Developmental Expression, Cellular Localization, and Testosterone Regulation of \( \alpha_1 \)-Antitrypsin in \textit{Mus caroli} Kidney

Jean J. Latimer\(^\ddagger\), Franklin G. Berger\(^\S\), and Heinz Baumann\(^\¶\)

Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263

\(^\ddagger\)Department of Biology, University of South Carolina, Columbia, South Carolina 29208

Abstract

\( \alpha_1 \)-Antitrypsin (\( \alpha_1 \)-protease inhibitor), an essential plasma protein, is synthesized predominantly in the liver of all mammals. We have previously shown that \textit{Mus caroli}, a Southeast Asian mouse species is exceptional in that it expresses abundantly \( \alpha_1 \)-antitrypsin mRNA and polypeptide, in the kidney as well as the liver (Berger, F. G., and Baumann, H. (1985) \textit{J. Biol. Chem.} 260, 1160–1165) providing a unique model for examination of the evolution of genetic determinants of tissue-specific gene expression. In the present paper, we have further characterized \( \alpha_1 \)-antitrypsin expression in \textit{M. caroli}. The extrahepatic expression of \( \alpha_1 \)-antitrypsin is limited to the kidney, specifically within a subset of the proximal tubule cells. The developmental pattern of \( \alpha_1 \)-antitrypsin mRNA expression in the kidney differs from that in the liver. In the kidney, \( \alpha_1 \)-antitrypsin mRNA is present at only 2–4\% adult level at birth and increases very rapidly to adult level during puberty between 26 and 36 days of age. There are no significant changes in liver \( \alpha_1 \)-antitrypsin mRNA levels during this period. Testosterone, while having only modest affects on \( \alpha_1 \)-antitrypsin mRNA accumulation in the adult kidney, causes a 20-fold induction of the mRNA in the pre-pubertal kidney. This suggests that the increase in \( \alpha_1 \)-antitrypsin mRNA expression during puberty is testosterone mediated. Southern blot analyses of \textit{Mus domesticus} and \textit{M. caroli} genomic DNA and a cloned \textit{M. caroli} \( \alpha_1 \)-antitrypsin genomic sequence, indicate that a single \( \alpha_1 \)-antitrypsin gene exists in \textit{M. caroli}, whereas multiple copies exist in \textit{M. domesticus}. These data show that the alteration in tissue specificity of \( \alpha_1 \)-antitrypsin mRNA accumulation that has occurred during \textit{Mus} evolution is associated with distinctive developmental and hormonally regulated expression patterns.

\( \alpha_1 \)-Antitrypsin (or \( \alpha_1 \)-protease inhibitor) is a serum glycoprotein (\( M_r = 55,000 \)) (1) that is synthesized primarily in the liver, and in limited amounts in human macrophages (2). It is present at high concentrations in human plasma (2.5 mg/ml) and functions in the nonenzymatic inactivation of neutrophil elastase (3). The essential nature of this function is shown by the existence of the human genetic disease, \( \alpha_1 \)-antitrypsin deficiency, which is

\(^\ddagger\)This work was supported by Grant AM33886 from the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases.

\(^\S\)Performed this work as partial fulfillment of a Ph.D. thesis.

\(^\¶\)Supported by an Established Investigator Award from the American Heart Association.
characterized by reduced levels of functional $\alpha_1$-antitrypsin in the circulation and results in the development of chronic obstructive pulmonary emphysema (4).

We have previously demonstrated that the wild derived mouse species, Mus caroli, exhibits an unusual $\alpha_1$-antitrypsin tissue specificity (5). While M. caroli produces $\alpha_1$-antitrypsin mRNA at normal levels in the liver (approximately 2000–4000 mRNA copies/cell), it also expresses the mRNA in the kidney, at levels ranging from 2000 to 6000 copies/cell. Although the $\alpha_1$-antitrypsin mRNA species from the kidney and liver encode a similar polypeptide, the mature proteins differ in their extents of secondary glycosylation (5). Liver $\alpha_1$-antitrypsin is secreted normally into the serum, whereas kidney $\alpha_1$-antitrypsin is excreted into the urine. Both the kidney and liver $\alpha_1$-antitrypsin are functional in vitro. A cis-acting genetic determinant has been postulated to be responsible for the interspecies difference in $\alpha_1$-antitrypsin tissue specificity (5).

The abundant expression of $\alpha_1$-antitrypsin in M. caroli kidney suggests that during the evolution of this mouse species, fixation of a genetic determinant which modified the tissue specificity of $\alpha_1$-antitrypsin expression occurred. In the present paper, we describe tissue-specificity and cellular localization of $\alpha_1$-antitrypsin mRNA within the kidney. In addition, we compare the developmental accumulation of $\alpha_1$-antitrypsin in liver and kidney and demonstrate that testosterone may be an important effector of expression in the kidney.

**EXPERIMENTAL PROCEDURES**

**Animals**

Inbred strains of Mus domesticus (strains C57BL/6J, BALB/c, DBA/2J) and random-bred M. caroli mice were obtained from the colonies of Dr. Verne Chapman of Roswell Park Memorial Institute. Male and female M. caroli, ranging in age from 1 day to 72 days postpartum, were utilized for the experiments presented in this report. Collection of organs was performed at approximately 10:00 a.m.

Testosterone was administered by subcutaneous implantation of 30-mg pellets at the nape of the neck of 20-day-old animals, and 15-mg pellets in 10-day-old animals. The length of treatment time was either 10 or 20 days. The presence of a residual testosterone pellet was verified at the time each animal was killed. Castrations were performed via midventral laparotomy, and ovariectomies were performed by dorsal laparotomy.

**Nucleic Acid Probes**

The following plasmids were used as probes in this study: p1796, a 900-bp$^1$ M. domesticus cDNA, which contains exons 2–5 of the BALB/c $\alpha_1$-antitrypsin gene (6, 7); p199 which contains a 424-bp cDNA complementary to one of the M. domesticus major urinary protein (MUP) mRNAs which is predominantly expressed in M. caroli (8); p7 which contains approximately 600 bp of the 28 S rRNA gene (9); pSlim which contains 911 bp of the murine renin-2 gene (10).

---

$^1$The abbreviations used are: bp, base pair; MUP, major urinary proteins; kb, kilobase pair.
p199 and p7 were labeled with \[^{32}P\]dATP and \[^{32}P\]dCTP by nicked translation of the entire plasmid (11). A 630-bp PstI fragment of the p1796 insert was isolated and labeled with \[^{32}P\]dCTP by the random priming reaction (12). Single stranded RNA probes labeled with either \[^{35}S\]uridine 5′-(thio)triphosphate or \[^{32}P\]rUTP were transcribed from the p1796 and pSlim inserts after subcloning them into Sp65 (13).

**Analysis of RNA**

Total RNA was extracted by either the guanidine HCl procedure (14) or the guanidine isothiocyanate procedure (15). RNA concentrations were determined by absorbance at 260 nm, dot blot hybridization to \[^{32}P\]-labeled genomic DNA complementary to 28 S rRNA, or densitometric scanning of photographic negatives of ribosomal RNA separated on agarose gels and stained with ethidium bromide. For standard analysis, 15 μg of total RNA were fractionated on a 1.5% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose (16). Northern blots probed with labeled cRNA probes were hybridized in 50% formamide, 0.3 M NaCl and washed in 0.03 M NaCl at 65 °C. All other Northern blots were hybridized and washed at standard stringency (0.3 M NaCl at 65 °C).

In order to compare hybridization between and among blots, each Northern analysis contained the same standard RNA sample (C57BL/6J adult male liver RNA) which had previously been determined to contain the equivalent of 6000 α1-antitrypsin mRNA copies/liver cell (6). Hybridization was quantitated by densitometry of the autoradiograms. The linear range of signal to RNA concentration was verified by serial dilutions of the standard RNA. Densitometry units were normalized to micrograms of RNA and compared to the standard. One hundred percent *M. caroli* adult liver α1-antitrypsin mRNA was defined as the mean value of the unit values from all liver preparations (n = 54). One hundred percent adult liver MUP mRNA was defined as the mean value of all unit values derived from animals at least 40 days old (n = 13).

RNAs were prepared from three littermates for the time points earlier than 10 days due to the paucity of tissue available from individual young animals. For all other time periods, RNAs from individual animals were utilized.

**In Situ Hybridization**

The livers and kidneys of adult *M. caroli* and *M. domesticus* males were dissected and frozen in 2-methylbutane. The tissues were then embedded and sectioned at 4–8 μm with a cryostat microtome. The slices were placed on poly-L-lysine coated glass slides (17).

Fixation and prehybridization treatments of the sections were performed according to the method of Hafen *et al.* (18). The RNAs were then cross-linked by a second paraformaldehyde fixation step and the remaining proteins acetylated in 100 mM triethanolamine and 25 mM acetic anhydride. Individual slides containing six sections each were then hybridized for 18–20 h at 45 °C in hybridization solution (50% formamide, 300 mM NaCl, 5 mM EDTA, 20 mM Tris, pH 8.0, 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl-pyrrolidone, and 100 μg/ml carrier tRNA), which contained 0.5 × 10^6 cpm
of the appropriate cRNA probe. Optimal hybridization was achieved when cRNA probes were reduced to 50–200 bp by hydrolysis in 1 M sodium carbonate for 20 min at 70 °C.

After hybridization, the sections were washed in 0.6 M NaCl, 0.06 M sodium citrate at room temperature, digested with RNase, rinsed, and then washed in 0.3 M NaCl, 0.03 M sodium citrate, followed by 15 mM NaCl, 1.5 mM sodium citrate at 45 °C (17). After dehydration, the slides were processed for autoradiography. Exposure time ranged from 7 to 10 days. After development of the photographic emulsion, the sections were stained with hemotoxin and eosin and were photographed at 16 and 100 × magnification under light- and dark-field microscopy.

Analysis of Genomic DNA

A genomic α1-antitrypsin sequence was isolated from a partial Sou3A M. caroli genomic liver DNA library cloned in Charon 30 (generously provided by Dr. M. Edgell, Dept. of Microbiology, University of North Carolina). The α1-antitrypsin clone was identified on the basis of hybridization to p1796 labeled insert, as well as by restriction endonuclease analysis. (Details of the characterization of the α1-antitrypsin gene will be presented elsewhere.)

Genomic DNA was isolated from M. domesticus and M. caroli livers (19). Restriction endonucleases were used according to the instructions of the suppliers. Digested DNA was fractionated on 0.8% agarose gels, transferred to nitrocellulose filters (20), and hybridized to 32P-labeled p1796 DNA. Hybridization and washes were performed at 65 °C at standard stringency (0.3 M NaCl).

The copy number(s) of α1-antitrypsin genes in M. domesticus versus M. caroli were determined by digesting genomic M. caroli, M. domesticus, and cloned α1-antitrypsin gene DNAs with PstI. These DNAs were then serially diluted and analyzed by Southern blotting using p1796 as probe. Hybridization was quantitated by densitometry, and the relative hybridization ratios of the various hybridizing fragments was then determined.

RESULTS

Tissue Specificity of α1-Antitrypsin Expression in M. caroli

In order to determine the sites of α1-antitrypsin mRNA expression in M. caroli, total RNA from 11 male M. caroli organs was examined by Northern analysis (Fig. 1). Hybridization was performed with an α1-antitrypsin cRNA probe which greatly increased the sensitivity of the analysis, relative to conventional cDNA probes. The limit of detection of this system was less than six mRNA copies/cell. Kidney and liver RNA show a predominant α1-antitrypsin mRNA of 1.5 kb which is in full agreement with the size reported by Krauter et al. (21). Two additional bands with sizes of 3.5 and 8–10 kb are consistently present and may represent precursor forms of the major α1-antitrypsin mRNA. The other organs do not contain any hybridizing RNA at the position of the mature α1-antitrypsin mRNA, although they do contain high molecular weight RNA bands. The significance, if any, of these bands

---

2 J.J. Latimer, F. G. Berger, and H. Baumann, unpublished data.
is unknown. The results of Fig. 1 indicate that the kidney is the only site of detectable extrahepatic $\alpha_1$-antitrypsin expression among the organs tested.

**Cellular Localization of $\alpha_1$-Antitrypsin Expression in M. caroli Kidney**

The cellular localization of the $\alpha_1$-antitrypsin transcripts within *M. caroli* organs was determined by *in situ* hybridization studies with cRNA probes. Controls included *M. caroli* liver and *M. domesticus* kidney. Strong hybridization of the $\alpha_1$-antitrypsin cRNA was observed uniformly in the *M. caroli* liver sections (Fig. 2B). In *M. caroli* kidney, intense hybridization was present in the tubule cells primarily in the cortical medullary region (Fig. 2A, and B). Fewer silver grains were present within the cells of the outer cortex, while only background levels were visible in the medulla. The position of the area of strongest hybridization within the kidney suggests that $\alpha_1$-antitrypsin transcription occurs in a subset of tubule cells probably including a portion of the Loops of Henle (22). In order to demonstrate cell type-specific hybridization, sections from the same kidney were hybridized with renin cRNA (Fig. 2A). Renin transcripts have been shown to be present in the juxtaglomerular cells of inbred mouse kidney at approximately 10,000 copies/cell (10). The renin cRNA probe hybridized specifically to the juxtaglomerular cells of *M. domesticus* kidney (data not shown), although no hybridization was observed with *M. caroli* kidney (Fig. 2B).

Original estimates of $\alpha_1$-antitrypsin mRNA copy number in *M. caroli* kidney ranged from 2,000 to 6,000 copies/average cell (5). Since the tubule cells which hybridized with the $\alpha_1$-antitrypsin cRNA probe represent approximately 20–30% of all kidney cells, we conclude that $\alpha_1$-antitrypsin mRNA in producing cells must be in the range of 6,000–30,000 copies/cell.

**Developmental Expression of $\alpha_1$-Antitrypsin mRNA**

It has previously been shown that $\alpha_1$-antitrypsin mRNA is expressed prenatally in the liver of *M. domesticus* (Ha/ICR) (6). At 14 days post-conception, the $\alpha_1$-antitrypsin mRNA concentration was approximately 240 copies/cell and increased to adult levels by the time of birth. Considering these data, the question of whether $\alpha_1$-antitrypsin expression in *M. caroli* kidney and liver exhibits a similar developmental regulation was addressed.

Northern blot analysis of liver RNA from *M. caroli* males at various ages (Figs. 3 and 4) indicates no significant changes in $\alpha_1$-antitrypsin mRNA level during postnatal development. Considerable variation was observed among individuals, similar to that previously observed by others (23). Adult male *M. caroli* $\alpha_1$-antitrypsin mRNA levels are only 33% of C57BL/6J adult male levels. Female *M. caroli* show a similar pattern of expression in liver (data not shown).

In *M. caroli* kidney, the $\alpha_1$-antitrypsin mRNA concentration is low at birth and remains constant until 26 days postpartum, at which time a sharp increase to adult levels occurs (Figs. 3 and 4). Adult kidney $\alpha_1$-antitrypsin mRNA levels average 110% of liver $\alpha_1$-antitrypsin mRNA. It has been shown that expression of the MUP in *M. domesticus* is induced in liver during puberty (24). The results shown in Figs. 3 and 4 indicate that the
onset of hepatic MUP mRNA accumulation coincides with the increase in α₁-antitrypsin mRNA levels in kidney.

Females undergo a developmental increase in the kidney α₁-antitrypsin mRNA levels similar to that seen in males. Although the α₁-antitrypsin mRNA levels are similar in males and females both prior to and following puberty, the rate of increase is significantly slower in females. Maximal adult levels are not reached until 60 days postpartum (data not shown; see also Table I for relative mRNA accumulation in 40-day-old animals).

The Effect of Testosterone on α₁-Antitrypsin Expression

The large induction of kidney α₁-antitrypsin mRNA concentrations during puberty prompted experiments aimed at determining whether or not α₁-antitrypsin expression was under the control of sex hormones. We therefore performed ovariectomy or castration of 20-day-old animals to determine if sex hormone levels affect the developmental induction in α₁-antitrypsin expression observed by day 40. As shown by Table I, castration of males nearly abolishes the normal increase in renal α₁-antitrypsin mRNA. There is no effect of ovariectomy on α₁-antitrypsin expression in females. MUP induction in the liver is not significantly affected by removal of the sex organs in males or females. When animals were treated with testosterone between 20 and 40 days of age, they exhibited enhanced levels of kidney α₁-antitrypsin relative to untreated animals (Table I). No modulating influence of testosterone on the α₁-antitrypsin or MUP mRNA level in liver was observed (Table I).

These experiments suggest a role for testosterone in the regulation of α₁-antitrypsin mRNA levels in postnatal kidney. More definitive proof for this was obtained by analysis of hormone effects in pre-pubertal animals prior to the time of the normal developmental induction. Male and female mice were treated with testosterone at 10 days of age and analyzed at 20 days, before the onset of α₁-antitrypsin mRNA induction in untreated animals. The α₁-antitrypsin mRNA accumulated to adult levels as a result of this treatment (Table I). Moreover there was no induction of MUP, indicating that kidney α₁-antitrypsin and liver MUP are not regulated identically, although the increases in their respective mRNAs are temporally coincident during normal development.

It should also be noted that males castrated prior to puberty showed restoration of adult kidney α₁-antitrypsin levels following testosterone administration (data not shown).

Analysis of α₁-Antitrypsin Genomic Sequences

It has been shown that inbred strains of M. domesticus possess multiple α₁-antitrypsin genes (21); M. caroli has been postulated to have a single gene (5). In order to determine whether differential expression of two separate α₁-antitrypsin genes explains the presence of α₁-antitrypsin in M. caroli kidney and liver, Southern blot analyses of restriction endonuclease digests of M. caroli and M. domesticus total cellular DNAs, together with that of an isolated genomic clone of the M. caroli α₁-antitrypsin gene, were performed (Fig. 5). The patterns for the M. caroli α₁-antitrypsin gene(s) are consistently simpler than those for the M. domesticus genes, indicating a lower gene copy number in the latter species. Each of the bands present in the cellular DNA of M. caroli can be accounted for in the cloned M. caroli
α1-antitrypsin gene sequence (see lanes labeled: PstI, PvuII, BglI, and EcoRI). To determine the copy numbers of α1-antitrypsin genes in the M. domesticus and M. caroli genomes, total cellular DNA as well as serial dilutions of the cloned α1-antitrypsin gene DNA were digested with PstI and analyzed by Southern blotting (see Fig. 5 for pattern). Comparison of the intensities of hybridizing bands indicates that the M. caroli genome contains but a single copy of the α1-antitrypsin gene (Fig. 5). In addition, densitometric scanning of the lanes containing cellular DNA indicates that M. domesticus (BALB/c) contains a 5-fold greater level of total hybridization to the α1-antitrypsin probe relative to M. caroli. Thus, while M. domesticus contains several copies of the α1-antitrypsin gene, M. caroli contains a single copy. Further evidence for a single α1-antitrypsin gene in M. caroli lies in the independent existence of a polymorphism which affects the pI of the α1-antitrypsin polypeptide and an EcoRI restriction fragment length polymorphism located approximately 500 bp upstream of the transcriptional start site, both of which segregate as a single mendelian gene.2

The comparison of restriction endonuclease patterns shown in Fig. 5 also revealed that in the case of all the enzymes, at least one M. domesticus band comigrates with one of the M. caroli bands. This may indicate that among the multiple α1-antitrypsin genes in M. domesticus, there is at least one which is more closely related to the M. caroli gene.

DISCUSSION

The expression of α1-antitrypsin mRNA in both liver and kidney in M. caroli, as opposed to the liver-specific expression in other Mus species, makes these mice useful in the study of genetic elements that govern tissue-specific gene expression and its evolution. In the present report, we have characterized renal α1-antitrypsin mRNA accumulation in detail. In M. caroli, as well as in other species, liver α1-antitrypsin mRNA begins to accumulate prenatally and reaches adult levels at birth. In contrast, there appear to be at least two aspects to renal α1-antitrypsin gene activity. The gene is activated relatively early in kidney development, resulting in a modest, but significant mRNA level at birth. The expression is stimulated some 30-fold at puberty and is subsequently maintained.

Our experiments provide strong evidence that testosterone plays a major role in the developmental induction of α1-antitrypsin mRNA. Interestingly, females undergo the induction as well as males, with only minor differences in the kinetics and magnitude of change. Other hormones, such as estrogens, may be involved. However, this is unlikely since females that were ovariectomized prior to the induction attained normal levels of α1-antitrypsin mRNA as adults (Table I). The low circulating levels of androgens in females may be sufficient to elicit the induction. More studies are needed to further clarify this point.

In situ hybridization experiments (Fig. 1) indicate that α1-antitrypsin mRNA synthesis occurs in tubule cells of the kidney. Interestingly, proximal tubule cells are androgen target cells in mice and manifest organ-specific induction of glucuronidase, alcohol dehydrogenase, ornithine decarboxylase, and other gene products in response to testosterone (25, 26).
The role of transcriptional versus post-transcriptional mechanisms in the regulation of kidney \( \alpha_1 \)-antitrypsin expression has yet to be determined. It is likely that early activation is a transcriptional phenomenon. The puberty-specific induction, however, may result from either induced transcription of the \( \alpha_1 \)-antitrypsin gene or stabilization of the \( \alpha_1 \)-antitrypsin mRNA. Recent studies suggested that mRNA stabilization is a major factor in androgen-regulated mRNA accumulation in the kidney (27). However, preliminary measurements of \( \alpha_1 \)-antitrypsin gene transcription rates by nuclear run off assays have indicated that the presence of \( \alpha_1 \)-antitrypsin mRNA in adult \( M. caroli \) kidney is closely correlated with transcriptional activity of the \( \alpha_1 \)-antitrypsin gene (data not presented).

Earlier studies identified a cis-active element as responsible for the species-specific difference in kidney \( \alpha_1 \)-antitrypsin mRNA levels in adult animals (5). On the genetic level, two models could be invoked to explain the acquisition of \( \alpha_1 \)-antitrypsin mRNA expression in \( M. caroli \) kidney. One model proposed that at least two \( \alpha_1 \)-antitrypsin structural genes were fixed in this species; one gene is liver specific while the other is kidney specific. Precedents for multiple \( \alpha_1 \)-antitrypsin genes exists, since quantitative hybridization (6) indicates that there are 3–4 copies of the \( \alpha_1 \)-antitrypsin gene in \( M. domesticus \). Our quantitative hybridization data (Fig. 5) supports this, as does the recent cloning of two expressed \( \alpha_1 \)-antitrypsin genes from \( M. domesticus \) (21). An alternative model involves fixation of a single \( \alpha_1 \)-antitrypsin gene which is expressed both in the kidney and in the liver. Analyses described in Fig. 5 indicate that \( M. caroli \) contains a single \( \alpha_1 \)-antitrypsin gene; thus the latter model is more likely.

Southern blot analyses of \( \alpha_1 \)-antitrypsin gene fragments in \( M. domesticus \) and \( M. caroli \) have suggested that at least one of the \( \alpha_1 \)-antitrypsin genes in \( M. domesticus \) has been conserved in \( M. caroli \). Thus after separation of the two lineages, \( M. domesticus \) may have acquired additional copies of the gene. Alternatively, \( M. caroli \) may have lost copies. Examination of other \( Mus \) species may shed light on this issue. Whatever the mechanism, it is clear that the single \( M. caroli \) \( \alpha_1 \)-antitrypsin gene evolved with an altered genetic regulatory element(s) which causes a broadened tissue specificity associated with a testosterone-mediated developmental pattern. Knowledge of the structural basis of these genetic elements may provide new insights into the function of regulatory sequences in DNA that determine tissue specificity and developmental expression of a specific mammalian genes.

**Acknowledgments**

We are grateful to Drs. L. Bowmann, N. Hastie, W. Held, and K. Gross for providing the cDNA probes used in this study; Dr. V. Chapman for the use of the \( M. caroli \) mouse colony; R. McGowan and C. Kane-Haas for their excellent instruction in the use of in situ hybridization; J. Black and D. Swiatek for technical assistance; Drs. S. G. Grant and D. A. Stephenson and Karen R. Prowse for helpful comments during the preparation of this manuscript; and M. Held for secretarial assistance.

**References**

1. Travis J, Salvesen GS. Annu Rev Biochem. 1983; 52:655–709. [PubMed: 6193754]

---

3K. Krauter, personal communication.
2. Rogers J, Kalsheker N, Wallis S, Speer A, Coutelle CH, Woods D, Humphries SE. Biochem Biophys Res Commun. 1983; 116:375–382. [PubMed: 6606425]
3. Minnich M, Kueppers F, James H. Comp Biochem, Physiol B. 1984; 78:413–419. [PubMed: 6331982]
4. Ohlsson, K. Proteases and Biological Control. Reich, E.; Rifkin, D.; Shaw, E., editors. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1975. p. 591
5. Berger FG, Baumann H. J Biol Chem. 1985; 260:1160–1165. [PubMed: 2981835]
6. Barth RK, Gross KW, Gremke LC, Hastie ND. Proc Natl Acad Sci U S A. 1982; 79:500–504. [PubMed: 6952203]
7. Hill RE, Shaw PH, Boyd PA, Baumann H, Hastie ND. Nature. 1984; 311:175–177. [PubMed: 6547997]
8. Sampsell BM, Held WA. Genetics. 1985; 109:549–568. [PubMed: 3979814]
9. Bowman LH, Rabin B, Schlessinger D. Nucleic Acids Res. 1981; 9:4951–4966. [PubMed: 7312622]
10. Dickinson DP, Gross KW, Piccini N, Wilson CM. Genetics. 1984; 108:651–667. [PubMed: 6389258]
11. Rigby PW, Diekmann M, Rhodes C, Berg P. J Mol Biol. 1977; 113:237–251. [PubMed: 881736]
12. Feinberg AP, Vogelstein B. Anal Biochem. 1983; 132:6–13. [PubMed: 6312838]
13. Johnson MT, Johnson BA. Biotechniques. 1984; 2:156–162.
14. Labarca C, Paigen K. Proc Natl Acad Sci U S A. 1977; 74:4462–4465. [PubMed: 270691]
15. Chirgwin JM, Przybyla AE, Rutter WJ. Biochemistry. 1979; 18:5294–5299.
16. Shaw PH, Held WA, Hastie ND. Cell. 1983; 32:755–761. [PubMed: 6831559]
17. McGowan RA, Kane-Haas C, Gross K. Biotechniques. 1985; 4:1–8.
18. Hafen E, Levine M, Garber RL; Gehring WJ. EMBO J. 1983; 2:617–623. [PubMed: 16453446]
19. Chapman V, Forrester L, Stanford J, Hastie N, Rossant J. Nature. 1984; 307:284–286. [PubMed: 6694730]
20. Southern EM. J Mol Biol. 1975; 98:503–517. [PubMed: 1195397]
21. Krauter KS, Citron BA, Hsu MT, Powell D, Darnell JE Jr. DNA (N Y). 1986; 5:29–36.
22. Tisher, C. The Kidney. 2. Brenner, B.; Rector, F., editors. Saunders Co; Philadelphia: 1981. p. 3-29.
23. Baumann H, Latimer J, Glibetic M. Arch Biochem Biophys. 1985; 246:488–493. [PubMed: 2421641]
24. Derman E. Proc Natl Acad Sci U S A. 1981; 78:5425–5429. [PubMed: 6946481]
25. Watson CS, Salomon D, Catterall J. Ann N Y Acad Sci. 1983; 371:101–113.
26. Swank RT, Paigen K, Davey R, Chapman V, Labarca C, Watson G, Ganschow R, Brandt E, Novak E. Recent Prog Horm Res. 1978; 34:401–436. [PubMed: 366684]
27. Berger F, Loose D, Meisner H, Watson G. Biochemistry. 1986; 25:1170–1175.
Total RNA was prepared from the organs of adult *M. caroli* males, fractionated by agarose gel electrophoresis (15 μg of RNA/lane except for liver and kidney), blotted onto nitrocellulose, and hybridized to 32P-labeled α1-antitrypsin cRNA. A 4-fold dilution series of liver RNA from C57BL/6J is included in lanes 1–3 (lane 1, 0.23 μg of RNA). The lane with a dilution 1/1024 is indicative of 6 α1-antitrypsin mRNA copies/cell. The lanes with *M. caroli* kidney and liver contains 0.23 μg of RNA. The positions of RNA size markers are indicated. The exposure time of this fluorograph was 11 days.
Fig. 2. Cellular localization of α₁-antitrypsin mRNA in *M. caroli* kidney and liver

A, sections of *M. caroli* kidney were hybridized with α₁-antitrypsin and renin cRNA probes. The area of the kidney sections indicated on the illustration is photographed at 16 × magnification under dark-field (upper panel) and light-field illumination (lower panel). B, the tissue distribution of α₁-antitrypsin transcripts is determined by *in situ* hybridization of α₁-antitrypsin cRNA to *M. caroli* liver and kidney (center of cortex) and to DBA/2 kidney (center of cortex). The sections were photographed under identical conditions at 100 × magnification under dark- and light-field illumination. Arrows in the kidney section indicate the cross-section of tubules containing α₁-antitrypsin in RNA.
Fig. 3. Developmental expression of α1-antitrypsin (AT) mRNA in the kidney and liver of M. caroli

Total kidney RNA were prepared from males, at the indicated postpartum day of life, were fractionated by agarose gel electrophoresis, blotted onto nitrocellulose, and hybridized to 32p-labeled p1796 insert DNA. Total liver RNA from the same animals was simultaneously blotted and hybridized to 32p-labeled p1796 insert and p199 cDNAs. C57BL/6J standard RNA dilutions were included for the purpose of quantitation.
In several experiments, different preparations of kidney and liver RNAs from *M. caroli* males at the indicated ages, were analyzed for the relative amounts of $\alpha_1$-antitrypsin and MUP mRNA via Northern blotting using $^{32}$P-labeled p1796 or p199 as probes. The autoradiograms were densitometrically scanned and quantitated relative to the C57BL/6J standard male liver RNA and ultimately expressed as percent adult *M. caroli* liver. Each point represents an independent RNA preparation.
Fig. 5. Southern analysis of *M. caroli* and *M. domesticus* α₁-antitrypsin gene(s)

Lung DNAs isolated from C57BL/6J and *M. caroli* male mice, and the cloned *M. caroli* α₁-antitrypsin gene sequence, were digested with the indicated restriction endonucleases, fractionated by agarose gel electrophoresis, and probed with the labeled p1796 insert. The positions of DNA size markers are indicated. This fluorograph was exposed for 5 days.
**Table I**

**Effect of testosterone on α₁-antitrypsin (AT) expression**

In each experimental group, littermates were subjected to the indicated treatments. Untreated animals from each experimental group were combined to constitute the control groups. When animals reached the indicated age, total RNA was extracted from the livers and kidneys and was analyzed by Northern blotting for the relative amounts of α₁-antitrypsin mRNA and MUP mRNA.

| Age   | Treatment                        | Sex | Relative mRNA amount (% adult liver) |
|-------|----------------------------------|-----|--------------------------------------|
| 20    | None                             | F   | 4 ± 2 (7) 110 ± 33 (7) ND             |
|       |                                  | M   | 4 ± 2 (4) 104 ± 38 (8) ND             |
| 40    | None                             | F   | 54 ± 22 (8) 138 ± 18 (8) 41 ± 24       |
|       |                                  | M   | 106 ± 29 (7) 136 ± 27 (7) 73 ± 25      |
| 40    | Ovarectomized at day 20          | F   | 53 ± 7 (2) 179 ± 3 (2) 37 ± 49         |
|       | Castrated at day 20              | M   | 13 ± 2 (3) 134 ± 45 (3) 45 ± 29        |
| 40    | Testosterone treated at day 20   | F   | 163 ± 16 (5) 149 ± 9 (5) 61 ± 12        |
|       |                                  | M   | 185 ± 62 (3) 131 ± 25 (3) 109 ± 4      |
| 20    | Testosterone treated at day 10   | F   | 81 ± 13 (2) 81 ± 12 (2) ND             |
|       |                                  | M   | 73 ± 7 (4) 83 ± 14 (4) ND             |

*a* These data are expressed as mean ± standard deviation. *n* is shown in parentheses and in the cases where *n* = 2, the data expressed as mean ± range of the two measurements. 

*b* ND, not detectable.