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Expression of Six Mouse Major Urinary Protein Genes in the Mammary, Parotid, Sublingual, Submaxillary, and Lachrymal Glands and in the Liver

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Mouse major urinary proteins (MUPs) are encoded by a family of about 35 to 40 highly conserved genes. In the preceding paper (K. Shahan, M. Gilmartin, and E. Derman, Mol. Cell. Biol. 7:1938–1946, 1987), we presented the sequences of the most abundant MUP mRNAs in the liver (MUP I, II, and III) and in the lachrymal (MUP IV) and submaxillary (MUP V) glands. We have shown that these five mRNAs are coded by five distinct genes, MUP I through V. In the present communication, we examine the expression of MUP genes in all of the six tissues in which MUP mRNAs are synthesized, the mammary, parotid, sublingual, lachrymal, and submaxillary glands and the liver. We show that gene MUP II is expressed in the liver and in the mammary gland, that gene MUP IV is expressed in the lachrymal and parotid glands, and that gene MUP V is expressed in the submaxillary, sublingual, and lachrymal glands. Furthermore, we present evidence that in addition to genes MUP I through V, another gene, MUP VI, is expressed in BALB/c mice in the parotid gland. The tissue-specific synthesis of MUP mRNAs is thus brought about by two major mechanisms: the expression, in different tissues, of different members of the family and the expression of a single gene at various levels in different tissues. When a particular MUP gene is expressed in several tissues, transcripts of this gene initiate at the same site and are spliced and polyadenylated in the same manner.

The mouse major urinary proteins (MUPs) are secretory proteins encoded by a family of genes that exhibit sequence conservation of at least 85%, both in the transcribed and in the flanking sequences (4; Y. Shi and E. Derman, unpublished data). MUPs are synthesized in six different tissues: the liver and the lachrymal, submaxillary, parotid, sublingual, and mammary glands (14, 16). In the liver, MUPs are synthesized in response to several hormones such as testosterone, growth hormone, glucocorticoid hormones, and thyroxine and only in post-pubescent mice (10, 16). In contrast, in the submaxillary and lachrymal glands, MUPs are synthesized in prepubescent mice as well as in adult mice (16). The hormonal regulation in these two glands, however, does vary. Whereas the synthesis of MUPs in the submaxillary gland does not appear to be hormonally controlled, the synthesis of the lachrymal gland MUPs is regulated by testosterone (16).

In the preceding paper (15), we described the sequences of the major species of MUP mRNAs in the liver and in the lachrymal and submaxillary glands, MUP I, II, III, IV, and V. These five mRNAs are encoded by five distinct members of the MUP gene family. The focus of the studies presented here is to examine the synthesis of MUP mRNAs in three additional tissues, the mammary, parotid, and sublingual glands, and to define the expression of each of the previously identified genes, MUP I to V. To this end, we characterized the expression of individual MUP genes with the synthetic oligonucleotide probes described in the preceding paper (15), in male and in female mice and at various stages of development. The oligonucleotides and their specificities are listed in Table 1. The major species of MUP mRNAs in the mammary, parotid, and sublingual glands were further characterized through direct sequencing of cDNA clones. In addition, by using synthetic oligonucleotide probes listed in Table 1, we estimated the copy number of genes MUP I to V and classified them with respect to the previously described subfamilies of MUP genes (2). Coordinately expressed genes of the MUP gene family were defined. The coordinately expressed genes are not all members of the same subfamily.

MATERIALS AND METHODS

Animals, preparation of RNA, primer extensions, end labeling of synthetic oligonucleotides, construction of cDNA clones, and DNA sequencing were done as described in reference 15. The mammary cDNA library was prepared from 2-week-pregnant mice. The sublingual gland and the parotid gland libraries were prepared from 3- to 4- week-old males.

Liver DNA was prepared from isolated nuclei (8) by deproteinization with proteinase K (200 μg/ml) for 15 min at 37°C in 10 mM Tris hydrochloride (pH 7.4), 50 mM EDTA, 0.1 M NaCl, and 0.2% sodium dodecyl sulfate, followed by phenol-chloroform extraction, RNase treatment, and additional phenol-chloroform extractions.

Hybridization reactions were done in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate), 10 mM EDTA, 0.1% Ficoll (Pharmacia Fine Chemicals), 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.2% sodium dodecyl sulfate, 0.2% sodium pyrophosphate, and 10% dextran sulfate. Hybridizations to synthetic oligonucleotides, end labeled as described in reference 15, were done at 45°C, with washes in 2× SSC, 0.2% sodium dodecyl sulfate at temper-

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TABLE 1. Synthetic oligonucleotide probes and their specificities[a]

Oligonucleotide probe	Hybridization for MUP[b]				
	I	II	III	IV	V
oLiv1-A	+	+	ND	−	−
oLiv1-C	+	+	−	−	−
oLiv1-E	+	−	−	−	−
oLiv6-B	−	+	−	−	−
oLiv6-E	+/−	+	−	−	+
oLiv7-D	−	−	+	−	−
oLac1-A	−	−	−	+	−
oLac1-E	−	−	−	+	−
oLac1-F	−	−	−	+	+
oLac1-G	−	−	+	+	−
oSmx1-A	−	−	−	−	+
oSmx1-G	−	−	−	−	+

[a] The table summarizes results of experiments shown in reference 15, Fig. 4, in which synthetic oligonucleotides were hybridized to replica slot blots of cDNA clones MUP 1 (cLiv1), MUP II (cLiv6), MUP III (cLiv7), MUP IV (cLac1), and MUP V (cSmx1). The specificities were established by hybridizations at 45°C in 2× SSC, with washes at 45°C (oLiv1-C, oLiv7-D, oLac1-A, oLac1-E, and oSmx1-A), 50°C (oLiv1-A, oLiv1-E, oLiv6-E, oLac1-F, oLac1-G, and oSmx1-G), or 62°C (oLiv6-B).

[b] Symbols: +, strong hybridization; −, no hybridization; +/−, weak hybridization; ND, not determined.

atures indicated in the figures. Hybridizations to nick-translated probes were done at 65°C.

For Northern blots the amounts of poly(A)$^+$ RNA were estimated by ethidium bromide staining alongside RNA standards and by hybridization to a cDNA clone, plivC-16 (8). These two estimates agreed to within of factor of 2 for all tissues, with the exception of the lachrymal gland. In the lachrymal gland, there is four to five times less mRNA complementary to clone plivC-16 than is in all other tissues. The Northern analyses were done as described in reference 8.

RESULTS

Mammary gland: synthesis of MUP II mRNA. MUP mRNA is about 0.02% of total mRNA in the mammary gland (see below and reference 14). Mammary gland mRNA hybridizes to the synthetic oligonucleotide probe oLiv6-B based on the sequence of liver MUP II mRNA (clone cLiv6 [15]; Fig. 1). The mammary gland mRNA and the liver mRNA are the only mRNAs that hybridize to this probe (Fig. 1).

To determine whether the liver and the mammary gland mRNAs could be encoded by the same gene, first, the copy number of the MUP II gene was estimated. MUP II mRNA is nearly (99.6%) identical to MUP I mRNA (15). On a Southern blot of mouse DNA, the MUP I-derived probes hybridize to 12 to 15 genes, referred to here as MUP I-like, most of which are in a single 4.2-kilobase (kb) *Eco*RI fragment (Fig. 2a). Because MUP I and MUP II mRNAs differ by only three nucleotides, MUP II-derived oligonucleotide probes cross-hybridize to various degrees with the MUP I-like genes (Fig. 2b). However, the MUP II gene can be identified because a point mutation at nucleotide 270 of MUP II mRNA (clone cLiv6; Fig. 1 in reference 15) results in the loss of an *Eco*RI site which is conserved in most other MUP genes (data not shown). Thus, whereas the probes based on the sequence of MUP I mRNA hybridize predominantly to a 4.2-kb *Eco*RI fragment (denoted by an arrow, Fig. 2a), to which the MUP II-specific probes also cross-hybridize (denoted by an open arrow, Fig. 2b), the MUP II gene is most likely contained within the 21.5-kb *Eco*RI fragment (denoted by an arrow, Fig. 2b). From the intensity of the hybridization signal, it seems that the MUP II gene is present in one or, at most, two copies in the BALB/c genome.

Because MUP II appears to be a single-copy gene, we have further investigated the possibility that this gene is expressed in both the liver and the mammary gland. To this end, a mammary gland cDNA library was constructed and screened with a liver MUP cDNA clone, plivS-1 (7, 8). Eighteen MUP cDNA clones were obtained from the library, consisting of ca. 100,000 recombinants. The five clones that were analyzed all hybridize to the MUP II-specific probes. The nucleotide sequences of two clones which include the 3′-untranslated and the coding regions were determined (data not shown); both sequences are identical to the sequence of the MUP II cDNA clone derived from liver mRNA (clone cLiv6; Fig. 1 in reference 15). The sequence data show that the MUP II mRNA is polyadenylated and spliced in the same manner in both tissues. Moreover, as determined by primer extension (data not shown), the transcription of MUP II mRNA is initiated at the same site in both the liver and the mammary gland, 66 nucleotides upstream of the initiator AUG (Fig. 1 and 2 in reference 15).

Parotid gland: synthesis of MUP IV mRNA in young mice and of MUP VI mRNA in adult mice. In the parotid gland, as in the mammary gland, MUP mRNA is synthesized at a low level, about 0.02% of total mRNA (Fig. 3; 14). To identify the MUP species synthesized in this tissue, Northern blots of parotid gland mRNA from young (3-week-old) and adult (8-week-old) animals were hybridized to the oligonucleotide probes listed in Table 1. Parotid gland mRNA from young animals and, to a much lesser extent, from adult animals hybridizes to synthetic oligonucleotide probes derived from the sequence of MUP IV mRNA. (As described previously [15], MUP IV is the major MUP mRNA species in the

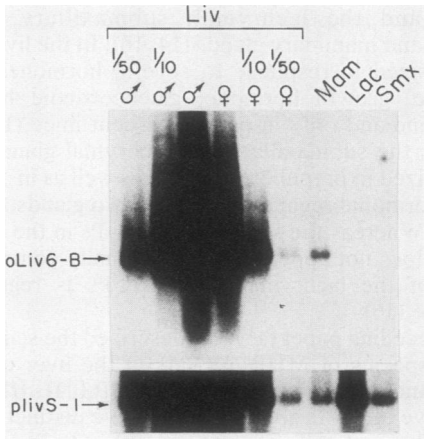

FIG. 1. Synthesis of MUP II mRNA in the mammary gland. Formaldehyde-denatured poly(A)$^+$ RNA from the liver (Liv; 3 μg) and the mammary (Mam; 6 μg), lachrymal (Lac; 1 μg), and submaxillary (Smx; 1 μg) glands was electrophoresed through a 1.4% formaldehyde agarose gel, blotted, and hybridized to the end-labeled oligonucleotide probe oLiv6-B (Table 1) in 5× SSC at 45°C. Wash conditions were 2× SSC at 62°C. Lachrymal and submaxillary gland RNA was extracted from tissues of 8-week-old male mice, liver RNA was extracted from 8-week-old mice of both sexes, and mammary gland RNA was extracted from 2-week-pregnant females. After exposure, the blot was stripped and rehybridized with nick-translated liver cDNA clone plivS-1 (8) to indicate the relative amount of MUP mRNA in each tissue.

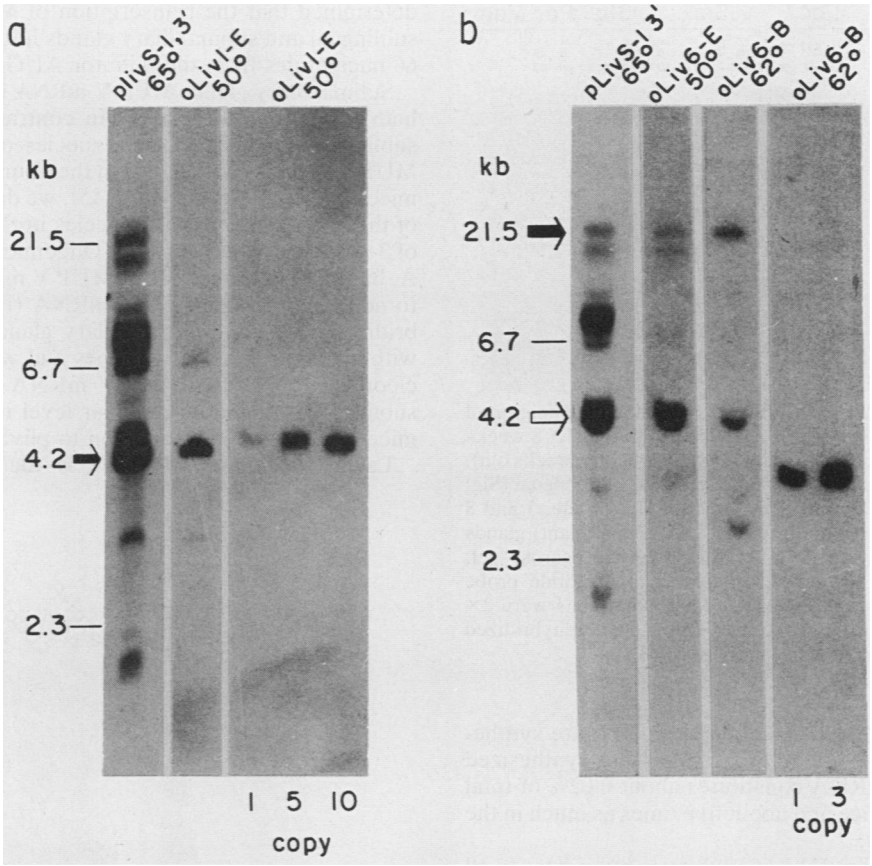

FIG. 2. Copy number of MUP I and MUP II genes in the BALB/c mouse. *Eco*RI-digested DNA (20 μg per lane) was electrophoresed through a 0.8% agarose gel and blotted. Individual lanes were cut apart and hybridized with the end-labeled oligonucleotide probes specific for genes MUP I (a) and MUP II (b). (The specificities of oligonucleotide probes are indicated in Table 1.) A control strip was hybridized to a nick-translated subclone of plivS-1 (8) containing sequences 3′ to an *Eco*RI site conserved in most MUP genes. (The *Eco*RI site is at amino acid position 50 [Fig. 1 in reference 15].) cDNA clones cLiv1 (a) and cLiv6 (b) in amounts equivalent to 1, 5, and 10 copies (cLiv1) and to 1 and 3 copies (cLiv6) of a gene were run in adjacent lanes as standards. The probes and wash temperatures (°C) used are indicated above each lane. Strips hybridized with oligonucleotide probes were exposed for 10 days. The control strip, hybridized with the nick-translated clone, was exposed for 1 day.

lachrymal gland.) Hybridization to MUP IV-derived probe oLac1-A is shown in Fig. 3.

The examination of a Southern blot of mouse DNA (Fig. 4) indicates that the MUP IV gene is present in a single copy in the genome. Each of the two oligonucleotide probes from the MUP IV-specific panel, oLac1-E and oLac1-F (Table 1), hybridizes to several *Eco*RI fragments of BALB/c DNA. (Some of these fragments do not correspond to MUP genes, since they do not hybridize to the MUP cDNA clone plivS-1. Others, e.g., the 7.2-kb band that hybridizes to the oLac1-F probe, are caused by cross-hybridization with genes other than MUP IV. The 7.2-kb band corresponds to MUP pseudogenes [data not shown].) However, only a single *Eco*RI fragment (denoted by an arrow, Fig. 4) hybridizes to both MUP IV-derived probes. Therefore, we conclude that the MUP IV gene is present within this 4.6-kb fragment. Since it appears that the MUP is a single-copy gene, the same gene must be expressed in both the lachrymal and the parotid glands.

To verify this conclusion, MUP cDNA clones were prepared from parotid gland mRNA of 3-week-old animals. Forty-seven MUP cDNA clones were isolated from a library of 200,000 clones. Twelve cDNA clones that were analyzed all hybridize to the MUP IV-derived probes (data not shown). Direct sequencing of three full-length clones (data not shown) has shown that in 3-week-old mice, the majority of parotid gland MUP mRNAs are indeed identical to lachrymal gland MUP IV mRNA (clone cLac1; Fig. 1 in reference 15). Moreover, the transcription of MUP IV mRNA initiates at the same site in both glands, 66 nucleotides upstream of the initiator AUG (Fig. 5).

Different MUP genes are expressed in the parotid glands of young and adult animals. Similar amounts of total MUP mRNA (determined by hybridization with a cDNA probe plivS-1) are synthesized in the parotid glands of young and adult mice (Fig. 3). However, the amount of MUP IV mRNA (determined by hybridization with the MUP IV-derived oligonucleotide probe oLac1-A) is much smaller in the adult mice. Since mRNA from the adult parotid gland does not hybridize to any other oligonucleotide probe, we infer that a MUP mRNA encoded by a gene distinct from genes MUP I to V is the major species in the parotid gland of adult mice. Because cDNA clones have not been constructed, the sequence of the major MUP mRNA species synthesized in this gland in the adult animals is not known. In this article, this putative species is referred to as MUP VI.

Sublingual gland: synthesis of MUP V mRNA in young mice and of MUP IV mRNA in adult mice. In the sublingual gland,

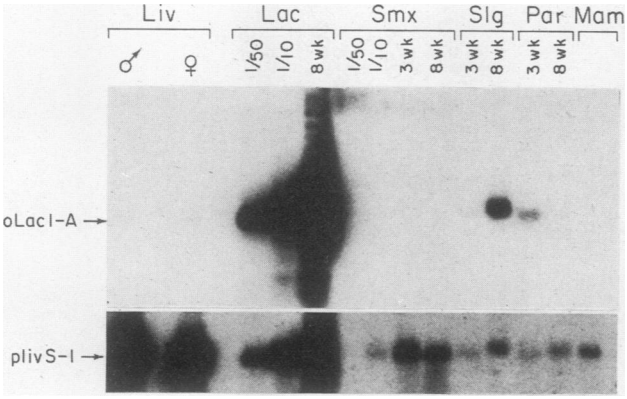

FIG. 3. Synthesis of MUP IV mRNA. Formaldehyde-denatured poly(A)$^+$ RNA from the liver (Liv; 3 μg; male and female, 8 weeks old) and the lachrymal (Lac; 12 μg and dilutions; male, 8 weeks old), submaxillary (Smx; 3 μg, male, 3 and 8 weeks old), sublingual (Slg; 3 μg; male, 3 and 8 weeks old), parotid (Par; 3 μg; male, 3 and 8 weeks old), and mammary (Mam; 6 μg; 2 weeks pregnant) glands was electrophoresed through a 1.4% formaldehyde agarose gel, blotted, and hybridized to the end-labeled oligonucleotide probe oLac1-A (Table 1) in 5× SSC at 45°C. Wash conditions were 2× SSC at 45°C. After exposure, the blot was stripped and rehybridized with nick-translated liver cDNA clone plivS-1 (8).

as in the parotid gland, species of MUP mRNA are synthesized in young mice that are different from those synthesized in adult mice. MUP mRNA constitutes about 0.02% of total mRNA in the young mice and about five times as much in the adult mice (Fig. 6; 14).

The sublingual gland mRNA of adult mice hybridizes to all the MUP IV-derived probes. (Hybridization to probe oLac1-A is shown in Fig. 3). As described above, the MUP IV gene appears to be a single-copy gene. Therefore, it is likely that the MUP IV gene is expressed in the sublingual glands of adult animals, in addition to the lachrymal and the parotid glands. In the sublingual gland, the transcription of this gene initiates at the same site as it does in the lachrymal and the parotid glands (Fig. 5). Note, however, that whereas the amount of MUP IV mRNA in both the lachrymal and parotid glands is larger in male than in female mice, the sex regulation is not evident in the sublingual gland (Fig. 6).

The sublingual gland mRNA from 3-week-old animals hybridizes to all of the MUP V-derived probes. (The signal is faint in hybridization to probe oSmx1-A as shown in Fig. 7.) The Southern blot of mouse DNA shown in Fig. 8 indicates that the MUP V gene is present also in one or, at most, two copies in the genome of BALB/c mice; only a single 20.5-kb band of *Eco*RI-digested mouse DNA (denoted by an arrow, Fig. 8) hybridizes to two different probes from the MUP V gene-specific panels, and the intensity of the hybridization signal corresponds to that expected from a single-copy gene. Thus, it seems likely that MUP mRNA in the sublingual glands of young animals is encoded by gene MUP V.

To unambiguously establish the identity of MUP mRNAs synthesized in the young mice, MUP cDNA clones from the sublingual gland mRNA of 3-week-old mice were constructed. All 32 clones isolated from a library of 100,000 recombinants hybridize to probes based on the sequence of MUP V mRNA. Moreover, sequencing of five cDNA clones (sequence not shown) has shown that in young mice the major species of sublingual gland MUP mRNA is identical to MUP V mRNA synthesized in the submaxillary gland (clone cSmx1; Fig. 1 in reference 15). By primer extension, we determined that the transcription of MUP V mRNA in the sublingual and submaxillary glands initiates at the same site, 66 nucleotides from the initiator AUG (data not shown).

Submaxillary gland: MUP V mRNA is the major species in both young and adult mice. In contrast to the parotid and sublingual glands, the same species of submaxillary gland MUP mRNA is synthesized in the young mice as in the adult mice. In the preceding paper (15), we described the structure of the major MUP mRNA species in the submaxillary gland of 3-week-old mice, MUP V. Oligonucleotide probe oSmx1-A, based on the sequence of MUP V mRNA, hybridizes also to adult submaxillary gland mRNA (Fig.7). Moreover, hybridization to adult submaxillary gland mRNA is observed with all MUP V-derived probes and with no other oligonucleotide probes. The MUP V mRNA is synthesized in the submaxillary gland at a similar level in male and in female mice (Fig. 6, see hybridization to plivS-1).

Lachrymal gland: MUP IV is the major MUP mRNA

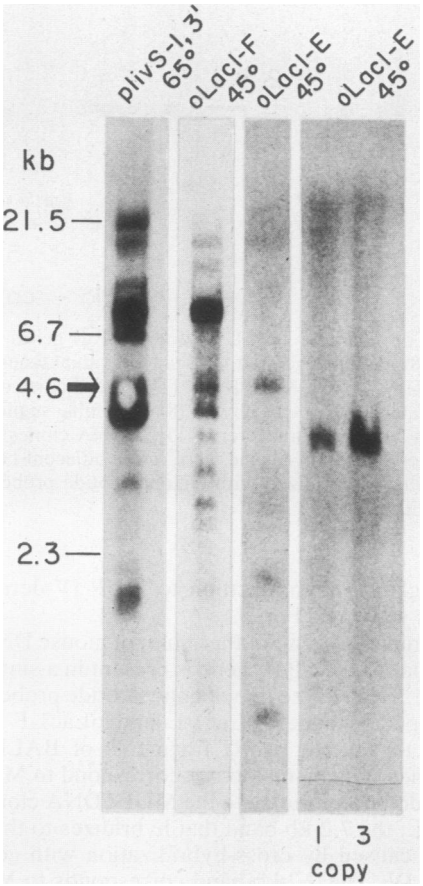

FIG. 4. Copy number of MUP IV gene in the BALB/c mouse. *Eco*RI-digested DNA (20 μg per lane) was electrophoresed through a 0.8% agarose gel and blotted. Individual lanes were cut apart and hybridized with the end-labeled oligonucleotide probes specific for MUP gene IV (Table 1). A control strip was hybridized to a nick-translated subclone of plivS-1 (8) containing only the sequences 3′ to the conserved *Eco*RI site. cDNA clone cLac1 (Fig. 1 in reference 15), in the amount equivalent to one and three copies of a gene, was run in an adjacent lane as a standard. The probes and wash temperatures (°C) used are indicated above each lane. Strips hybridized with oligonucleotide probes were exposed for 10 days. The control strip, hybridized with the nick-translated clone, was exposed for 1 day.

species, and MUP V is the minor MUP mRNA species. In the preceding paper (15), we presented the sequence of the major MUP mRNA species in the lachrymal gland, MUP IV. This is, however, not the only species in this tissue. As shown, the lachrymal gland mRNA also hybridizes, even though weakly, to probes derived from MUP V mRNA. (Hybridization to the probe oSmx1-A is shown in Fig. 7). To show that this is not caused by cross-hybridization with MUP IV mRNA, primer extension experiments were done.

By primer extensions, MUP IV and V mRNAs can be distinguished because of their differently sized signal peptide sequences. The primer extension products of the major lachrymal gland species, MUP IV, with primer oLac1-A, is six nucleotides shorter than the primer extension product of MUP V mRNA with primer oSmx1-A (Fig. 1 and 2 in reference 15). By this method, we determined that in the lachrymal gland, MUP V mRNA is synthesized at a level about 100-fold lower than that for the MUP IV mRNA (data not shown).

Liver: synthesis of MUP I, II, and III. Studies presented here and in the preceding paper (15) give evidence of three species of liver mRNA, MUP I, II, and III. The synthesis of liver MUP mRNA is sex regulated; the total amount of MUP mRNA is 5- to 10-fold larger in male than in female mice (7, 10). Because none of the oligonucleotide probes unambiguously discriminates between MUP I and MUP II mRNAs, and because MUP I- and MUP II-derived cDNA clones have not yet been isolated from female and male livers, respectively, it is not known whether MUP I and II mRNAs are synthesized in both sexes. That MUP III mRNA is synthesized in both male and female mice, at a level 5- to 10-fold higher in male mice, is seen in Fig. 9.

It has been documented that, in the liver, MUP mRNA is synthesized only in postpubescent animals and that the onset of MUP mRNA synthesis is paralleled by the onset of transcription of MUP genes (7). All species of liver MUP mRNA begin to be synthesized around the start of puberty, in 4- to 5-week-old mice (data not shown). Thus, the synthesis of MUP I, II, and III mRNAs appears to be coordinately regulated.

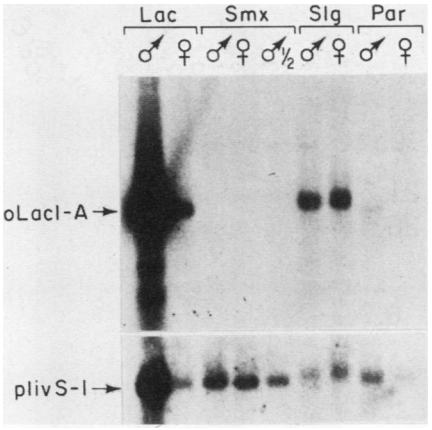

FIG. 6. Sex-related expression of MUP IV gene. Formaldehyde-denatured poly(A)$^+$ RNA from the lachrymal (Lac; 12 μg; male and female, 8 weeks old), submaxillary (Smx; 3 μg; male and female, 8 weeks old), sublingual (Slg; 3 μg; male and female, 8 weeks old), and parotid (Par; 3 μg, male and female, 8 weeks old) glands was electrophoresed through a 1.4% formaldehyde agarose gel, blotted, and hybridized to the end-labeled oligonucleotide probe oLac1-A (Table 1) in 5× SSC at 45°C. Wash conditions were 2× SSC at 45°C. After exposure, the blot was stripped and rehybridized with nick-translated liver cDNA clone plivS-1 (8).

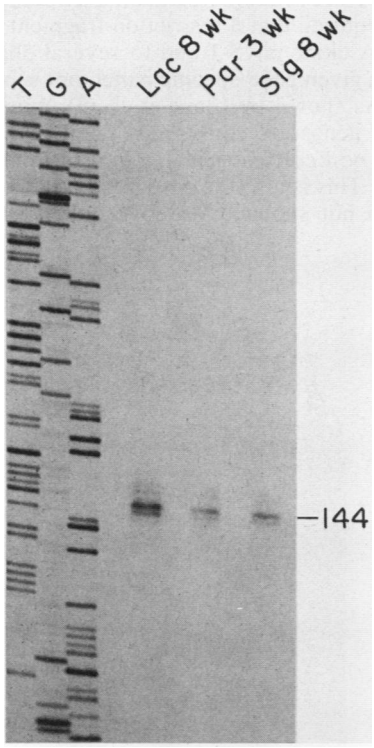

FIG. 5. Transcription initiation site of MUP IV mRNAs. Synthetic oligonucleotide primer (2 ng) oLac1-A (Table 1) was hybridized to lachrymal (Lac; 0.1 μg; male, 8 weeks old), parotid (Par; 5 μg; male, 3 weeks old), and sublingual (Slg; 0.5 μg male, 8 weeks old) gland poly(A)$^+$ RNA. Annealed RNA was extended with reverse transcriptase, and the extended products were electrophoresed through a sequencing gel along with sequencing reactions of clone cSmx1, all as described in Materials and Methods. The major primer extension products are 144 nucleotides long, inclusive of the primer.

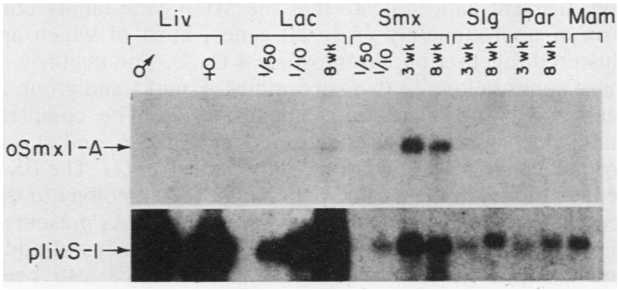

FIG. 7. Synthesis of MUP V mRNAs. Formaldehyde-denatured poly(A)$^+$ RNA from the liver (Liv; 3 μg; male and female, 8 weeks old) and the lachrymal (Lac; 12 μg and dilutions; male, 8 weeks old), submaxillary (Smx; 3 μg; male, 3 and 8 weeks old), sublingual (Slg; 3 μg; male, 3 and 8 weeks old), parotid (Par; 3 μg; male, 3 and 8 weeks old), and mammary (Mam; 6 μg; 2 weeks pregnant) glands was electrophoresed through a 1.4% formaldehyde agarose gel, blotted, and hybridized to the end-labeled oligonucleotide probe oSmx1-A (Table 1) at 45°C. Wash conditions were 2× SSC at 45°C. After exposure, the blot was stripped and rehybridized with nick-translated liver cDNA clone plivS-1 (8) in 5× SSC at 65°C.

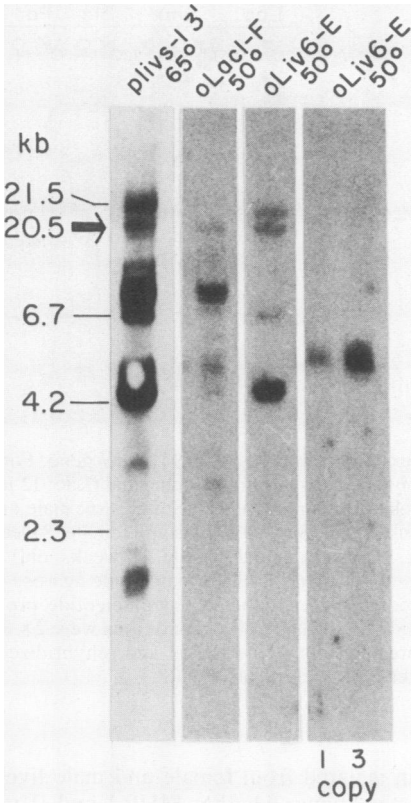

FIG. 8. Copy number of MUP V gene in the BALB/c mouse. *Eco*RI-digested DNA (20 μg per lane) was electrophoresed through a 0.8% agarose gel and blotted. Individual lanes were cut apart and hybridized with the end-labeled oligonucleotide probes specific for MUP gene V (Table 1). A control strip was hybridized to a nick-translated subclone of plivS-1 (8) containing only the sequences 3′ to the conserved *Eco*RI site. Clone cSmx1 (Fig. 1 in reference 15), in the amount equivalent to one and three copies of a gene, was run in an adjacent lane as a standard. The probes and wash temperatures (°C) used are indicated above each lane.

DISCUSSION

MUP genes and MUP pseudogenes: number and organization. Current data indicate that the MUP gene family consists of approximately 35 to 40 genes, most of which are clustered together on chromosome 4 (1, 2). The majority of these genes belong to two subfamilies, group 1 and group 2, each consisting of 12 to 15 members (2). The complete nucleotide sequences of one group 1 gene, BS-6, and of one group 2 gene, BS-2,3, were recently published (4). The BS-6 gene exhibits, in the exonic regions, 99.6% homology to the sequence of the liver MUP I and MUP II mRNAs presented in the accompanying paper (15). Partial sequences of addition group 2 genes besides gene BS-2,3 have also been determined (9). In all group 2 genes, a nonsense mutation at amino acid position 7 disrupts the coding frame (9). The group 2 genes have consequently been considered to be pseudogenes. It has been shown that the group 1 and group 2 genes are arranged in head-to-head-oriented gene pairs (5).

Our estimate of the number of MUP I-like genes (Fig. 2a) agrees with the estimate of the number of group 1 genes determined by Bishop et al. (2); thus, the group 1 genes are likely all MUP I-like genes. The number of expressed MUP I-like genes (group 1) is not known (15). Because of the extreme sequence homology among the individual members of this subfamily, the question of how many members of this family are expressed, and at what level, can be answered only by sequencing a large number of cDNA clones. Likewise, the close homology of MUP I-like genes makes it difficult to distinguish the expressed genes from other genes of this subfamily. Since probes based on sequences of MUP I mRNA hybridize only to liver mRNA and not to mRNA from any other tissues (data not shown), if genes of this subfamily other than MUP I and MUP II are expressed in BALB/c mice, these genes are expressed only in the liver.

It has been suggested that although the nonsense mutation in group 2 genes does not allow these genes to encode MUPs, the group 2 genes could encode a small peptide (4). However, when an oligonucleotide probe derived from a sequence conserved in five group 2 genes (9), spanning the nonsense mutation, is hybridized to the Northern blot shown in Fig. 1, no complementary RNA is detected in any of the six tissues examined (data not shown). Thus, it appears that the group 2 genes are not transcribed into stable RNA.

Not all MUP genes can be classified as group 1 or group 2 genes. In the accompanying paper (15), in addition to nucleotide sequences of two mRNAs transcribed from group 1 genes, the MUP I and the MUP II, we present the sequences of three mRNAs, MUP III, IV, and V, transcribed from genes that do not belong to either group 1 or group 2. MUP III, IV, and V appear to be encoded by single-copy genes.

In this paper, the MUP II, IV, and V genes are assigned to particular *Eco*RI restriction fragments on Southern blots. To do this, we required that a restriction fragment hybridize to a MUP cDNA clone plivS-1 and to several oligonucleotide probes from a given gene-specific panel, not separated by an *Eco*RI site. As shown by Clark et al. (3), many restriction sites in MUP genes are conserved. They have also shown that there are no *Eco*RI sites in any introns in the nine genes they mapped. This is also the case in 10 MUP genes that we mapped (data not shown). Moreover, the cloned MUP V

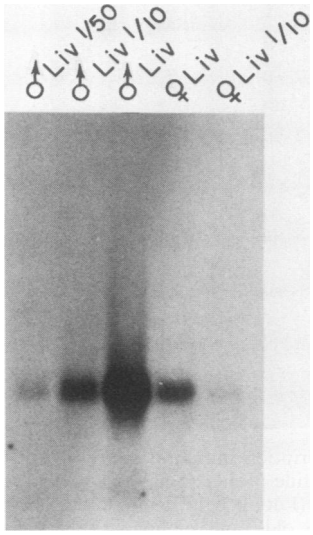

FIG. 9. Sex-related synthesis of MUP III mRNA. Poly(A)$^+$ RNA (3 μg) from livers of 7-week-old male and female mice and dilutions as indicated were electrophoresed through a 1.4% formaldehyde agarose gel, blotted, and hybridized to the end-labeled oligonucleotide probe oLiv4-D in 5× SSC at 45°C and washed under the same conditions.

TABLE 2. Percentage of total mRNA[a]

mRNA	% of total mRNA in:								
	Liver		Mammary gland	Lachrymal gland (8 wk)	Submaxillary gland (8 wk)	Parotid gland		Sublingual gland	
	Male	Female				3 wk	8 wk	3 wk	8 wk
MUP I	5	ND[b]	*[c]	*	*	*	*	*	*
MUP II	ND	1	0.02	*	*	*	*	*	*
MUP III	1	0.1	*	*	*	*	*	*	*
MUP IV	*	*	*	1	*	0.02	0.005	*	0.1
MUP V	*	*	*	0.01	0.2	*	*	0.02	*
MUP VI	ND	ND	ND	ND	ND	*	0.04	ND	ND

[a] Shown are estimates of the abundancies of MUP I to VI mRNAs in paroid, sublingual, submaxillary, and lachrymal glands of male mice, in the liver of both sexes, and in the mammary gland. The abundancies of MUP mRNAs were derived from the following data: frequncies of isolation of a particular MUP cDNA clone from a cDNA library (stated in the text), Northern blots (Fig. 1, 3, and 7), and primer extension experiments (Fig. 5). The estimates obtained by the different methods agree to within a factor of 2 and agree within this factor with our earlier data in the NCS strain (14).

[b] ND, Not determined.

[c] *, Undetected, i.e., less than 0.005%.

gene has already been obtained, and no intronic *Eco*RI sites were found.

Studies described here and in the preceding paper (15) have shown that there are six distinct MUP genes expressed in BALB/c mice. The sequences of five of the six MUP mRNAs (MUP I to V) have been established (15). The existence of MUP VI mRNA synthesized in the parotid glands of adult animals has been inferred from hybridization experiments. With the possible exception of additional MUP I-like genes expressed in the liver, we have most likely accounted here for most, or all, of the expressed genes in the BALB/c strain of mice.

Tissue-specific expression of MUP genes I to VI. Data presented here and in reference 15, summarized in Table 2, indicate that the MUP I to VI genes are differentially expressed. As described above, genes MUP II, IV, and V are expressed in several tissues. We have shown, by direct sequencing of MUP cDNA clones, that the gene MUP II is expressed in the liver and in the mammary gland, that the gene MUP IV is expressed in the lachrymal and in the parotid gland, and that the gene MUP V is expressed in the submaxillary gland and in the sublingual gland. In addition, hybridization and primer extension experiments indicate that that the gene MUP IV is also expressed in the sublingual gland of adult animals and that the gene MUP V is also expressed in the lachrymal gland.

Genes MUP II, IV, and V are expressed at a different level in different tissues (Table 2). The differences in the level of expression may be caused by differences in the number of cells in each tissue in which MUPs are synthesized. Alternatively, the differences in the level of expression could be due to differences in the transcription rates, efficiency of mRNA processing, or mRNA stability. We have shown that in all tissues in which a particular gene is expressed, most transcripts initiate at the same site. However, whether the expression of a given gene is controlled in different tissues by distinct regulatory elements and distinct *trans*-acting regulatory factors is not yet known. Likewise, even though the MUP II, IV, and V mRNAs are processed in the same manner in all tissues, the same species of MUP mRNAs may, in different tissues, exhibit different stability (6, 12).

In the parotid and sublingual glands, species of MUP mRNA are synthesized in the young mice that are different from those synthesized in the adult mice. In both of these tissues, major developmental events occur after birth. During postnatal development, the acinar cells, in which synthesis of secretory proteins occurs, first increase in number, and later they mature, as manifested by cell enlargement and accumulation of rough endoplasmic reticulum and secretory granules (11, 13). In 3-week-old mice, toward the end of the proliferative phase, MUP IV and MUP V mRNAs are synthesized in the parotid and the sublingual glands, respectively. After 7 to 8 weeks, which corresponds to the completion of the second developmental stage, MUP VI and MUP IV mRNAs are synthesized in the parotid and sublingual glands, respectively. In the parotid gland, the MUP IV can thus be considered an "early" gene and the MUP VI can be considered a "late" gene. In contrast, in the sublingual gland, the MUP IV is the late gene.

Coordinately and divergently expressed genes. The MUP genes I, II, and III are expressed in the liver beginning at puberty. By the criteria of tissue specificity and developmental regulation, these genes can thus be considered to be coordinately regulated. These genes are therefore likely to share a common regulatory element(s). The sequences of MUP I and MUP II mRNAs are highly conserved (99.6%). The MUP III mRNA has, however, diverged from the MUP I and MUP II mRNAs to the same extent as the MUP IV and MUP V mRNAs, which are not synthesized in the liver. It seems unlikely that the MUP I, II, and III genes have independently evolved to be regulated in an analogous manner. Rather, it seems more likely that the regulatory sequences required for expression of these genes in the liver have been conserved during evolution. Alternatively, the coordinate regulation of the MUP I, II, and III genes may have been brought about by gene conversion-like events which have included the regulatory elements. Recombinatorial events seem to contribute to the evolution of this gene family (15).

Sequencing studies presented in the preceding paper (15) allowed us to prepare panels of oligonucleotide probes with which differentially expressed MUP genes can be identified. The availability of these probes allows us to isolate the individual genes, determine their structure, and study their expression in transgenic mice. Through the experiments reported here, expression of the differentially regulated genes is now characterized in vivo. We can thus begin to investigate the processes by which the closely related members of this gene family have acquired, during evolution, differential and coordinate regulation. The tissue-specific regulation of MUP genes may be brought about by concerted interaction of several *trans*-acting factors with several regulatory elements, some of which may be common to all MUP genes. Alternatively, distinct regulatory factors and distinct regulatory elements may bring about tissue-specific expression of individual members of the family. The studies pre-

sented here provide the basis on which we can begin to approach these questions.

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