Structural Genes of the Mouse Major Urinary Protein Are on Chromosome 4

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ABSTRACT The major urinary proteins (MUPs) of mouse are a family of at least three major proteins which are synthesized in the liver of all strains of mice. The relative levels of synthesis of these proteins with respect to each other in the presence of testosterone is regulated by the Mup-a locus located on chromosome 4. In an effort to determine the mechanism of this regulation in molecular terms, a cDNA clone containing most of the coding region of a MUP protein has been isolated and identified by partial DNA sequence analysis. Using a combination of hybridization analysis and somatic cell genetics, the structural gene family has been unambiguously mapped to mouse chromosome 4. These data suggest that Mup-a regulation operates in a *cis* fashion and that models proposing *trans* regulation of MUP protein synthesis are unlikely.

The differential expression of members of a multigene family in the course of the life cycle of an organism is a well known phenomenon, and describing the regulation of this expression in molecular terms is a popular topic in eucaryotic developmental biology. One widely used model for such regulation proposes the existence of a constant family of structural genes, dependent upon a regulatory gene (or genes) that modulates expression of the various members of the family at the appropriate points in development. Polymorphisms in the regulatory gene would further cause heritable variations in the expression of the family even though the structural genes themselves were unchanged. Such a model has been discussed for the expression of the family even though the structural genes themselves were unchanged. Such a model has been discussed for the expression of H-2 and TL surface antigens on normal and neoplastic mouse tissues (1), and for the expression of immunoglobulin allotype markers in rabbits and mice (17). The model might also be applied to the regulation of synthesis of the group of major urinary proteins (MUPs) in mice (15, 16). However regulatory elements of gene families in mammalian cells have not been identified as independent genetic loci separable from the gene families they might control as have such loci in yeast, for example (2).

The MUPs are a series of at least three closely related electrophoretically separable proteins, MUP1, 2, and 3 (6, 7, 12), synthesized in large amounts in the liver of males and testosterone-treated females, secreted into the blood, and excreted with the urine. All inbred strains of mice tested can produce all three MUP proteins (15). Thus, BALB/c mice produce 80% of MUP1, barely detectable amounts of MUP2, and 20% of MUP3, whereas C57BL/6 mice produce 20% MUP1, 40% MUP2, and 40% MUP3 (14, 16). The difference in the levels of the MUP1 and 2 proteins produced by these different strains of mice has been hypothesized to be the result of an element termed Mup-a that segregates with the markers known to be on chromosome 4. If an animal produces mostly MUP1 and little MUP2 it has been described as Mup-a^1 and if it produces more MUP2 than MUP1 it is described as Mup-a^2. It however has not been possible in crosses of inbred strains to separate genetically the presumed alleles of the Mup-a locus from the "structural" MUP1 and 2 proteins. This is because no genetic assay (e.g. polymorphism of MUP-proteins) for the structural genes exists. With the advent of recombinant DNA it is possible to determine chromosome locations of genes by molecular techniques.

To determine the basis for MUP expression in different mouse strains we have begun an isolation and characterization of the mouse DNA that encodes the MUP gene family in the two strains. In this report we describe characterization of a recombinant clone containing double-stranded cDNA corresponding to the mRNA encoding one of the MUPs. Using this clone as a hybridization probe, we have investigated the MUP

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structural gene family and shown that the structural genes encoding the MUP proteins are located on the same chromosome as the putative regulatory locus.

MATERIALS AND METHODS

Liver cDNA clones were isolated, characterized, and cloned DNA prepared as previusly described (5). DNA for sequence analysis was cleaved with appropriate restriction endonucleases and labeled at the 3′ termini with cordycepin triphosphate using a kit supplied by New England Nuclear Corp. (Boston, MA). DNA sequencing was performed by the chemical method of Maxam and Gilbert (10).

Mouse hamster cell hybrids were prepared, characterized, and maintained as previously described (3, 13). DNA for mapping experiments was cleaved with EcoR1 according to supplier's instructions. Gel electrophoresis, transfer of DNA from gels to nitrocellulose, and hybridization of filters has been previously described (3, 5).

RESULTS

Characterization of the MUP cDNA Clone

Recombinant DNA clones consisting of plasmid pBR322 linked to double-stranded cDNA derived from unfractionated poly(A) containing mRNA of male adult mouse livers were constructed and screened as previously described (5). ~20% of the clones obtained in this "shotgun" cloning experiment corresponded to a highly abundant mRNA that in turn represented 5–10% of total mRNA in male mice (4). One clone of the group was selected for further analysis. The clone, p19, contained a cDNA insert of ~0.9 kB as determined by gel electrophoresis. When male and female poly(A) containing mRNAs isolated from mouse liver were resolved by gel electrophoresis on a denaturing gel, transferred to nitrocellulose and hybridized to nick-translated plasmid p19 DNA, a single diffuse mRNA band of ~0.95 kB was found to hybridize. The intensity of the band in the male was consistent with the 5- to 10-fold difference in the mRNA concentration between male and female (unpublished observations). In addition, in vitro translation of liver mRNA selected by hybridization to p19 produced at least two polypeptides that were precipitable with anti-MUP antiserum and that comigrated with authentic MUPs on SDS polyacrylamide gels (4).

Proof that the cDNA insert in clone p19 contained sequences of one of the MUP structural genes was achieved by partial sequence analysis by the Maxam and Gilbert method (10). Fig. 1 shows the partial DNA sequence obtained from one end of the cDNA insert. Only one possible open reading frame was found in the sequence of 72 nucleotides immediately following the poly(G) tail, and it corresponded exactly to amino acids 2 through 26 of mature MUP1 and MUP2 (8). The plasmid p19 insert contained nearly the entire mRNA sequence beginning near the 5′ end and continuing through and including poly(A) at the 3′ end. However, it has not yet been possible to determine which gene the plasmid corresponds to, MUP1 or MUP2.

Chromosomal Organization of MUP Genes

The close homology of the known MUP amino acid sequences (8) led us to expect that the P19 probe would cross-hybridize extensively with the other members of the MUP structural gene family. In addition, Hastie et al. (11) have used data obtained by hybridization of a partially purified MUP cDNA to mouse liver mRNA from BALB/c (Mup-a[1]) and C57BL/6 (Mup-a[2]) to argue that the sequences of MUP1, 2, and 3 are indistinguishable by standard hybridization conditions. When nick-translated p19 DNA was hybridized with a Southern blot of EcoRI digested mouse genomic DNA at least 14 clearly defined fragments larger than the mRNA were detected (lane *1*, Fig. 2). Since our probe probably cannot distinguish one MUP gene from another, this observation indicated that the MUPs are a multiple family of genes. Simple counting of the bands which hybridize to the MUP cDNA probe on an EcoRI blot of mouse genomic DNA (Fig. 2, lane *1*) shows at least 14 major bands, 6 of which surely represent 2 or more copies because they are present in greater than equimolar amounts. This sets an upper limit on the number of MUP structural genes at 15 to 20. This number is in agreement with the previously mentioned cDNA reassociation data (11) and is similar to the number of $\alpha 2\mu$ globulin genes in rat (9).

Since the putative Mup-a regulatory locus is mapped to chromosome 4 and since we had an identified cDNA clone of one of the MUP mRNA sequences we could use chromosomal mapping by molecular techniques to determine if structural and regulatory elements were on the same chromosome. Somatic cell hybrids formed between primary mouse cells and the Chinese hamster fibroblastoid cell line E36 retain a complete set of hamster chromosomes together with smaller numbers of mouse chromosomes. Different hybrid cell lines retain different sets of mouse chromosomes. By correlating the presence or absence of a mouse gene sequence, detected by the Southern blotting procedure, with the presence or absence of given mouse chromosomes in a panel of such lines, a gene can

	glu	ala	ser	ser	thr	gly	arg	asn	phe	asn
G(14)	GAA	GCT	AGT	TCT	ACG	GGA	AGG	AAC	TTT	AAT
	val	glu	lys	ile	asn	gly	glu	trp	his	thr
	GTA	GAA	AAG	ATT	AAT	GGG	GAA	TGG	CAT	ACT
	ile	ile								
	ATT	ATT								

FIGURE 1 Partial DNA Sequence of p19. The 0.9 kb cDNA insert in p19 was excised with PstI and its 3′ OH ends were labeled with P-32 cordycepin triphosphate. After cutting the insert with EcoRI, which cuts the insert once asymetrically, the two labeled insert fragments were resolved on a 1.7% agarose gel. The DNA sequence of each labeled fragment was determined using the Maxam and Gilbert (10) DNA sequencing technique.

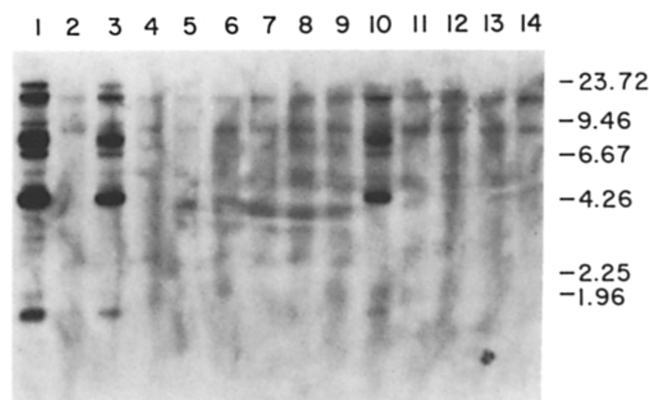

FIGURE 2 Chromosomal localization of MUP-specific DNA fragments. DNA was prepared from mouse, hamster, and somatic cell hybrid cells as described (3). 30 μg of each DNA were digested with restriction endonuclease EcoRI, then loaded onto lanes *1–14* of a 0.7% agarose gel and subjected to electrophoresis. After electrophoresis, the DNA was transferred to nitrocellulose and hybridized to nick-translated p19 DNA. Lanes *1* and *2* contain mouse and hamster DNA. Lanes *3–14*, containing DNA from hybrid cells, correspond to the columns identified in Table I. Size markers show the position of kB of HinDIII digested phage lambda DNA.

TABLE I

*Hybrid Cell Lines Tested for MUP Structural Genes**

Mouse chromosome number	BEM 1-6	BEM 1-4	MACH 7A13-3B3	4A63	4A64A1	4B31Az3	3B9C4-1	2A2	MAE28A	MAE32	ECM4e	R44-1
1	0.61	0.35	0.00	0.00	0.34	0.00	0.39	0.53	0.00	0.00	0.00	0.00
2	1.03	0.83	1.50	0.86	0.41	0.65	0.94	0.90	0.00	0.00	0.00	0.00
3	0.94	0.70	0.01	0.00	0.01	0.00	0.09	0.22	0.00	0.00	0.00	0.00
4	0.97	0.03	0.00	0.00	0.02	0.00	0.00	0.25	0.00	0.00	0.00	0.00
5	0.00	0.22	0.54	0.00	0.00	0.00	0.35	0.02	0.00	0.00	0.00	0.00
6	1.97‡	1.09	0.04	0.00	0.06	0.03	0.85	0.69	0.00	0.00	0.00	0.00
7	0.13	0.00	0.20	0.69	0.25	0.84	0.29	0.69	0.00	0.00	0.00	0.00
8	0.87	0.35	0.00	0.00	0.00	0.81	0.30	0.33	0.00	0.00	0.00	0.00
9	0.23	0.00	1.35	0.00	0.01	0.00	0.76	0.65	0.00	0.00	0.00	0.00
10	0.27	0.25	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.74	0.83	1.77	0.11	0.23	0.16	0.88	0.73	1.03‡	0.00	0.00	0.00
13	0.77	0.52	0.26	0.44	0.00	0.00	0.21	0.22	0.00	0.00	0.00	0.00
14	1.03	0.78	0.27	0.11	0.02	0.10	0.70	0.82	0.00	0.00	1.00	0.00
15	1.74	1.00	1.30	0.92	1.05	0.16	1.30	0.90	0.00	0.00	1.00	0.00
17	0.87	0.65	0.17	0.97	0.06	0.77	1.15	0.80	0.00	0.86	0.00	0.00
18	0.87	1.09	0.70	0.25	0.20	0.26	0.91	0.82	0.00	0.00	0.00	1.00‡
19	0.55	0.00	1.09	0.33	0.03	0.06	1.03	0.55	0.00	0.00	0.00	0.00
X	1.26	0.96	1.07	1.86	0.27	0.90	1.15	0.82	0.00	0.00	0.00	0.00
	1.85‡	0.48	0.00	0.00	0.02	0.00	0.00	0.41	1.03‡	0.86	0.00	0.00
Number scored	31	60	33	50	101	31	50	50	31	100	50	18
MUP positive	+	–	–	–	–	–	–	+	–	–	–	–

* Mouse chromosomes were identified in metaphase spreads subjected to sequential Giemsa-Viokase-Hoechst "33258" staining technique. The number shown is the mean number of copies of the chromosome per cell.

‡ Includes copies of the chromosome occuring in the form of translocations.

be mapped to a chromosome. A panel of cell lines that allows the unambiguous mapping of mouse DNA sequences to almost all mouse chromosomes has been described previously (3, 13). When we tested this panel for the presence of the MUP gene family, we found a perfect correlation between the presence of MUP DNA fragments on a Southern blot and the presence of chromosome 4 (Fig. 2). Note that the pattern of mouse DNA fragments complementary to MUP cDNA in hybrid cells containing chromosome 4 was very similar to that found in total mouse DNA, suggesting that this group of DNA sequences was not altered in the course of its passage through the somatic cell hybrids. Thus we can assign most or perhaps all of the MUP structural genes to chromosome 4.

DISCUSSION

Szoka and Paigen (16), using recombinant inbred strains, have shown that the type of regulation governed by the Mup-a locus is a variation in the relative levels of production of the MUP proteins in the presence of testosterone. Recombinant inbred strains displayed only parental levels of MUP1 or MUP2 in the presence of testosterone. This argues but does not prove that the Mup-a locus is a single genetic locus. Any model to explain the MUP phenotype must account for the fact that regulation of MUP1 is difficult to segregate from regulation of MUP2.

Several models can explain the MUP phenotype. In one the Mup-a gene is physically separate from the structural locus and produces a product which acts in *trans* to regulate an array of structural genes. Polymorphisms in the Mup-a gene product could then evoke the observed heritable MUP phenotype. The Mup-a locus is on chromosome 4. Had the structural MUP genes been on another chromosome, then this model would be favored. It is, of course, possible that although the Mup-a and structural loci map to the same chromosome the Mup-a locus is still a *trans* acting regulatory locus. However, since the putative regulatory element is on the same chromosome as most, if not, all the structural genes, there need be no separate, controlling locus. The reported differences in MUP gene expression might simply be dependent on a polymorphism in the DNA structure at the hormone binding site or promotors of MUP1 and MUP2 genes.

The first step to test any of these models is to score whether MUP1 and MUP2 are controlled differently at the levels of transcription. It is already known that the entire set of MUP genes is controlled at the level of transcription from tissue to tissue in adults (4, 5). The aim of present work is to obtain genomic clones that distinguish between MUP1 and MUP2 mRNA sequences and score for transcriptional rates in different strains. This will be followed by sequence studies of MUP1 and MUP2 in the two strains.

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Note in Added Proof: While this manuscript was in review, Bennett et al. (*Proc. Natl. Acad. Sci. U. S. A.* 79:1220–1224) reported the mapping of the MUP structural genes to chromosome 4 using a different set of somatic cell hybrids and recombinant inbred strains.

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