Species- and tissue-specific expression of human α_1 -antitrypsin in transgenic mice

Gavin D. Kelsey,^{1,3} Sue Povey,¹ Anne E. Bygrave,² and Robin H. Lovell-Badge²

¹MRC Human Biochemical Genetics Unit and ²MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK

α_1 -Antitrypsin (α_1 AT) is an abundant serum protein whose major site of synthesis is in the hepatocyte. α_1 AT transcripts are also present, albeit at a lower level, in a variety of other human tissues. This pattern of expression is partly related to initiation of transcription at sites with distinct tissue specificities. The mouse α_1 AT gene, in contrast, is more strictly liver specific in its expression. To explore the regulation of the α_1 AT gene we have microinjected a cosmid insert carrying the human gene into fertilized mouse eggs. In three lines obtained from transgenic mice, inheritance of copies of the human gene is accompanied by a high serum concentration of the human protein. Human α_1 AT RNA accumulates to the highest level in liver of transgenic animals. The presence of transcripts in other tissues indicates that the human pattern of expression is maintained, whereas the temporal activity of the introduced gene parallels that of the endogenous one during mouse embryogenesis.

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α_1 -Antitrypsin (α_1 AT) is a major serum protease inhibitor whose physiological target is neutrophil elastase (Travis and Salvesen 1983). Over 30 electrophoretic variants of human α_1 AT have been described (Fagerhol and Cox 1981) and these are allelic at a single locus, designated *Pi*, on chromosome 14 (Darlington et al. 1982). The clinically most important variant, *PiZ*, is characterized by a single amino acid substitution (Jeppsson 1976; Yoshida et al. 1976), and determines a serum deficiency of α_1 AT. Inheritance of two *Z* alleles is associated with early-onset pulmonary emphysema, most probably as a consequence of a failure to regulate adequately alveolar elastase activity (Carrell et al. 1982). The *PiZ* phenotype is also associated with liver damage, which is life-threatening in a minority of individuals (Sveger 1978). The molecular basis of this disease has not been resolved, especially the variation in its severity, but it is likely to be related to the impaired secretion of α_1 AT (Carrell et al. 1982).

In common with many serum proteins, the major site of synthesis of α_1 AT is in the hepatocyte (Fagerhol and Cox 1981), and its high serum concentration would suggest that the α_1 AT message is an abundant mRNA species in liver. Extrahepatic synthesis of human α_1 AT has also been reported. The ability of alveolar macrophages and peripheral blood monocytes to produce α_1 AT might represent a locally important source of the protease inhibitor (van Furth et al. 1983; Perlmutter et al. 1985). The presence of translatable α_1 AT mRNA in the

brain is more difficult to interpret (Dziegielewska et al. 1986). The protein is detectable in human fetal serum, and liver slices and yolk sac from first-trimester human embryos synthesize α_1 AT in culture (Gitlin and Biasucci 1969; Gitlin and Perricelli 1970). In laboratory mice there are four or five α_1 AT genes clustered at a single locus, although it is not known how many of these are active (Hill et al. 1985; Krauter et al. 1986). The expression of α_1 AT is, however, considered to be liver specific in the adult mouse, although in one wild-derived species, *Mus caroli*, α_1 AT mRNA is also present at a high level in the kidney (Berger and Baumann 1985). In the mouse embryo, the visceral yolk sac is a significant source of α_1 AT (Meehan et al. 1984).

The ability to introduce cloned genes into the mouse germ line by microinjection of fertilized eggs has provided a powerful technique for the study of gene expression (Palmiter and Brinster 1985). The influence of putative tissue-specific regulatory elements can be assessed in all possible cell types, and changes in the expression of an introduced gene can be followed throughout development. Transgenic mice have now been made with a number of genes, and, in many cases efficient and appropriate expression has been observed (e.g., Grosschedl et al. 1984; Storb et al. 1984; Swift et al. 1984; Chada et al. 1985; Krumlauf et al. 1985; Townes et al. 1985).

We have made transgenic mice with the human α_1 AT gene to investigate whether the hepatic and extrahepatic synthesis of α_1 AT depend on the same *cis*-acting regulatory element. The observation that the tissue specificities of the human and mouse genes differed added an extra dimension to the experiment. Furthermore, the

³ Present address: Institut für Zell-und Tumorbologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

potential might exist to explore PiZ-determined liver disease by the introduction into mice of a mutant α_1 AT gene. In this paper we report our findings on a series of mice carrying copies of a cosmid insert containing the human α_1 AT gene. The human α_1 AT gene is expressed in transgenic mice in a pattern very similar to that found in human tissues, including the appropriate use of tissue-specific initiation sites. During mouse development, the activity of the human gene parallels that of the mouse gene.

Results and discussion

Tissue-specific initiation for human α_1 AT gene transcription

To confirm in which human tissues the α_1 AT gene is normally active, a sensitive RNase protection assay using a single-stranded RNA probe prepared from SP6 vectors (Melton et al. 1984) was chosen, because this could also be used to identify unequivocally human α_1 AT transcripts in tissues of transgenic mice.

A probe specific for the α_1 AT message was transcribed from a 220-bp *Bam*HI–*Pst*I genomic fragment encompassing exon 1 of the α_1 AT gene. [The first exon is variously reported as 45 or 50 nucleotides in length (Long et al. 1984; Ciliberto et al. 1985).] In the presence of human liver RNA, the major fragments of the probe protected from RNase digestion were 45 and 49 nucleotides long

(Fig. 1). The presence of multiple bands in this region might indicate heterogeneity in the α_1 AT mRNA or that the RNase digestion conditions were not optimal. In addition, however, a second set of RNase-resistant fragments of 80–85 nucleotides was consistently observed. These were a minor component in liver RNA, but in adult lung RNA both sets were equally abundant, and in the total white cell sample the larger fragments were the only ones detected. Alveolar macrophages and peripheral blood monocytes are known to synthesize α_1 AT (Perlmutter et al. 1985), and monocytes comprised 10% of the white cell population from which the RNA was prepared. This argued that the larger fragments must represent functional transcripts, and led us to speculate that they arise either from initiation upstream of the limits of the probe used, or from utilization of an alternative splice site within intron 1.

To distinguish between these possibilities, an RNA probe was prepared from a 500-bp *Pst*I–*Pst*I genomic fragment comprising the original *Bam*HI–*Pst*I probe with an additional 300 nucleotides 5' to the *Bam*HI site. As expected, when this probe was hybridized to liver RNA the major RNase-resistant products were again 45–49 nucleotides long. However, the 80- to 85-nucleotide fragment of the *Bam*HI–*Pst*I probe protected by lung RNA was not reproduced with the longer probe (Fig. 2). Instead, several larger bands were observed, with the most abundant clustered at 105 nucleotides. Since the two probes differed only in upstream sequences, it

Figure 1. Distribution of α_1 AT RNA in human tissues. Total RNA, 10 μ g unless indicated, was hybridized to 2.5×10^5 cpm of *Bam*HI–*Pst*I probe specific for exon 1 of the α_1 AT gene. RNase-resistant fragments were resolved by denaturing polyacrylamide gel electrophoresis. Samples were as follows: (L) liver; (SG) small intestine; (K) kidney; (Lu) lung; (Sp) spleen; (M) skeletal muscle; (W) white cell; (B) brain; (G) intestine; and (Y) yolk sac. The gestational ages of fetal tissues are indicated in weeks. HL and HU designate the major liver and upstream initiated fragments, respectively. Molecular weight markers were end-labeled *Msp*I fragments of pAT153.

Further experiments will be necessary to characterize fully the upstream initiation. The possibility that multiple sites exist has not been explored fully, nor have we excluded the presence of an additional upstream exon specific for the monocyte and macrophage transcripts.

As expected, liver contained the highest concentration of α_1 AT RNA in the adult (4600 copies per cell). The liver was also the major source of α_1 AT in the fetus, with transcripts essentially at adult levels by 6–12 weeks. Transcripts were also abundant in the yolk sac

Table 1. Relative amounts of α_1 AT RNA in different tissues

^g 13.5 days, 15.5 days, and newborn, respectively.

RNA, downstream initiation being predominant in kidney. Figure 1 also illustrates that the two initiation sites are differentially regulated in liver: Upstream initiation was more prominent in adult than fetal liver. This could represent an accumulation of nonparenchymal cells expressing α_1 AT RNA from the upstream site in the adult liver.

The tissue distribution of mouse α_1 AT RNA was similarly determined with an RNA probe originating from a *Sau3A*–*PstI* fragment at the 3' end of a mouse cDNA clone (Hill et al. 1984). The major fragment of this probe protected by mouse α_1 AT RNA was 91 bases. (Smaller fragments were also detected, but these were only observed in tissues in which the 91-nucleotide band was also present.) Amongst adult tissues, transcripts were detected almost exclusively in liver (Fig. 3). The concentration of α_1 AT RNA increased in liver from 13.5-day embryos to adult mice (6 weeks), and the message was also abundant in yolk sac (10% of that found in liver). This is in agreement with previous findings (Meehan et al. 1984), but the specificity of the RNase protection assay allows us to extend these data by concluding that the yolk sac and liver transcripts are most likely the products of the same gene. Trace amounts were present in other tissues, such as lung, spleen, and sternum, sug-

gestive of α_1 AT synthesis in monocytes and macrophages.

The assay employed is specific for a single mouse gene. It is possible that other α_1 AT genes are active and have evolved distinct tissue specificities. Our estimate of the abundance in liver of the transcripts we assay, 1900–5500 copies per cell, is in reasonable agreement with previous determinations (Barth et al. 1982), suggesting that these represent the products of the major liver-expressed gene. Thus, the major mouse and human α_1 AT genes are regulated differently.

The significance of α_1 AT expression in the human but not the mouse fetal intestine is not clear. The human yolk sac is a less substantial tissue than that of the mouse, and it might be that the gut provides an additional source of α_1 AT in the human embryo for this reason. If this were the case, it would be interesting to see whether the expression of other human yolk sac products is also shared by this organ.

Construction and identification of transgenic mice

A cosmid containing the human α_1 AT gene has been isolated from a library of 350,000 recombinants. Serum from the donor of the DNA from which the library was

Figure 3. Distribution of α_1 AT RNA in adult and embryonic mouse tissues. Total RNA from each tissue was assayed with 2.5×10^5 cpm of the *PstI*–*Sau3A* probe specific for mouse α_1 AT. The age of the embryonic samples is given in days. Liver samples were 10 μ g, and those of other tissues 20 μ g, except where indicated. (Sk) Skin; (E) whole embryo; other tissues are identified as in Fig. 1.

built was available, and this allowed the cloned α_1 AT gene to be identified as encoding the M₁ allele, the most frequent at the *Pi* locus.

The restriction map of α ATc1, as shown in Figure 4, indicates that the cosmid contains, in addition to the α_1 AT gene, 8 kb of DNA upstream and more than 20 kb downstream of the gene. This cosmid is capable of directing the synthesis of α_1 AT RNA in a transient assay in mouse L cells (data not shown). A gene sharing considerable homology with α_1 AT has been identified on this cosmid 10 kb downstream of the α_1 AT gene. This is revealed as a 7.6- or 5.6-kb genomic *Eco*RI fragment, depending upon a polymorphic restriction site, when a Southern blot is hybridized with an α_1 AT probe. Transcripts from this related sequence have not been detected in any human tissue, although the gene can be transcribed in L cells (G. Kelsey and S. Povey, unpubl.). For microinjection into mouse eggs, the human DNA insert was liberated from the cosmid by *Sal*I digestion.

Forty-nine mice were born following microinjection. Of these, seven were identified by Southern blotting to contain copies of the human α_1 AT gene (Fig. 4; Table 2).

Table 2. Human α_1 AT in transgenic mice

Mouse	Gene copy number	Serum α_1 AT concentration ^a
16	10–15	7.5
32	2	4.8
33	<1	ND ^b
37 ^c	<1	— ^d
39	15–20	8.0
41	<1	0.1
45 ^c	<1	ND

^a Concentration in mg ml⁻¹.

^b (ND) Not done.

^c Mice 37 and 45 contain incomplete copies of the cosmid insert.

^d (—) Not detected.

Mice 16 and 39 have integrated between 10 and 20 copies of the cosmid per cell and mouse 32 has 2 copies. Mice 37 and 45 contained copies of apparently truncated α_1 AT genes, whereas mice 33, 37, 41, and 45 are probably mosaic animals since tail DNA from these mice contained less than a single copy per genome.

Figure 4. The human α_1 AT gene in transgenic mice. (A) DNA digested with *Eco*RI was electrophoresed, blotted onto nitrocellulose, and hybridized to the 6.5-kb *Bam*HI fragment of α ATc1. This probe hybridizes to a 9.5-kb *Eco*RI fragment of the α_1 AT gene, and 7.6- or 5.6-kb fragments of the α_1 AT-related gene. (C) Control mouse DNA. Mouse 37 does not contain a complete copy of the cosmid as revealed by hybridization to additional probes from α ATc1. (B) The human insert within α ATc1 shown in terms of *Eco*RI sites. The polymorphic site, not present on this cosmid, is indicated in brackets. α_1 AT exons are designated by shaded blocks, and the approximate position of the related gene by an open block. The origin of the 6.5-kb *Bam*HI probe used for the Southern analysis in A is indicated below the cosmid.

Synthesis of human serum α_1 AT by transgenic mice

If the human α_1 AT gene was expressed in the hepatocytes of the transgenic animals and the gene product correctly processed and secreted, human α_1 AT should be detectable in the sera of these mice. Using a polyclonal antiserum in a double-diffusion assay, we have identified an antigen indistinguishable from human α_1 AT in the serum of four of the founder mice carrying the intact α_1 AT gene. Quantification of the antigen concentration by rocket immunoelectrophoresis (Fig. 5 and Table 2) revealed that three of these animals, 16, 32, and 39, contained levels of the human protein well in excess of those normally found in human serum (1.3 mg ml^{-1}).

Of the first 15 offspring of mouse 16, seven were found to have inherited copies of the human α_1 AT gene. All seven of these mice had also inherited the ability to synthesize similar high levels of the human protein. We now have an extended pedigree from mouse 16 in which the expression of human serum α_1 AT behaves as a normal Mendelian marker, being passed on to half of the offspring.

Figure 5. Identification of human serum α_1 AT in transgenic mice. (A) Double immunodiffusion assay. Human serum, or sera from CBA, C57BL/6, and transgenic mouse 16, was allowed to diffuse against goat anti-human α_1 AT (center well), and precipitin arcs stained with Coomassie Blue. Serum samples were diluted 1 in 8. (B) Rocket immunoelectrophoresis was performed in agarose containing 5% goat anti-human α_1 AT until completion. Standards were human serum containing α_1 AT at 8 mg ml^{-1} diluted to 1 in 10, 15, 25, 60, and 180. Mice 16, 32, and 39 sera were diluted 1 in 20; mice 41 and 37 sera were diluted 1 in 2; and mouse control (C) serum was diluted 1 in 10. The secondary peak specific to transgenic mice has not been characterized.

Table 3. Human α_1 AT RNA in transgenic mice

	16-1/2		32-1/2		39	
	HL ^a	HU ^b	HL	HU	HL	HU
Liver	100 ^c	1.3	100 ^c	2.3	100 ^c	2
Small intestine	13.3	7.4	8	1.9	9.9	2.9
Lung	0.8	3.2	0.2	2.2	0.4	3.4
Kidney	6.6	3.7	4	1.4	7.7	2.6
Spleen	0.05	2.3	2.3	1.4	0.05	2.8
Brain	— ^d	0.02	—	0.02	—	0.1
Muscle	—	0.01	—	0.05	0.05	0.1
Skin	—	0.05	—	0.1	—	0.1
Sternum	0.1	2.6	ND ^e	ND	0.25	5.0

^a Major liver initiation site.

^b Upstream initiation site.

^c 100 represents 11,900 copies per liver cell for mouse 16-1/2, 23,900 for 32-1/2, and 22,700 for mouse 39.

^d (—) Not detected.

^e (ND) Not done.

A human pattern of expression of human α_1 AT in transgenic mice

RNA was prepared from at least eight tissues each from mice 16-1/2, 32-1/2, and 39 as representative of the three transgenic lines expressing the highest levels of serum α_1 AT. In assaying these RNAs both human- and mouse-specific probes were combined in the same reaction, permitting the direct comparison of human and mouse α_1 AT RNA in each tissue.

In all three transgenic mice assayed, the highest concentrations of human α_1 AT were present in liver (Fig. 6; Table 3). The levels of human RNA were six times greater than those of the mouse transcript and four times that detected in the human liver sample. Human α_1 AT RNA was present in other tissues in the transgenic mice, but at lower levels than in liver. The distribution of these transcripts more closely resembled that seen in human tissues than the pattern of expression of the mouse gene. In addition, the abundance in other tissues relative to liver was remarkably similar in all three mice, implying an effect inherent to the human gene, rather than a consequence of the site of integration into the mouse chromosome. The pattern diverged most notably in the case of intestinal expression, where transcripts were relatively more abundant in transgenic mice than in the adult human tissue. Thus, in all three mice, small intestine contained 10–20% of the concentration of human α_1 AT RNA found in liver, whereas kidney contained 5–10%, lung 2–4%, and spleen 3%. Mouse α_1 AT RNA was almost or totally undetectable in these tissues, both in control and transgenic animals. Sections of small intestine were assayed from transgenic mice 16-1/1, 32, and 39-1/2, and this indicated that the abundance of human α_1 AT transcripts was uniform along the length of the jejunum and ileum, and the level was about twice that in the duodenum. In contrast, the trace of murine α_1 AT RNA we detected was confined to the upper, most vascularized portion (data not shown).

Figure 6. Expression of human α_1 AT RNA in transgenic mice 16-1/2 and 32-1/2. Total RNAs were hybridized to 2.5×10^5 cpm human probe (*Bam*HI-*Pst*II) and 0.5×10^5 cpm mouse probe (*Pst*II-*Sau*3A). Samples were 20 μ g unless stated otherwise. Probe fragments protected by mouse α_1 AT RNA are indicated by Mo, and human α_1 AT-specific fragments by HL (liver promoter), HU (upstream initiation), or H. Tissues are identified as in Fig. 1. A signal from 1 μ g of lung RNA from mouse 16-1/2 was apparent on a longer exposure of the gel.

Tissue-specific initiation for human α_1 AT RNA was also apparent in the transgenic mice, and the utilization of upstream and downstream sites was similar to that seen in human tissues. Thus, the downstream site predominated in liver RNA. At the same time, consistent differences in site selection existed between human and transgenic mouse tissues. For example, whereas both sites were equivalent in human lung, upstream initiation was preponderant in lung RNA from all three mice. Conversely, upstream initiation was more noticeable in transgenic than human kidney.

Since both human initiation sites are employed in transgenic mice, it would be interesting to discover whether the mouse gene has a similar mechanism for determining liver and monocyte-macrophage expression. Alternative initiation sites can be conserved precisely between species, as illustrated by the multiple transcripts of both the avian and murine myosin alkali light-chain genes [Nabeshima et al. 1984; Roberts et al. 1984].

Developmental regulation of the human α_1 AT gene in transgenic mice

To ascertain at which point in embryological development the human α_1 AT gene was activated in the transgenic mice, DNA was isolated from tail and hind limbs of embryos of line 16 mice at 11.5, 13.5, and 15.5 days, and from 1-day postpartum animals. Individuals that had inherited the human α_1 AT genes were identified by Southern blotting. RNA was then prepared from the tissues of transgenic embryos and, separately, from their nontransgenic littermates. The RNAs prepared from the transgenic embryos were assayed with the human α_1 AT probe, and the control samples were assayed with the mouse probe.

The expression of the human α_1 AT gene was found to follow closely that of the mouse (compare Figs. 3 and 7). The α_1 AT RNA increased in concentration in the liver over the 13.5-day to newborn period, and was higher still in the adult (6-week) sample. The appearance of pro-

Figure 7. Developmental regulation of human α_1 AT in transgenic mice. RNA from transgenic embryos, newborn mice, and adults from line 16 mice were assayed with 2.5×10^5 cpm *Bam*HI–*Pst*II human probe. Liver samples were 10 μ g, and those of other tissues were 20 μ g unless indicated.

tected fragments in the whole embryo at 11.5 days probably indicates that both human and mouse genes are already active in the liver at this age. Transcripts of both species were also detectable in the yolk sac at a constant level throughout the 11.5- to 15.5-day period analyzed. The major difference was, again, in intestinal expression. A high level of human α_1 AT RNA was present in 15.5-day embryos, and this was maintained in newborn and adult animals. This contrasted both with the absence of the mouse transcript in these tissues, and with the apparent suppression of α_1 AT in human adult small intestine.

Taken together, these results clearly indicate that the segment of DNA introduced into the mice contained sufficient information to reproduce the human pattern of expression at α_1 AT. This can be compared with experiments with murine α -fetoprotein (AFP). AFP is expressed in embryonic liver, gut, and yolk sac, but the gene is normally inactive in adult tissues. Appropriately regulated expression of an AFP minigene flanked by 7 kb of 5' DNA has been observed in transgenic mice (Krumlauf et al. 1985). Recent transient expression assays in human hepatoma cells have shown that this flanking region contains a proximal sequence indispensable for expression, and three enhancers which are maximally ac-

tive in hepatoma cells (Godbout et al. 1986). In transgenic mice the proximal element is by itself incapable of eliciting expression of the AFP minigene. At least one enhancer is found to be necessary, and each has a distinct effect on the pattern of expression (Hammer et al. 1987). Transient expression assays have suggested that 720 nucleotides of DNA upstream of the human α_1 AT gene are sufficient to confer hepatoma-specific expression. In these experiments, however, the analysis was restricted to 1200 nucleotides of 5' DNA (Ciliberto et al. 1985). In the light of the AFP results, it would be interesting to see whether sequences further upstream of the α_1 AT gene contribute to the high level of expression in the hepatocyte. By introducing α_1 AT genes with less flanking DNA into mice, we hope to be able to investigate how regulatory elements influence the activity of the human α_1 AT gene in various tissues.

Conclusions

We have established the normal pattern of expression of the human and the mouse α_1 AT gene. Two points have come out of this analysis. First, α_1 AT RNA, although most abundant in liver, is present in a variety of human tissues. This regulation is twofold: α_1 AT RNA initiates

at sites with distinct tissue specificities, and the strengths of the promoters presumed to govern these sites must also vary between cell types. Second, the expression of the human and the mouse gene differs. The mouse gene is more strictly liver-specific than the human, the most striking divergence being the abundance of α_1 AT RNA in the human fetal intestine. This difference may reside in the controlling elements linked to the genes, or in the *trans*-acting factors present in human and mouse tissues. We have found that the human α_1 AT gene was expressed efficiently and appropriately when introduced into mice as a cosmid insert which contained, in addition, 8 kb of DNA 5' and 20 kb 3' to the gene. The expression took on characteristics of both the human and mouse situation, but also some novel aspects. Thus, human α_1 AT RNA accumulated to the highest level in liver of transgenic mice. Moreover, the expression of the human gene in embryonic mouse liver indicated that it could be activated correctly upon the differentiation of this tissue. The distribution of transcripts in other tissues showed that the human pattern of expression was largely maintained. This must be the consequence of *cis*-acting sequences associated with the introduced DNA. The persistence of high levels of human α_1 AT RNA in adult transgenic mouse small intestine was the most significant departure from the normal human pattern, and may be indicative of the absence of appropriate down regulators in this mouse tissue.

These results are consistent with regulation by multiple elements linked to the α_1 AT gene. They may also tell us something about the evolution of these *cis*-acting signals. The sequences conferring liver and yolk sac expression are conserved, since the human gene in mice responds in a manner identical to the mouse gene. At the same time, sequences have evidently diverged to support the expression of the human gene in tissues in which the mouse gene is inactive.

Finally, the observation that the three transgenic mouse lines attain a high serum concentration of the human α_1 AT protein encourages us to think that some aspects of the liver disease associated with the PiZ variant could be reproduced in transgenic mice.

Methods

Isolation of the human α_1 AT gene

A cDNA clone encoding α_1 AT was isolated from a human liver cDNA library with a synthetic oligonucleotide [sequence AC-C ATC GAC GAC AAA GGG A (Celltech)], using the screening conditions described (Woods et al. 1982). An unamplified cosmid library of human lymphoid line DNA was made available by S. Carson (Meunier et al. 1986). Approximately 350,000 recombinants were screened with nick-translated cDNA insert in the manner described by Meunier et al. (1986). The restriction map of the most intensely hybridizing recombinant, α ATc1, was established by a partial digestion strategy derived from Wolfe et al. (1984). Comparison with published data (Leicht et al. 1982; Long et al. 1984) revealed that this recombinant contained the entire α_1 AT gene.

Microinjection and identification of transgenic mice

The human insert of α ATc1 was purified from the cosmid by elution from agarose gels following *Sal*I digestion. DNA was injected at 2 μ g ml⁻¹ into the pronuclei of (C57BL/6 $\times$ CBA)F₁ $\times$ F₁ fertilized eggs (Hogan et al. 1986). Injected eggs were cultured overnight and transferred to day-0.5 pseudo-pregnant F₁ foster mothers; 25% of embryos developed to term. DNA was prepared from sections of tail at weaning (Lovell-Badge 1986). For the identification of transgenic embryos, DNA was prepared from nuclei isolated from tail and hind limbs (Lovell-Badge 1986). Approximately 3 μ g of each DNA was digested in the presence of 4 mM spermidine with *Eco*RI in accordance with the manufacturer's recommendations. After electrophoresis in 0.8% agarose/TAE (40 mM Tris:acetate acid, pH 8, 4 mM EDTA), DNA was transferred to nitrocellulose (Southern 1975). Filters were prehybridized in 5 $\times$ SSC, 5 $\times$ Denhardt's, 0.1% SDS, 0.1% sodium pyrophosphate, and 50 μ g ml⁻¹ herring sperm DNA for 4 hr at 65°C. Hybridization was to a gel-purified 6.5-kb *Bam*HI fragment from α ATc1 which contained the coding exons of the human α_1 AT gene. Probe, labeled with [α -³²P]dCTP by nick-translation, was hybridized overnight at 65°C in the same solution as above, except that Denhardt's was reduced to 1 $\times$ and dextran sulfate was included to 10%. Filters were washed to a final stringency of 0.1 $\times$ SSC at 65°C.

Immunodiffusion and rocket immunoelectrophoresis

Double immunodiffusion assays were performed in 1.5% agarose in phosphate-buffered saline. The central well contained goat antiserum to human α_1 AT (Atlantic Antibodies) and was surrounded by six antigen wells. Diffusion was allowed to continue for at least 24 hr at room temperature. Rocket immunoelectrophoresis (Laurell 1966) was performed in 1% agarose containing antibody at 5% for at least 3 hr at 10 V cm⁻¹, with cooling. Human serum containing α_1 AT at 8 mg ml⁻¹ served as a standard (Serotec).

Preparation of RNA

RNA was prepared immediately from fresh mouse tissues or from material that had been stored at -70°C after flash-freezing in liquid nitrogen. Human adult tissues were mainly obtained from renal transplant donors or surgery. The adult kidney came from a PiZ individual. Fetal tissues were provided by the MRC Foetal Tissue Bank at the Royal Marsden Hospital. RNA from early human fetuses was the kind gift of C. Graham. Tissues were homogenized in 6 M urea, 3 M LiCl for at least 60 sec. After allowing overnight precipitation at 4°C, RNA was collected by centrifugation at 10,000 rpm and 4°C for 30 min. Pellets were washed in the same solution and then taken up in 10 mM Tris-HCl (pH 7.5), 0.5% SDS. Following extraction with phenol and chloroform, RNA was precipitated with ethanol, and finally dissolved in diethyl pyrocarbonate-treated water. The integrity of RNA samples was assessed by the presence of 28S and 18S ribosomal bands upon agarose gel electrophoresis.

RNAse protection assays

A 220-bp *Bam*HI-*Pst*I fragment from α ATc1 encompassing exon 1 of the human α_1 AT was subcloned into the vector SP64 by standard techniques (Maniatis et al. 1982). The plasmid was linearized at the *Eco*RI site and a single-stranded RNA probe was transcribed from this template in the presence of [α -³²P]UTP as described by Melton et al. (1984). Samples of up to 20 μ g total RNA in hybridization buffer were mixed with an

excess, 2.5×10^5 cpm, of probe. Hybridization and RNase protection assays were carried out as described [Melton et al. 1984], with hybridization at 45°C and digestion at 30°C. After precipitation with ethanol and 20 µg of carrier tRNA, RNase-resistant fragments were resolved on 7 M urea, 6% polyacrylamide gels. Dried gels were set down for autoradiography at -70°C against Fuji RX film, with Dupont Lightning Plus intensifying screens. The relative intensities of bands were measured by densitometric scanning, and mRNA abundance estimated by comparison with known amounts of labeled transcript run on the same gel, and by assuming an average of 15 pg mRNA per cell. The estimates should be regarded as approximate, since single determinations were made on some of the samples. A further exon 1-specific probe was derived from a 500-bp *Pst*I fragment of α ATc1 in the same way; its orientation with respect to the SP6 promoter of the vector was established by the location of a *Bam*HI site within the human insert. A mouse-specific α_1 AT probe was prepared after subcloning into SP64 a 100-bp *Pst*I-*Sau*3A fragment at the 3' end of the cDNA insert of plv1796 [Hill et al. 1984].

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References

- Barth, R.K., K.W. Gross, L.C. Gremke, and N.D. Hastie. 1982. Developmentally regulated mRNAs in mouse liver. *Proc. Natl. Acad. Sci.* **79**: 500–504.
- Berger, F.G. and H. Baumann. 1985. An evolutionary switch in tissue-specific gene expression: Abundant expression of α_1 -antitrypsin in the kidney of a wild mouse species. *J. Biol. Chem.* **260**: 1160–1165.
- Carrell, R.W., J.-O. Jeppsson, C.-B. Laurell, S.O. Brennan, M.C. Owen, L. Vaughan, and D.R. Boswell. 1982. Structure and variation of human α_1 -antitrypsin. *Nature* **298**: 329–334.
- Chada, K., J. Magram, K. Raphael, G. Radice, E. Lacy, and F. Costantini. 1985. Specific expression of a foreign β -globin gene in erythroid cells of transgenic mice. *Nature* **314**: 377–380.
- Ciliberto, G., L. Dente, and R. Cortese. 1985. Cell-specific expression of a transfected human α_1 -antitrypsin gene. *Cell* **41**: 531–540.
- Darlington, G.J., K.H. Astrin, S.P. Muirhead, R.J. Desnick, and M. Smith. 1982. Assignment of human α_1 -antitrypsin to chromosome 14 by somatic cell hybrid analysis. *Proc. Natl. Acad. Sci.* **79**: 870–873.
- Dziegielewska, K.M., N.R. Saunders, E.J. Schejter, H. Zakut, D. Zevin-Sonkin, R. Zisling, and H. Soreq. 1986. Synthesis of plasma proteins in fetal, adult, and neoplastic human brain tissue. *Dev. Biol.* **115**: 93–104.
- Fagerhol, M.K. and D.W. Cox. 1981. The Pi polymorphism: Genetic, biochemical, and clinical aspects of human α_1 -antitrypsin. *Adv. Hum. Genet.* **11**: 1–62.
- Gitlin, D. and A. Biasucci. 1969. Development of γ G, γ A, γ M, β_{1C}/β_{1A} , C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1 -antitrypsin, orosomucoid, β -lipoprotein, α_2 -macroglobulin, and prealbumin in the human conceptus. *J. Clin. Invest.* **48**: 1433–1446.
- Gitlin, D. and A. Perricelli. 1970. Synthesis of serum albumin, prealbumin, α -foetoprotein, α_1 -antitrypsin and transferrin by the human yolk sac. *Nature* **228**: 995–997.
- Godbout, R., R. Ingram, and S.M. Tilghman. 1986. Multiple regulatory elements in the intergenic region between the α -fetoprotein and albumin genes. *Mol. Cell. Biol.* **6**: 477–487.
- Grosschedl, R., D. Weaver, D. Baltimore, and F. Costantini. 1984. Introduction of a μ immunoglobulin gene into the mouse germ line: Specific expression in lymphoid cells and synthesis of functional antibody. *Cell* **38**: 647–658.
- Hammer, R.E., R. Krumlauf, S.A. Camper, R.L. Brinster, and S.M. Tilghman. 1987. Diversity of alpha-fetoprotein gene expression in mice is generated by a combination of separate enhancer elements. *Science* **235**: 53–58.
- Hill, R.E., P.H. Shaw, P.A. Boyd, H. Baumann, and N.D. Hastie. 1984. Plasma protease inhibitors in mouse and man: Divergence within the reactive centre regions. *Nature* **311**: 175–177.
- Hill, R.E., P.H. Shaw, R.K. Barth, and N.D. Hastie. 1985. A genetic locus closely linked to a protease inhibitor gene complex controls the level of multiple RNAs. *Mol. Cell. Biol.* **5**: 2114–2122.
- Hogan, B., F. Costantini, and E. Lacy. 1986. *Manipulating the mouse embryo: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Jeppsson, J.O. 1976. Aminoacid substitution Glu $\rightarrow$ lys in α_1 -antitrypsin PiZ. *FEBS Lett.* **65**: 195–197.
- Krauter, K.S., B.A. Citron, M.-T. Hsu, D. Powell, and J.E. Darnell, Jr. 1986. Isolation and characterization of the α_1 -antitrypsin gene of mice. *DNA* **5**: 29–36.
- Krumlauf, R., R.E. Hammer, S.M. Tilghman, and R.L. Brinster. 1985. Developmental regulation of α -fetoprotein genes in transgenic mice. *Mol. Cell. Biol.* **5**: 1639–1648.
- Laurell, C.-B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15**: 45–52.
- Leicht, M., G.L. Long, T. Chandra, K. Kurachi, V.J. Kidd, M. Mace, Jr., E.W. Davie, and S.L.C. Woo. 1982. Sequence homology and structural comparison between the chromosomal human α_1 -antitrypsin and chicken ovalbumin genes. *Nature* **297**: 655–659.
- Long, G.L., T. Chandra, S.L.C. Woo, E.W. Davie, and K. Kurachi. 1984. Complete sequence of the cDNA for human α_1 -antitrypsin and the gene for the S variant. *Biochemistry* **23**: 4828–4837.
- Lovell-Badge, R.H. 1986. Introduction of genes into embryonic stem cells. In *Embryonic stem cells: A practical approach* (ed. E. Robertson). IRL Press, Oxford. (In press.)
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Meehan, R.R., D.P. Barlow, R.E. Hill, B.L.M. Hogan, and N.D. Hastie. 1984. Pattern of serum protein gene expression in mouse visceral yolk sac and foetal liver. *EMBO J.* **3**: 1881–1885.

- Melton, D.A., P.A. Krieg, M.R. Rebagliati, T. Maniatis, K. Zinn, and M.R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**: 7035–7056.
- Meunier, H.F., S. Carson, W.F. Bodmer, and J. Trowsdale. 1986. An isolated β_1 exon next to the DR α gene in the HLA-D region. *Immunogenetics* **23**: 172–180.
- Nabeshima, Y.-O., Y. Fujii-Kuriyama, M. Muramatsu, and K. Ogata. 1984. Alternative transcription and two modes of splicing result in two myosin light chains from the same gene. *Nature* **308**: 333–338.
- Palmiter, R.D. and R.L. Brinster. 1985. Transgenic mice. *Cell* **41**: 343–345.
- Perlmutter, D.H., F. Cole, P. Kilbridge, T.H. Rossing, and H.R. Colten. 1985. Expression of the α_1 -proteinase inhibitor gene in human monocytes and macrophages. *Proc. Natl. Acad. Sci.* **82**: 795–799.
- Roberts, B., P. Daubas, M.-A. Akimemko, A. Cohen, I. Garner, J.-L. Guenet, and M. Buckingham. 1984. A single locus in the mouse encodes both myosin light chains 1 and 3, a second locus corresponds to a related pseudogene. *Cell* **39**: 129–140.
- Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**: 503–517.
- Storb, U., R.L. O'Brien, M.D. McMullen, K.A. Gollahon, and R.L. Brinster. 1984. High expression of cloned immunoglobulin κ gene in transgenic mice is restricted to B lymphocytes. *Nature* **310**: 238–241.
- Sveger, T. 1978. Alpha-1 antitrypsin deficiency in early childhood. *Pediatrics* **62**: 22–25.
- Swift, G.H., R.E. Hammer, R.J. MacDonald, and R.L. Brinster. 1984. Tissue-specific expression of rat pancreatic elastase gene in transgenic mice. *Cell* **38**: 639–646.
- Townes, T.M., J.B. Lingrel, H.Y. Chen, R.L. Brinster, and R.D. Palmiter. 1985. Erythroid-specific expression of human β -globin genes in transgenic mice. *EMBO J.* **4**: 1715–1723.
- Travis, J. and G.S. Salvesen. 1983. Human plasma proteinase inhibitors. *Annu. Rev. Biochem.* **52**: 655–709.
- van Furth, R., J.A. Kramps, and M.M.C. Diesselhof-Den Dulk. 1983. Synthesis of α_1 -anti-trypsin by human monocytes. *Clin. Exp. Immunol.* **51**: 551–557.
- Wolfe, J.W., R.P. Erickson, P.W.J. Rigby, and P.N. Goodfellow. 1984. Cosmid clones derived from both euchromatic and heterochromatic regions of the human Y chromosome. *EMBO J.* **3**: 1997–2003.
- Woods, D.E., A.F. Markham, A.T. Ricker, G. Goldberger, and H.R. Colten. 1982. Isolation of cDNA clones for the human complement protein factor B, a class III major histocompatibility complex gene product. *Proc. Natl. Acad. Sci.* **79**: 5661–5665.
- Yoshida, A., J. Lieberman, L. Gaidulis, and C. Ewing. 1976. Molecular abnormality of human α_1 -antitrypsin variant (Pi-ZZ) associated with plasma deficiency. *Proc. Natl. Acad. Sci.* **73**: 1324–1328.

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G D Kelsey, S Povey, A E Bygrave, et al.

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