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Genes That Modify Expression of Major Urinary Proteins in Mice

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A survey of major urinary proteins (MUPs) from eight BALB/c mouse substrains by isoelectric focusing identified a common pattern with about 10 protein bands in males. One substrain, BALB/cJPt, differed in that it expressed two variant MUP patterns, designated 4.1^{lo} and null. To find the chromosomal location of the gene which determines the 4.1^{lo} phenotype, BALB/cJPt-MUP-4.1^{lo} was crossed with a wild-derived *Mus musculus domesticus* inbred strain (CLA) that expresses the common BALB/c MUP pattern. The F_1 phenotype revealed that the gene(s) controlling the MUP-4.1^{lo} trait was recessive. A restriction fragment polymorphism between these strains found with a MUP cDNA probe allowed us to establish that a gene determining the MUP-4.1^{lo} trait was not linked to the MUP structural genes on chromosome 4. Assays for other chromosomal marker loci revealed that a gene determining the MUP-4.1^{lo} trait, designated *Mupm-1*, was closely linked to *Myc-1* on chromosome 15. To determine the genetic basis of the null trait, BALB/cJPt-MUP-null mice were crossed with BALB/cJPt-MUP-4.1^{lo} mice. A MUP restriction fragment polymorphism between these two lines was tightly linked to a gene or genes involved in determining the MUP-null phenotype. The two variant MUP phenotypes in BALB/cJ mice are determined by separate genes, one of which is located on chromosome 4 and the other on chromosome 15. The chromosomal location of *Mupm-1* suggests that it produces a *trans*-acting factor which regulates MUP expression.

The major urinary proteins (MUPs) of the mouse are a group of 19,000-molecular-weight, structurally and antigenically related proteins with isoelectric points (pIs) in the range of pH 4.2 to 4.7. MUPs are synthesized predominantly in the liver but also in salivary, mammary, and other secretory glands. The MUPs produced in the liver are secreted into the plasma and excreted through the kidneys into the urine (9). Normal males excrete 5 to 20 times as much MUP as females. Gonadectomy reduces the quantity of MUP excreted by males to the female level (10). Administration of testosterone induced females and castrated males to excrete levels of MUP characteristic of normal males, indicating the regulatory role of this hormone.

A genetic locus originally designated *Mup-a* (now called *Mup-1*) which determines the pattern of MUPs expressed in the urine is located on chromosome 4 (14). The *Mup-1*^{a} allele in BALB/c and most other strains of mice determines the expression of a characteristic set of electrophoretically distinguishable urinary proteins. Other inbred strains (e.g., C57BL/6) have an alternate allele, *Mup-1*^{b}, which results in a set of MUPs electrophoretically distinguishable from those found in *Mup-1*^{a} strains.

The MUPs are encoded by a multigene family estimated to contain approximately 30 genes per haploid genome (3, 12). The structural genes are organized in a tandem array on chromosome 4 (6). Utilizing a MUP cDNA probe and Southern hybridization, Bennett et al. (2) reported a restriction fragment length polymorphism (RFLP) among inbred strains. One restriction fragment (RF) pattern was found in all *Mup-1*^{a} strains and another pattern was found in all *Mup-1*^{b} strains. Among crosses between *Mup-1*^{a} and *Mup-1*^{b} strains, no recombination was found between the *Mup-1* protein pattern and the RF pattern, indicating that the structural genes and the DNA sequences which determine the *Mup-1* phenotype are very tightly linked.

Recently, one of us (28) reported that BALB/cJ mice showed a significantly lower excretion of MUP than BALB/cBy mice on cellulose-acetate electrophoresis. These two BALB/c sublines have been separated over 50 years (25). This finding suggested that a major mutation affecting MUP expression has occurred in BALB/cJ. Isoelectric focusing (IEF) of MUPs (11) reveals more components than the original three described by agar or paper electrophoresis (10), although a correspondence exists between the sex and strain differences revealed by either method. Utilizing IEF, we screened eight BALB/c substrains and found two novel phenotypic patterns in the BALB/cJPt substrain. Furthermore, using RFLPs of MUP genes and other marker loci, we sought to determine the genetic basis of these two MUP phenotypes. We present evidence that one of these phenotypes is controlled by a regulatory locus on chromosome 15. The other phenotype is partly controlled by a gene linked to the MUP structural genes on chromosome 4.

(Portions of this research are from a dissertation to be submitted to the Graduate School, University of Maryland, College Park, by Robert Duncan in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Mice. All of the mice used in this study were bred at the conventional mouse colony of the National Cancer Institute, maintained at Hazleton Labs America, Rockville, Md., under contract number N01-CB2-5584. The origins of the BALB/c substrains have been described previously (see reference 25 for references).

Due to difficulties in maintaining a breeding colony of BALB/cJ mice, males obtained from the Jackson pedigree stock and females obtained from the Jackson production stock were crossed, and the progeny were selected for breeding efficiency. This line of the BALB/cJ substrain is designated BALB/cJPt to distinguish it from other BALB/cJ lines. BALB/cJPt mice have been brother-sister mated for eight generations. BALB/cJPt mice have the distinguishing

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markers of BALB/cJ mice, including high adult levels of alpha-fetoprotein, aggressiveness in males, and presence of the *Qa2* cell surface marker gene. Centreville Light A (CLA), a wild-derived strain (7), has been inbred for over 20 filial generations. BALB/cJPt females were mated with CLA males to produce F_1 and F_2 progeny.

Urine was obtained from individual mice, 5 weeks old or older, by exerting slight pressure over the bladder and collecting the urine in a clean test tube or restraining the mouse on a stainless steel mesh screen placed on a polyethylene sheet. Urine which dropped onto the sheet was pipetted into a clean test tube. Urine was dialyzed overnight against deionized water and stored at −20°C. In some cases the dialyzed urine of females was lyophilized and resolubilized in a smaller volume to obtain a sufficient concentration of MUP for IEF. Samples (50 to 120 μl) of dialyzed urine were lyophilized to dry powder and then reconstituted to 20 μl with deionized water.

IEF. A 7.5% acrylamide gel with pH 3.5 to 5 ampholines was cast 0.3-mm thick on an LKB Ultromould. The gel was loaded onto an LKB Multifor apparatus with the electrodes laid along the 25-cm dimension of the gel, 11 cm apart. H_3PO_4 buffer (1 M) was used in the anode and 1 M glycine was used in the cathode. The apparatus was cooled with refrigerated water to 4°C. The gel was prefocused for 1 h at 10 mA. Paper tabs (5 by 10 mm) were applied to the surface of the gel about 1 cm from the cathode. Dialyzed urine (20 μl) was applied to each tab, and 10 mA or 1,400 V, whichever was the limiting factor, was applied for 3 h. The gel was removed, fixed, and stained with Coomassie R250.

A relative measure of the concentration of MUP in the urine of an individual mouse was obtained by scanning densitometry of stained IEF gels. Scanning was done with a Hoefer G300 densitometer and integrated with the Hoefer GS360 software on an IBM PC computer.

Isozyme analysis. Kidney and erythrocyte tissue previously stored at −70°C was shipped on dry ice to Animal Genetic Systems, Rockville, Md., where enzymes were extracted and electrophoresed and polymorphic forms were identified.

Southern blot techniques. Purified DNA extracted as described previously (16) was digested with a two- to sixfold excess of the appropriate enzyme for 4 to 10 h at 37°C as specified by the manufacturer. Digested DNA was loaded onto agarose gels at approximately 10 μg per lane. The gels (17 by 17 by 0.5 cm) were electrophoresed in 40 mM Tris-acetate (pH 7.4)–20 mM sodium acetate–1 mM EDTA at 1 V/cm for 24 h. Gels were ethidium bromide stained and photographed under UV light. Gel was soaked in 0.25 N HCl for 7 min and in 0.5 N NaOH–1 M NaCl for 0.5 h and then neutralized in 0.5 M Tris-HCl (pH 7.2)–3 M NaCl for 1 h. DNA was transferred to nitrocellulose filters by the method of Southern (31) in 20× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate). Filters were rinsed in 3× SSC and baked in a vacuum at 80°C for 2 h. Before hybridizing, filters were soaked for 2 h at 65°C in 3× SSC–10× Denhardt solution (8)–5 MM EDTA–0.1% sodium dodecyl sulfate (SDS)–50 μg of sonicated salmon sperm DNA per ml. Hybridizations were carried out at 65°C for 12 to 18 h in the prehybridization mix plus 10% dextran sulfate (1) and probe (10 ng/ml).

Hybridization probes. To label MUP structural genes, filters were probed with an 850-kilobase (kb) *Pst*I insert cut from the p499 cDNA clone (20) which was kindly given to us by William Held, Roswell Park Memorial Institute.

Several molecular probes were used to identify loci known to reside on chromosome 15. The *gdc-1* probe was generously provided by L. Kozak and encodes the isoenzyme glycerol-3-phosphate dehydrogenase (19). A *gdc-1* probe was derived by *Pst*I digestion and isolation of a 700-base-pair (bp) insert. The v-*sis* probe is a 1.2-kb *Pst*I-digested insert isolated from the p3040 plasmid (Oncor, Inc., Gaithersburg, Md.). The *Ly-6* probe is a 750-bp *Eco*RI insert isolated from the cDNA clone pKLy6.1-2R, kindly provided by K. LeClair (21). The c-*myc* exon I probe is a 578-bp fragment derived by *Bam*HI-*Pst*I digestion of the cDNA clone pMcmyc54, which encodes most of exon 1 and a small portion of exon 2 (32).

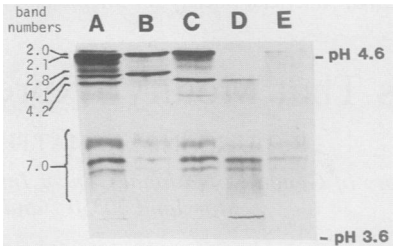

FIG. 1. MUP IEF patterns. Equal volumes of dialyzed urine were loaded onto each lane and focused by the procedure given in Materials and Methods. pH values mark the positions of pI standards (Sigma Chemical Co., St. Louis, Mo.), soybean trypsin inhibitor (pH 4.6), and *Aspergillus* amyloglucosidase (pH 3.6). Lane A is typical of the pattern observed in males of BALB/cAn, CLA, other *Mup-1*^{a} type strains, and intercrossed males which express the MUP-4.1^{hi} phenotype. Lane B is typical of the pattern observed in females of BALB/cAn, CLA, and others which express the MUP-4.1^{hi} phenotype. Lane C is typical of the pattern observed in males of BALB/cJPt family 1 and other males which express the MUP-4.1^{lo} phenotype. Lane D is the pattern observed in males of BALB/cJPt family 2 and other males which express the MUP-null phenotype. Lane E is the pattern typical of BALB/cJPt family 1 and family 2 females and intercrossed females that express the BALB/cJ phenotype.

The probes were labeled to 1×10^8 to 2×10^8 cpm/μg with [^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.) (27). After hybridization, the filters were washed for 5 min in 3× SSC–0.1% SDS–5 mM EDTA and for 10 min in 1× SSC–0.1% SDS–5 mM EDTA at 65°C. Filters were then given a final low-salt wash in 0.2× SSC–0.1% SDS–5 mM EDTA for 30 min at 65°C, blotted dry, covered with Saran wrap, and autoradiographed for 1 to 5 days at −70°C by exposure to Kodak XR-2 film.

RESULTS

IEF differences in BALB/c mice. With a pH gradient from 3.5 to 5.0, the typical BALB/c MUP IEF pattern contained seven to nine major bands, which we have designated by numbering in order from cathode to anode (Fig. 1) following the numbering system for MUP IEF bands of Knopf et al. (17). The correspondence between these bands and the MUP components identified by agar gel electrophoresis (10) was established by running purified MUP components (obtained from John Finlayson) on an IEF gel alongside urine samples from BALB/cAn mice (data not shown). Purified agar gel component 1 focused as three bands, numbers 2.8, 2.1, and 2.0, on IEF. Purified agar gel component 3 focused as four IEF bands, labeled 7. Neuraminidase digestion of whole MUP (data not shown) and other evidence (5; W. A. Held, personal communication) indicated that the component 7 bands were glycosylated, as after neuraminidase digestion, the pIs of these bands were all more basic. The pIs of the other IEF components remained unchanged.

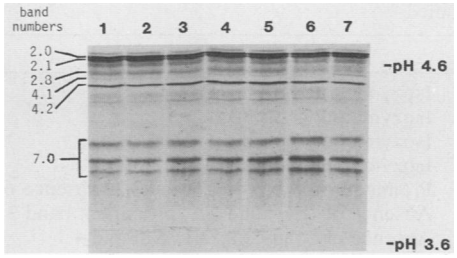

FIG. 2. Series of MUP-4.1^{lo} males. Dialyzed urine from seven different BALB/cJPt family 1 males was focused on the same gel. The range in intensity of band 4.1 seen among these samples from the high level in lane 7 to the low level in lane 3 is typical of the range of variation in MUP-4.1^{lo} phenotype males.

Urine samples from male and female mice of eight BALB/c sublines and CLA mice were analyzed by IEF. Six BALB/c sublines (Arg, HeA, ORNL, SK, Wm, and Wt) are identical to BALB/cAn (Fig. 1). Urine from strains AKR/Rb6;15, C3H/He, SJL/JA, PDB/Pt, DBA/2N, CLA (a strain inbred from wild mice), and other strains with the *Mup-1*^{a} wild phenotype also gave a pattern identical to that of BALB/cAn. BALB/cJPt was exceptional in that males lacked or had a faintly staining IEF band 4.1 (Fig. 1C). This phenotype was designated MUP-4.1^{lo} to reflect the fact that band 4.1 is consistently reduced in BALB/cJPt mice, although it ranged in intensity from no band at all to approximately half the intensity seen in a BALB/cAn mouse (Fig. 2). To maintain this trait distinct from a second MUP phenotype observed in BALB/cJPt mice (see below), we established a separate breeding stock designated BALB/cJPt family 1. Examination of 14 BALB/cJPt family 1 males from four generations which all had reduced-intensity 4.1 bands indicated that the BALB/cJPt family 1 is breeding true for this phenotype. The total quantity of MUP excreted by MUP-4.1^{lo} males was not significantly less than that by BALB/cAn males (Table 1).

Some BALB/cJPt males had a second unusual phenotype characterized by absence of IEF bands 2.0, 2.1, 2.8, and 4.1. We call this the null phenotype and designated the individuals BALB/cJPt-MUP-null (Fig. 1D). A breeding stock of BALB/cJPt-MUP-null mice has been established, which we designated BALB/cJPt family 2. Examination of 27 family 2 males which spanned four generations that were all null type indicated that family 2 is breeding true for the null phenotype. The total quantity of MUP excreted by MUP-null

TABLE 1. Quantitation of MUP excretion

Strain	Sex	No. of mice	Mean relative MUP concn[a]	95% confidence limits (upper, lower)
BALB/cAn	M	11	2.80*	4.09, 1.92
BALB/cJPt family 1	M	9	3.50*	5.31, 2.31
BALB/cJPt family 2	M	9	0.97†	1.48, 0.64
BALB/cAn	F	11	1.23†	1.79, 0.81
BALB/cJPt[b]	F	16	0.20‡	0.27, 0.15

[a] Data are relative absorbance units, obtained by dividing total absorbance units for an individual MUP sample on an IEF gel by total absorbance units of a prepared 1-mg/ml protein sample run on the same gel. Means with different superscripts are significantly different ($P < 0.05$) by the T' test for multiple, unplanned comparisons among pairs of means performed on log-transformed data (29).

[b] BALB/cJPt family 1 and family 2 were not significantly different, so data were pooled.

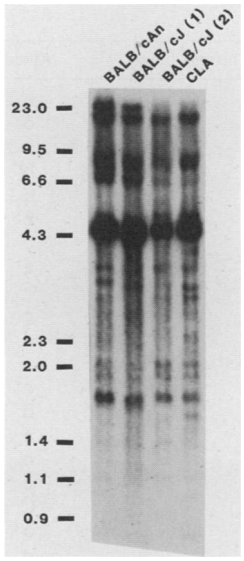

FIG. 3. MUP Southern blot. Kidney DNA from an individual of the strain listed above each lane was digested with *Eco*RI, electrophoresed in an agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized with an 850-bp insert containing the p499 MUP cDNA clone (20). Standards indicate the size (in kilobases) of fragments of *Hin*dIII-digested lambda DNA (23 to 2 kb) and *Hae*III-digested ϕX174 DNA (1.4 to 0.9 kb). BALB/cJ(1) and BALB/cJ(2) indicate BALB/cJPt family 1 and family 2, respectively.

males was significantly lower than the quantity excreted by BALB/cAn or MUP-4.1^{lo} males (Table 1). This difference may correspond to the difference in MUP excretion observed with cellulose-acetate electrophoresis between BALB/cJ and BALB/cBy mice (28).

BALB/cJPt females from both family 1 and family 2 excreted very little MUP at all, far less than BALB/cAn females (Fig. 1E and Table 1). To visualize IEF bands in females, the urine was concentrated. In eight BALB/cJPt females tested from within family 1, band 4.1 was expressed but there was considerable variation in band intensity. Twenty-two females from BALB/cJPt family 2 spanning four generations were screened. Three patterns of MUP expression were identified in these females: three individuals were like the MUP-4.1^{lo} females and expressed all the bands seen in BALB/cAn females. Nine individuals only expressed bands 7, similar to the null phenotype, and 10 mice expressed bands 2.0 and 7 but not 4.1. Thus, BALB/cJPt family 2 females consistently showed a striking reduction in total urinary MUPs, concentrated samples showed considerable variability in the bands expressed, and the phenotype did not differ consistently from that of the BALB/cJPt family 1 females.

RFLPs in MUP structural genes on chromosome 4. To evaluate the genetic context of the new MUP phenotypes described, we examined the DNA directly from three individual BALB/cAn, six BALB/cJPt family 1, six BALB/cJPt family 2, and six CLA mice. We used a liver cDNA probe which cross-hybridizes with many MUP structural genes to label Southern blots of DNA from each of the types. The multiple genes of the MUP family were cut into a variety of RF sizes, resulting in a complex pattern of 10 to 15 bands. Digestion with the restriction enzyme *Eco*RI, *Stu*I, *Sac*I, or *Eco*RV generated RF patterns which distinguished BALB/cJPt family 2 from both BALB/cJPt family 1 and BALB/cAn

TABLE 2. Loci and alleles studied

Locus	Chromosome	Allele	Strain of origin	Description
IDH-1	1	a	BALB/cJ	Isozyme polymorphism
		b	CLA	Isozyme polymorphism
Car-2	3	a	CLA	Isozyme polymorphism
		b	BALB/cJ	Isozyme polymorphism
Mup-1	4	a	BALB/cAn, CLA	Prominent IEF bands 2.0 and 2.1, absence of band 3
		b	C57BL/6	Absence of IEF band 2.1, prominent band 3
		c	BALB/cJPt family 2	Absence of bands 2.0, 2.1, 2.8, and 4.1
MUP RFLP	4	a	BALB/cAn	MUP cDNA probe hybridizable
			BALB/cJPt family 1	23-kb *Eco*RI fragment present
		c	BALB/cJPt family 2	Absence of 23-kb *Eco*RI fragment
		d	CLA	Absence of 23-kb *Eco*RI fragment, unique 1.6-kb *Eco*RI fragment present
Brown	4	b	BALB/cJ	Brown coat color
		B	CLA	Black coat color
Gus	5	a	BALB/cJ	Isozyme polymorphism
		b	CLA	Isozyme polymorphism
Color	7	c	BALB/cJ	Albino coat color
		C	CLA	Coat color expression
Es-3	11	a	BALB/cJ	Polymorphic isozyme
		c	CLA	Polymorphic isozyme
Myc-1	15	a	BALB/cJ	2.3-kb band with c-*myc* exon 1 probe *Taq*I RFLP
		b	CLA	3.3-kb band with c-*myc* exon 1 probe *Taq*I RFLP
Ly-6	15	a	BALB/cJ	RFLP with multiple unique *Xba*I fragments
		b	CLA	RFLP with multiple unique *Xba*I fragments
Sis	15	a	CLA	1.3-kb band with v-*sis* *Taq*I RFLP
		b	BALB/cJ	1.0-kb band with v-*sis* *Taq*I RFLP
Gdc-1	15	b	CLA	3.8-kb band with *gdc-1* *Pst*I RFLP
		c	BALB/cJ	3.3-kb band with *gdc-1* *Pst*I RFLP

mice (Fig. 3 and the MUP RFLP in Table 2). BALB/cAn and BALB/cJPt family 1 mice had a 23-kb *Eco*RI band which was absent in BALB/cJPt family 2. DNA from BALB/cAn, BALB/cJPt family 1, and the remaining six BALB/c sublines was digested with a variety of restriction enzymes, but no differences were found. *Eco*RI digestion of CLA DNA revealed an RF pattern which distinguished CLA from all other mice of the *Mup-1*^{a} group. The unique *Eco*RI RF pattern in CLA mice was characterized by the absence of a 23-kb *Eco*RI band and the presence of a 1.6-kb band.

The change in DNA sequence which results in the change in the RF patterns may be due to changes in the exons, introns, or flanking regions of a MUP gene. With available evidence it is not possible to conclude that the DNA differences determine the different MUP phenotypes. However, since it has been demonstrated that all the DNA sequences hybridizing with a MUP cDNA probe are on chromosome 4 (2, 3), the RFLPs provide a marker to indicate the strain or substrain of origin of an individual mouse's MUP structural genes on chromosome 4.

Genetic crosses. To test whether the genes determining the MUP-4.1^{lo} phenotype were linked to the MUP structural genes, CLA mice (MUP-4.1^{hi}) were crossed with BALB/cJPt family 1. F_1 offspring were all MUP-4.1^{hi}, but among the F_2 generation 124 males expressed the MUP-4.1^{hi} trait and 37 males expressed the MUP-4.1^{lo} phenotype. The observed ratio was not significantly different from the 3:1 ratio ($0.1 < P < 0.5$ in a chi-squared test) expected for a single locus with MUP-4.1^{lo} being the recessive phenotype. DNA was extracted from a sample of F_2 individuals and analyzed on Southern blots. Table 3 shows the distribution of MUP RFLP genotypes among mice expressing the 4.1^{lo} MUP phenotype. The data clearly indicate that the gene determining the 4.1^{lo} trait was not linked to the MUP structural genes, as 20 of 29 MUP-4.1^{lo} phenotype males, which must be homozygous for a BALB/cJ allele to express this trait, carried one or two copies of the CLA MUP RFLP haplotype.

To determine the chromosomal location of the gene responsible for the MUP-4.1^{lo} trait, we tested 10 additional loci known to be polymorphic between BALB/cJ and CLA mice on eight different chromosomes (Table 2). (CLA × BALB/cJ)F_2 individuals which are of the MUP-4.1^{lo} phenotype should be homozygous for BALB/cJ alleles at the locus which determines this trait. Therefore, in individuals with

TABLE 3. Observed number of mice of each genotype at 11 marker loci for (BALB/cJPt × CLA)F_2 males that express the MUP-4.1^{lo} phenotype

Locus	Chromosome	Observed genotypic frequency[a]			Fit with expected value (χ^2)[b]
		J/J	J/C	C/C	
Idh-1	1	7	11	5	0.11
Car-2	3	4	12	5	0.21
MUP RFLP	4	9	19	1	5.88
Brown[c]	4	7	26		0.09
Gus	5	3	14	6	1.24
Color[c]	7	2	33		5.95*
Es-3	11	5	11	7	0.11
Gdc-1	15	20	9	3	21.70**
Sis	15	19	6	0	32.18**
Ly-6	15	20	5	0	37.30**
Myc-1	15	30	3	0	72.26**

[a] J, BALB/cJPt allele; C, CLA allele.

[b] Values were determined for fit with a genotypic ratio of 1:2:1 where heterozygotes were observed and 3:1 where dominance was observed. The value was adjusted for small sample size (29). Significance: *, $P < 0.05$; **, $P < 0.001$.

[c] For loci which show dominance, only the recessive homozygote is listed; the other frequency is that of the dominant phenotype.

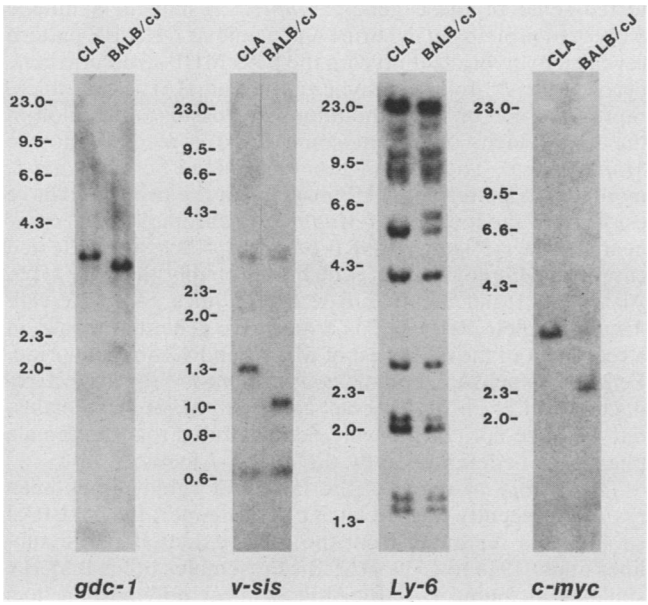

FIG. 4. Chromosome 15 RFLPs between CLA and BALB/cJ mice. Genomic DNA from CLA and BALB/cJPt mice was digested and subsequently hybridized with the probes indicated. The restriction enzyme used for each probe was as follows: *gdc-1* probe, *Pst*I; v-*sis* probe, *Taq*I; *Ly-6* probe, *Xba*I; c-*myc* probe, *Taq*I. Sizes are indicated in kilobases.

this phenotype, the genotypic frequencies expected at a second locus would be in a 1:2:1 ratio if the second locus assorts independently or 3:1 if one allele is dominant and independent assortment is maintained. The genotype at the 10 additional marker loci was determined for as many MUP-4.1^{lo} (CLA × BALB/cJPt)F_2 mice as possible, and the frequencies were tested against those expected (Table 3). Six of the 11 loci tested (*Idh-1*, *Car-2*, MUP-RFLP, Brown, *Gus*, and *Es-3*) showed independent assortment from the gene determining the MUP-4.1^{lo} trait. The lack of F_2 mice with the C/C genotype at the MUP-RFLP (Table 3) made the genotypic frequencies deviate somewhat from the expected, but the BALB/cJ allele at the MUP RFLP did not segregate with the MUP-4.1^{lo} trait significantly. These results suggest that a gene determining MUP-4.1^{lo} is not on chromosome 1, 3, 4, 5, or 11. The four loci located on chromosome 15 showed highly significant linkage with the MUP-4.1^{lo} trait, suggesting that a gene which determines that trait is located on chromosome 15. One other locus, Color, deviated from the expected genotypic ratios (Table 3). The deviation at the Color locus resulted from a deficiency of BALB/cJ homozygotes carrying *c/c* (albino) on chromosome 7, which suggests differential viability of this genotype or some affinity between the BALB/cJ chromosome bearing the locus for MUP-4.1^{lo} and the CLA chromosome 7. In either case, linkage of the gene which determines MUP-4.1^{lo} to chromosome 7 is not indicated, as the predominant phenotype was a nonparental combination for these two loci. Considering the data from all 11 loci, the most likely position of a gene determining the MUP-4.1^{lo} trait is chromosome 15.

We have named the gene controlling the MUP-4.1^{lo} phenotype MUP modifier 1 (*Mupm-1*) because its chromosomal location requires that it act at a distance to modify the pattern of MUPs which appear in the urine. The wild-type or common allele of this gene is *Mupm-1*^{a}, and the BALB/cJPt variant is *Mupm-1*^{b}.

TABLE 4. Recombination frequencies between *Mupm-1* and four chromosome 15 marker loci[a]

Marker locus	Frequency of recombination with *Mupm-1* ± SE	No. of haplotypes observed
Myc-1	0.05 ± 0.027	66
Ly-6	0.10 ± 0.042	50
Sis	0.12 ± 0.046	50
Gdc-1	0.22 ± 0.052	64

[a] The recombination frequencies between these four loci were published in Huppi et al. (15).

To determine a more precise map location of *Mupm-1*, the frequency of recombination with four chromosome 15 loci was determined (Fig. 4 and Table 4). These recombination frequencies, along with other chromosome 15 mapping studies (13, 15, 21, 23), suggest the gene order centromere–*Mupm-1*–*Myc-1*–*Ly-6*–*Sis*–*Gdc-1*–telomere.

To test the genetic basis of the MUP-null trait, BALB/cJPt family 1 mice were crossed with BALB/cJPt family 2. Since the MUP pattern of family 1 females cannot be distinguished from that of family 2 females, only the male offspring were followed in the cross. Of 31 F_1 males examined, all expressed the MUP-4.1^{lo} phenotype of family 1. This phenotype in the F_1 males can be explained by one of two hypotheses. MUP-null could be determined by a third allele at the *Mupm-1* locus which is recessive to *Mupm-1*^{b}. A second hypothesis is that the BALB/cJPt family 2 mice are homozygous *Mupm-1*$^{b/b}$, as are the BALB/cJPt family 1 mice, and the null phenotype of the family 2 males is determined by a recessive allele at a second locus. Either hypothesis could account for the F_1 results. Among the F_2 males, the two parental phenotypes were expressed: 33 individuals were MUP-4.1^{lo} type and nine individuals were MUP-null type. The phenotypic ratio is consistent with a recessive allele at a single locus determining the null phenotype, but whether that locus is *Mupm-1* cannot be determined from these data.

The chromosomal linkage of the gene determining the null phenotype could confirm whether it is a separate locus from *Mupm-1*. To investigate linkage, DNA was extracted from the kidneys of a sample of F_2 offspring from the BALB/cJPt family 1 × BALB/cJPt family 2 cross. Individuals were tested for their MUP RFLP on Southern blots. Of four null phenotype individuals tested, all four were homozygous for the MUP RF pattern observed in the BALB/cJPt family 2 parents. Of the 13 MUP-4.1^{lo} individuals tested, all carried the MUP RF pattern of the BALB/cJPt family 1 parents (the RF pattern of heterozygotes is indistinguishable from the family 1 RF pattern).

These F_2 males showed complete coincidence of the MUP-null phenotype and the MUP RF pattern of the parental stock, which expresses the null phenotype. This correspondence suggests that the DNA sequence determining the null trait is linked to the MUP structural genes on chromosome 4 and is not a new allele at the *Mupm-1* locus on chromosome 15.

To test whether there is interaction between the *Mupm-1* locus and the sequence determining the null trait on chromosome 4, MUP-null mice must be bred with a strain which differs in the chromosome 4 region and at the *Mupm-1* locus. This was achieved by crossing BALB/cJPt family 2 with BALB/cAn mice. All of the F_1 offspring (10 males and 12 females) expressed the BALB/cAn phenotype, indicating that it is dominant. The hypothesis that the MUP pattern is

controlled by the locus on chromosome 4 and the locus on chromosome 15 and that BALB/cAn and BALB/cJPt family 2 mice have alternate alleles at both loci predicts the four phenotypic classes of a dihybrid cross in F_2. The double dominant phenotype would be identical to BALB/cAn (4.1^{hi}, Fig. 1A). One single recessive phenotype would be homozygous for the *Mupm-1* locus and therefore express the BALB/cJPt family 1 pattern (4.1^{lo}, Fig. 1C). The second single recessive phenotype would be homozygous for the BALB/cJPt family 2 chromosome 4 and dominant at the *Mupm-1* locus. The phenotype of these individuals cannot be predicted because this genetic combination has not been seen prior to this cross. The double recessive class would have the genotype of the BALB/cJPt family 2 and should excrete the MUP-null pattern (Fig. 1D). Four phenotypes were in fact observed in the F_2 males. The three predicted phenotypes and a novel phenotype were represented in the expected proportions. The novel phenotype was lacking MUP band 2.1, showed variable expression of band 4.1, and otherwise resembled that of BALB/cAn mice.

Further confirmation of the hypothesis was obtained by testing a sample of F_2 individuals for their MUP RFLP. All of the 4.1^{hi} males tested carried the MUP RFLP of BALB/cAn. We did not type the 4.1^{lo} 2.1^{+} individuals, but all of the novel-phenotype and the null-phenotype individuals were homozygous for the BALB/cJPt family 2 RFLP. Thus, the RFLP data support the hypothesis that a locus on chromosome 4 and a second locus on another chromosome together determine the pattern of MUP expression in the urine.

Among the (BALB/cAn × BALB/cJPt family 2) F_2 females, only two phenotypes were clearly distinguishable: 20 individuals excreted the higher total quantity of MUP and showed a prominent band 4.1 as in BALB/cAn females, and 10 individuals excreted the low quantity of all MUPs characteristic of BALB/cJPt females. This phenotypic ratio is consistent with a single locus at which the BALB/cJ allele is recessive, but we were unable to establish the genetic basis of this phenotype.

There are many levels at which the mutation on chromosome 4 could affect the excretion of MUPs in male urine, requiring further investigation. A deletion of MUP structural genes is suggested by the absence of excreted proteins coinciding with the absence of DNA fragments revealed in the MUP RF pattern. MUP RF patterns of BALB/cJPt family 2 and BALB/cAn mice on the same autoradiogram were scanned, and the same filters were rehybridized with a *gdc-1* probe and scanned to standardize the quantity of DNA present. However, the results were too variable to conclusively demonstrate deletion of MUP genes in the BALB/cJPt family 2 mice. Additionally, a deletion is hard to reconcile with the fact that females of the same breeding stock were able to excrete all the MUPs of $Mup\text{-}1^{a}$ strains, though at a very low level. The MUP-null trait is equivalent to other alleles at the *Mup-1* locus in that it determines a novel MUP protein pattern and is tightly linked to the MUP structural genes. Considering the current understanding of the complex MUP locus at the molecular level, the genetic differences assigned to the *Mup-1* locus may not be alleles in the classical sense. Therefore, we suggest that MUP-null is a third haplotype in the MUP complex, $Mup\text{-}1^{c}$.

DISCUSSION

We have found two novel allelomorphic genes in BALB/cJPt mice that affect MUP excretion. Both of these genes differ from their counterparts in all other BALB/c sublines tested. One of these genes, $Mup\text{-}1^{c}$, results in a unique pattern of proteins in the urine and a unique DNA RF pattern revealed by hybridization with the p499 MUP structural gene probe. $Mup\text{-}1^{c}$, found in some but not all BALB/cJPt mice, represents one or more mutations or possibly a deletion in the *Mup-1* locus on chromosome 4 and is recessive to the $Mup\text{-}1^{a}$ allele. The second gene designated here, *Mupm-1*, modifies the pattern of MUP proteins in the urine. We have established the location of *Mupm-1* on chromosome 15 very near the *Myc-1* locus. BALB/cAn and other strains tested carry the $Mupm\text{-}1^{a}$ allele, which is probably the wild type. All family 1 and family 2 BALB/cJPt mice carry the rare $Mupm\text{-}1^{b}$ allele. $Mupm\text{-}1^{b}$ is a recessive gene that results in a reduction in the excretion of MUP band 4.1 in male urine. Females which are homozygous $Mupm\text{-}1^{b/b}$ excrete a reduced level of all MUPs compared with $Mupm\text{-}1^{a}$ females, but we have not conclusively demonstrated that the female phenotype is determined by the *Mupm-1* locus.

The history of the BALB/c family of sublines has been reviewed recently (25). Relevant to this paper, the BALB/cJ subline was separated from the other extant BALB/c sublines about 1938 to 1939. BALB/cJ resembles other BALB/c sublines including BALB/cAnPt at most polymorphic loci tested (28). The current breeding stock of the BALB/cJPt strain carries several novel mutations that distinguish BALB/cJ from other BALB/c inbred lines. The $Raf\text{-}1^{b}$ (or $Afr\text{-}1^{b}$) allele in BALB/cJ is associated with very high serum levels of alpha-fetoprotein (24). High levels of three enzymes in catecholamine biosynthesis, tyrosine hydroxylase, dopamine β-hydroxylase, and phenylethanolamine *N*-methyl transferase in the adrenal glands (4), are unique to the Jackson substrain. BALB/cJ mice also express other quantitative differences in liver enzymes (18). Pt19, a variable tandem repeat DNA sequence probe, hybridizes to multiple RFs representing homologous sequences throughout the genome. Some of the fragments are found in both BALB/cAn and BALB/cJ mice; some fragments found in BALB/cAn are not present in BALB/cJ mice (16). Other more complex phenotypes which distinguish BALB/cAn and BALB/cJ include differential susceptibility to diabetes induction (22) and to pristane-induced plasmacytomas (26). Genetic differences that distinguish BALB/cJ from other BALB/c sublines could be due to segregating genes that were not made homozygous until after many of the sublines were split off or mutations which occurred in separate sublines. It would appear that the BALB/cJ subline has accumulated a much larger number of mutations than other BALB/c sublines. However, it has been suggested that the multiple BALB/cJ phenotypic differences may be related to a single mutational event in a gene that regulates the transcription of many different genes (18). Our chromosomal positioning of *Mupm-1* bears on this discussion in that its map location is very close to *Afr-1* (E. Blankenhorn, R. Duncan, K. Huppi, and M. Potter, submitted for publication). Further studies will be required to determine whether *Mupm-1* and *Afr-1* are the same or two distinct loci, thereby either supporting or weakening the hypothesis of a single mutation of pleiotropic effect. A single mutation cannot account for both the new mutations we have shown here, $Mup\text{-}1^{c}$, and the *Mupm-1*/*Afr-1* complex which distinguishes BALB/cJ from all other BALB/c sublines, because they map to chromosomes 4 and 15, respectively. These additional genetic differences between BALB/cJ and all other BALB/c sublines suggest that BALB/cJ mice are more divergent from a common BALB/c ancestor than the other sublines.

The product of the *Mupm-1* gene has not been established.

Several hypotheses can be proposed about its function. First, the product of the *Mupm-1* gene could act as a regulator of MUP gene transcription. In males the *Mupm-1*^{b} allele may produce a transcription factor which fails to activate a gene; the other genes are apparently activated to normal levels. In females, the *Mupm-1*^{b} transcription factor may fail to activate fully all MUP genes, or other loci may be involved.

The suggested reduction in testosterone sensitivity of BALB/cJ males (28) may be responsible for reduced excretions of band 4.1. However, band 4.2 was excreted in large amounts in BALB/cAn males but not at all in BALB/cAn females (Fig. 1), suggesting testosterone induction of this band. Band 4.2 is also excreted in large amounts in all BALB/cJ males, suggesting that testosterone induction of MUP genes is not inhibited in BALB/cJ. Consequently, the band 4.1 MUP gene(s) appears to require specific regulators for maximal transcription, and we currently favor the interpretation that the product of the *Mupm-1* gene supplies this *trans*-acting regulator.

A second hypothesis is that the product of *Mupm-1* acts at a posttranscriptional level, either modifying the mRNA, making it more unstable, inhibiting translation of the MUP-4.1 mRNA, modifying the 4.1 protein, or preventing its arrival in the urine at some point. Glycosylation or noncovalent binding of a ligand are not likely to be posttranscriptional modifications which account for the 4.1^{lo} phenotype. Neuraminidase digestion of total urinary MUPs does not affect the quantity or pI of band 4.1 in urine from BALB/cAn or BALB/cJPt. Extensive dialysis of total urinary MUP against 8 M urea and subsequent return to deionized water does not change the relative quantity or pI of band 4.1 in urine from BALB/cAn or BALB/cJPt mice (data not shown). Further investigation will be necessary to rule out other posttranscriptional mechanisms before it will be possible to conclude that *Mupm-1* regulates the expression of specific MUP genes by synthesis of a *trans*-acting regulatory factor.

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