://www.ncbi.nlm.nih.gov/locuslink/list.cgi). Abpa, Abpb, Abpg, Abpe, and Abpz each map to the same region of mouse chromosome 7, 7A3-7B1. BLAST alignments of Abpb with mouse genomic sequence did not find an exact match. However, several additional related secretoglobin sequences are clustered in mouse chromosome region 7A3-7B1.

Alignment of (cDNA) Abpb with the mouse genomic contig NT_081228 indicated that Abpb is composed of three exons, bases 1-81, 82-269, and 270-429. BLAST alignment of (cDNA) Abpb with (cDNA) Abpa (GenBank accession no. BC016132) demonstrated two regions of high sequence homology at the ends of the cDNAs (90% and 87% identity), corresponding to the 5′ and 3′ untranslated regions and the signal peptide. The middle portion of the alignment, encompassing the mature protein coding region (bases 87-299), exhibited only 69% identity (Fig. 2A). More sequence conservation was apparent in cDNA regions flanking the mature protein (approximately exons 1 and 3), than in the sequence encoding the protein product itself (exon 2).
We observed the same pattern of sequence conservation in comparing sequences within the Fel dI, chain 2 branch of the secretoglobin family. We aligned the nucleic acid sequences of Abpb, Abpg, Abpd, Abpe, and Abpz using Clustal W (data not shown). We noted that the 5' and 3' untranslated regions and the signal peptide encoding segments had more sequence identities than the mature protein encoding regions. In paired comparisons, the 5' 100 to 110 bases of each sequence are at least 90% identical with each of the others. Likewise, from the stop codon to the poly(A) tail of each sequence, encompassing the 3' 160 bases, 85% or more of the bases are identical. Within the coding regions for the mature proteins, bases 110 to 365, the five sequences are only 65% to 70% identical with each of the others (Fig. 3A). The untranslated regions and the signal peptides of these mouse secretoglobin mRNA sequences are more highly conserved than the mature protein encoding sequences.

Lacrimal Gland Specificity/Gender Specificity

To determine the gender and tissue expression patterns of the four secretoglobin sequences, we sequentially hybridized labeled fragments of each clone to a nylon blot of male and female mouse poly(A) RNA from harderian, lacrimal, submandibular, sublingual, and parotid glands and from liver. We stripped and reused the same nylon RNA blot for the hybridizations presented in this study.

We initially labeled clone fragments of Abph and Abpd, containing 5' or 3' untranslated regions, for hybridization to the mouse RNA blot. Each of these probes hybridized to lacrimal and salivary gland RNA. 32P-labeled 5' abp-η, bases 1-307 of (cDNA) Abph, hybridized to a prominent 470-base RNA band in male and female lacrimal gland and to a less intense 470-base band in male and female submandibular gland (Fig. 2B). 5' abp-η also hybridized weakly to 1.5-kb bands in the lacrimal gland RNA lanes (data not shown). The salivary gland hybridization may have been due to cross-hybridization with (mRNA) Abpa or other related secretoglobins.

To address this possibility, we next isolated a probe fragment devoid of 5' and 3' conserved sequences. Using the default parameters for nucleic acid BLAST alignments, we selected an internal segment of abp-η, bases 1-307 of (cDNA) Abph, that did not align with other mouse sequences in the GenBank mouse expressed sequence tag (EST) database. 32P-labeled internal abp-η hybridized to a 470-base band in both

FIGURE 1. Clustal W alignment of the deduced amino acid sequences of ABP, β, γ, δ, ε, and ζ (GenBank accession no. AY293278, AY293281, AF272844, AY370634, and AY370635). Identical amino acids are shaded when common to three or more sequences. Underlined columns are those in which all five amino acids are identical. Arrow: predicted cleavage site of signal peptides, after amino acid 23.

FIGURE 2. (A) Diagram of a BLAST alignment between the η subunit of lacrimal ABP and the corresponding regions of the α subunit of salivary ABP. Percent sequence identities are indicated above the line representing the mRNAs. (B) Northern blot of rabbit RNA hybridized to abp-η and to α-actin. Left: size of each hybridizing RNA band; right: labeled cDNA fragment; numbers in parentheses: bases represented in each probe.
male and female lacrimal gland RNA (Fig. 2B). Among the mouse orbital and salivary glands investigated, Abph mRNA expression appeared specific to the lacrimal gland.

To verify RNA loads in each lane, we stripped the blot and re-probed the same nylon RNA blot with -actin. The actin probe hybridized to 1.5-kb -actin and cross-hybridized to 1.9-kb and -actins in every lane (Fig. 2B). The actin hybridization demonstrated the presence and integrity of RNA in each sample.

Next we addressed the expression of the remaining secretoglobins, belonging to the Fel dI, chain 2 subfamily. -labeled 3' abp-3, bases 123-524 of (cDNA) Abpd, hybridized to a prominent 540-base RNA band in male lacrimal gland, a less intense 2.2-kb band in male lacrimal gland, and a weak 540-base band in female lacrimal gland (Fig. 3B). 3' abp-3 also hybridized to prominent, slightly larger 590-base RNA bands of equivalent intensity in male and female submandibular gland.

We also observed a weak hybridization signal of 540 bases in male harderian gland and of 590 bases in male parotid gland (Fig. 3B). On longer exposures, we detected 590-base bands in both male and female sublingual glands (data not shown).

Again we were concerned that cross-hybridization with other secretoglobins may have contributed to the lacrimal or salivary gland signals. To minimize this potential we carefully chose another probe fragment, avoiding the 5' and 3' end conserved sequences. We selected an internal segment of abp-3, bases 123-347 of (cDNA) Abpd, that did not align with mouse salivary gland EST sequences. The shorter -labeled internal abp-3 hybridized strongly to a 540-base band in male lacrimal gland and very weakly to a 540-base band in female lacrimal gland (Fig. 3B). Abpd mRNA was much more highly expressed in male than in female lacrimal gland. Abp-3 hybridization was lacrimal-gland–specific among the mouse tissues examined.

We stripped and re-probed the same nylon RNA blot with internal -labeled fragments of abp-3 and abp-3. -labeled internal abp-3, bases 169-355 of (cDNA) Abpe, hybridized to a prominent 540-base band in male and female mouse lacrimal glands (Fig. 3B), and to a weak band at 2.7 kb (data not shown). Abp-3 hybridization was approximately equivalent in male and female lacrimal glands. -labeled internal abp-3, bases 157-350 of (cDNA) Abpz, hybridized strongly to a 540-base band in male lacrimal gland and weakly to the same sized band in female lacrimal gland (Fig. 3B). On longer exposures, abp-3 also hybridized weakly to a 1.4-kb band in male lacrimal gland (data not shown). Both Abpe and Abpz mRNA expression was specific for the lacrimal gland among the mouse RNA samples examined. Abpz mRNA, like abpd mRNA, was more highly expressed in male than in female lacrimal gland.

**DISCUSSION**

**Sexual Dimorphism**

We performed a differential display screening, comparing male and female mouse lacrimal gland poly(A) RNAs. As a result of the screening, we cloned four lacrimal-gland–specific mouse mRNAs, two of which were expressed primarily in male lacrimal gland (Abpd and Abpz). Database sequence comparisons indicated that the four clones were members of the secretoglobin family.

Gender-based differential expression has been demonstrated for secretoglobins in other species. In the rabbit lacrimal gland, lipophillin AL2 mRNA expression was male specific (Remington SG, et al. IOVS 2002;43:ARVO E-Abstract 3117). In cat salivary glands and skin, the major allergen Fel dI, although present in both sexes, was more abundant in male cats. Fel dI production was regulated by androgens.

In rat, the C3 component of prostatein was expressed in male lacrimal glands, but not in female glands and not in the lacrimal glands of castrated males or testicular feminized animals. Androgen administration induced C3 mRNA and protein expression in female and in castrated male lacrimal glands. In cultures of female rat lacrimal glands, C3 expression was also inducible by androgens.

Hamster harderian gland expression of heteroglobin mRNA was female specific and under hormonal regulation. Castration of males resulted in transient harderian gland expression which was stimulated by administration of estradiol. Heteroglobin mRNA and protein were also expressed in parotid and submandibular glands of both sexes.

Mouse salivary proteins, later known as ABPs, exhibited different electrophoretic mobilities in males and females. The female protein pattern was present in both sexes before puberty. Sexual development of male mice or administration of testosterone to female mice resulted in the conversion of the female pattern to that of the male. These electrophoretic mobility differences may have reflected the expression of different ABP protein subunits.

In the mouse lacrimal glands, differential display analyses from another laboratory supported our result that Abpd was expressed in the male, but not in the female. These researchers also reported salivary Abpa mRNA in lacrimal glands, primarily in female mice.
of lacrimal ABP subunits, secretoglobin composition probably differs between male and female mouse lacrimal glands.

**Submandibular Gland Cross-Hybridization**

In Northern blot analysis of mouse glandular tissues, our two longest secretoglobin probes cross-hybridized with mouse submandibular gland RNA. Removal of the conserved 5’ or 3’ ends of each probe resulted in lacrimal gland-specific hybridization of the remaining coding region sequence.

The 5’ end of (cDNA) Abpb is 90% identical with salivary gland Abpa, and the 3’ untranslated region of (cDNA) Abpd is 87% identical with Abpb and Abpz. These regions of high sequence homology were probably responsible for our observed submandibular gland hybridization signals, which in each case were approximately equivalent in intensity in both males and females. Our cross-hybridization data are in agreement with a recent report that there are no sex differences in mRNA expression of Abpa in mouse submandibular glands.41

Whereas we demonstrated lacrimal-gland-specific hybridization of secretoglobin probes abp-δ, -ε, -ζ, and -η, other researchers found evidence for another secretoglobin subunit, LGP10 of the Scgb1B branch, in both the lacrimal and submandibular glands of mouse.50,51 The amino acid sequence of LGP10 was 75% identical with the η subunit of lacrimal ABP. LGP10 was identified as an autoantigen in the NOD mouse model of Sjogren’s syndrome.

In the GenBank mouse EST database, we found evidence for the expression of two of the four lacrimal gland secretoglobins in other mouse tissues. Gastric epithelial progenitor cells contained a sequence match for mouse Abpd, and female skin contained sequence matches for Abpd and Abpz, the two secretoglobin mRNAs that exhibited gender-based differential expression in lacrimal gland. These database findings imply that ABPs in different mouse tissues may vary in subunit composition and hence in specific functions.

Similarly, lacrimal gland, but not salivary gland, expression of mouse Abpd, Abpe, Abpz, and Abpb mRNA suggests unique aspects to the roles that secretoglobins have in tissues and saliva.

**Conservation of 5’ and 3’ untranslated regions**

The secretoglobin probes that cross-hybridized with mouse submandibular gland RNA included conserved sequence in the 5’ untranslated region/signal peptide for Abpb and in the 3’ untranslated region for Abpd. The sequence identity was 85% to 90% in the mRNA ends of lacrimal-gland-specific secretoglobins compared with the paralogous submandibular gland secretoglobins, whereas the sequence identity was approximately 65% to 69% in the internal protein coding regions between lacrimal gland and salivary gland secretoglobins. These comparisons hold for the Scgb1 branch (Abpa and Abpb), as well as for the Feλt branch, chain 2 branch (Abpb, Abpg, Abpd, Abpz, and Abpz). Most related genes exhibit more sequence conservation within the protein coding region than in the 5’ and 3’ flanking regions.52 High sequence conservation of untranslated mRNA regions is an uncommon finding.

Although our observations encompass paralogous secretoglobin sequences expressed in different mouse tissues, others have noted strong sequence conservation in the untranslated regions between closely related secretoglobin subunits, or among allelic versions of a single subunit. For rat prostatein C1 and C2,19 heteroglobin B1 and B2,10 and mouse salivary Abpb and Abpg, the 5’ and 3’ untranslated regions and the signal peptide exhibited higher sequence homology than the mature protein-coding region.

A corollary to high conservation in untranslated mRNA regions is higher than expected sequence divergence within protein coding regions. Abpz alleles among different mouse species demonstrated high rates of substitution within the coding region, suggesting positive selection at this genetic locus.53 Rat prostatein genes were also identified in a database screen looking for high rates of nonsynonymous over synonymous substitutions—that is, high rates of nucleic acid substitutions that resulted in amino acid changes compared with substitutions that resulted in no amino acid change.54

Other genes that exhibited higher than expected sequence variability within protein coding regions include immune function molecules, such as major histocompatibility complex (MHC) loci55 and β-defensins,56,57 as well as reproductive proteins.59 Among immune surveillance and reproductive proteins, a common theme is molecular recognition of a separate organism, either an invader or a mate. It remains to be determined whether secretoglobins participate in analogous molecular recognition events.

**Sex Steroid Binding?**

Mouse salivary ABP constituted the major steroid-binding protein in mouse saliva.60 Sequence similarities suggest that lacrimal-gland-specific mouse ABP may also bind androgens. Because the lacrimal gland proteins are likely secreted, lacrimal ABP may be predicted to bind androgens in the tears. Androgens have been shown to ameliorate pathologies in mouse models of Sjögren’s syndrome,61,62 and topical androgen administration has been suggested as a possible treatment for ocular surface autoimmune diseases.63 It is possible that immune modulatory activities of androgens on the ocular surface are mediated through androgen-binding proteins in the tears.

**Acknowledgments**

The authors thank Jean M. Crow for a critical reading of the manuscript.

**References**

Secretoglobins in Mouse Lacrimal Glands


45. Laukaitis CM, Dlouhy SR, Kern RC. The mouse salivary androgen-binding protein (ABP) gene cluster on chromosome 7: character-


