Expression of the Human α-1-Antitrypsin Gene in Transgenic Rats

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A genetic-engineering construction is developed to provide expression of the full-size cDNA of human α-1-antitrypsin controlled by the promoter elements of the murine metallothionein-1 gene. Transgenic animals were obtained by microinjection of pAT-MT plasmid into fertilized rat oocytes. The genome of such animals contains the sequences of human α-1-antitrypsin-cDNA revealed by blot- and dot-hybridization. Integrated human α-1-antitrypsin-cDNA copies are found with a high frequency (9 out of 22 animals) in the second-generation offspring obtained after crossings of transgenic animals. A protein with antigenic specificity of human a-1-antitrypsin is found in the serum of transgenic rats by the immunoblotting method.

Key Words: *α-1-antitrypsin; transgenic animals*

One of the most widespread monogenic hereditary diseases is a deficiency of α-1-antitrypsin (AAT), a serum protein from the family of inhibitors of serine protease (serpins) [4,5]. Analysis of AAT gene expression in heterologous systems is of interest for the development of effective methods of treatment of the main manifestation of AAT deficiency, namely primary pulmonary emphysema [9], which should be based on the substitutive introduction of recombinant AAT or on the transfection of somatic target cells by genetic-engineering constructions providing effective expression of AAT. The expression of such recombinant DNAs was studied by us previously in *E. coli* cells [2] and in cultured animal cells [1]. Experiments on transgenic laboratory animals expressing the human AAT gene have mainly centered on the mapping of functional elements of the promoter region in the AAT gene [8] and on the elucidation of the mechanisms of intracellular accumulation in hepatocytes of mutant AAT, a product of the Z-allele of the human AAT gene [6]. It would be useful to obtain and analyze transgenic animals expressing the human AAT gene controlled by heterologous promoter elements which might provide its universal expression in the cells of different organs. Here we describe the construction of recombinant DNA which contains the regulatory zone of the murine metallothionein-1 gene and the full-size coding sequence of human AAT-cDNA and the production of transgenic rats containing stably integrated and expressed copies of this construction in their genome.

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MATERIALS AND METHODS

Plasmids pMTI with promoter of the murine metallothionein-1 gene, polAT3 with full-size human AAT-cDNA, and bacterial vector pTZ19 were used as an source material to obtain the expressive genetic-engineering construction containing full-size human AAT-cDNA. *E. coli* cells JM109, restriction endonucleases EcoRI, BglII, and HindIII, and DNA-ligase (Amersham) were also used in the study.

All experiments on the isolation of plasmid DNA, its restriction and electrophoretic analysis,

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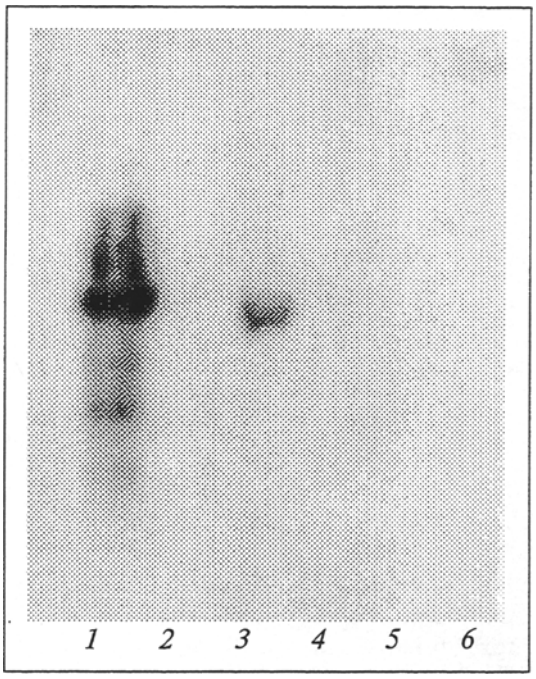

Fig. 1. Blot–hybridization of rat chromosomal DNA with human ^{32}P–AAT–cDNA. *1*) positive control (10 μg DNA of control rat+60 pg DNA of pAT–MT plasmid); *2*) negative control (10 μg DNA of nontransformed rat); *3–6*) DNA from animals transformed by pAT–MT (10 μg of DNA per each track).

the obtaining and cloning of recombinant DNA, and on the isolation of animal chromosomal DNA were carried out according to protocols described in a manual on molecular cloning [7]. Zygotes from outbred albino rats were used to obtain transgenic animals. Cleavage of chromosomal DNA with restriction endonucleases, electrophoresis of DNA fragments in agarose gel, their transfer to nitrocellulose filters (Schleicher-Shuell), and hybridization with ^{32}P-DNA probes were performed with scrupulous attention to the recommendations described in the manual [7]. The procedure of dot-hybridization [7] was used to determine the sequences of the human AAT transgene in the genomic DNA of offspring from transgenic rats of the second generation. ^{32}P-DNA of recombinant plasmids was obtained in the reaction of statistical priming with a Klenow fragment of DNA-polymerase-1 with the use of the Multiprime DNA Labeling System (Amersham) kit according to the manufacturer's instructions. The content of human AAT antigen was measured in the serum of transgenic rats by the immunoblotting technique [3] using rabbit monoclonal antibodies against human AAT (obtained by M. M. Shavlovskii) and as secondary antibodies goat IgG against rabbit IgG (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow) labeled with ^{125}I as described elsewhere [3].

RESULTS

Full-size human AAT-cDNA, promoter of the murine metallothionein-1 (MT) gene, and bacterial vector pTZ19 were used to develop the genetic-engineering construction providing a high level of AAT expression. Fragments of initial plasmid DNAs containing human AAT-cDNA (1.36 kilobase pairs, kbp) and MT gene promoter (0.8 kbp) were obtained by a twofold cleavage with restriction enzymes BglII and EcoRI of polAT3 and pMTI plasmids, respectively. These fragments were ligated and incorporated at the EcoRI-site in pTZ19 vector (2.9 kbp). The recombinant pMT-AT DNA (5.06 kbp) odtained contains AAT-cDNA sequences controlled by the MT-promoter. The orientation (5'→3') of the conjugated MT-AAT sequence vis-a-vis the bacterial promoter was determined by the analysis of sets of recombinant DNA fragments obtained by simultaneous cleavage with of BglII and HindIII. The construction chosen for further study had its constituent elements (5'→3') oriented as follows: bacterial promoter - MT-promoter - AAT-cDNA.

Recombinant DNA was administered with microinjections in pronuclei of rat zygotes at 1500 copies per cell. After the microinjections, 8 zygotes were grafted to one female recipient which gave birth to 4 male pups. DNA isolation from tails of the newborn animals with subsequent blot-hybridization with the AT-cDNA-probe was performed for the analysis of integration of the MT-AT construction into the genomic DNA. DNA from nontransformed rats was used as a control. The isolated DNA preparations were treated with EcoRI, and the DNA fragments were separated by electrophoresis in 1% agarose gel, transferred to nitrocellulose filters, and hybridized with ^{32}P-AAT-cDNA. The results of these experiments, presented in Fig. 1, show that in 1 out of 4 newborn rats the genomic DNA contains sequences hybridizing with human AAT-cDNA.

A protein with antigenic specificity of human AAT, determined by immunoblotting, appeared in the bloodstream of a transgenic rat as a result of human AAT gene expression. The serum from nontransgenic rats was used as the negative control, while human serum was the positive control. The results of these experiments, depicted in Fig. 2, show that a protein with antigenic properties of human AAT and with similar electrophoretic mobility appears in the bloodstream of transgenic animals. Some peculiarities of glycosylation and processing of the AAT carbohydrate chains which are the products of transgene expression in rat organs not expressing the endogenous AAT gene

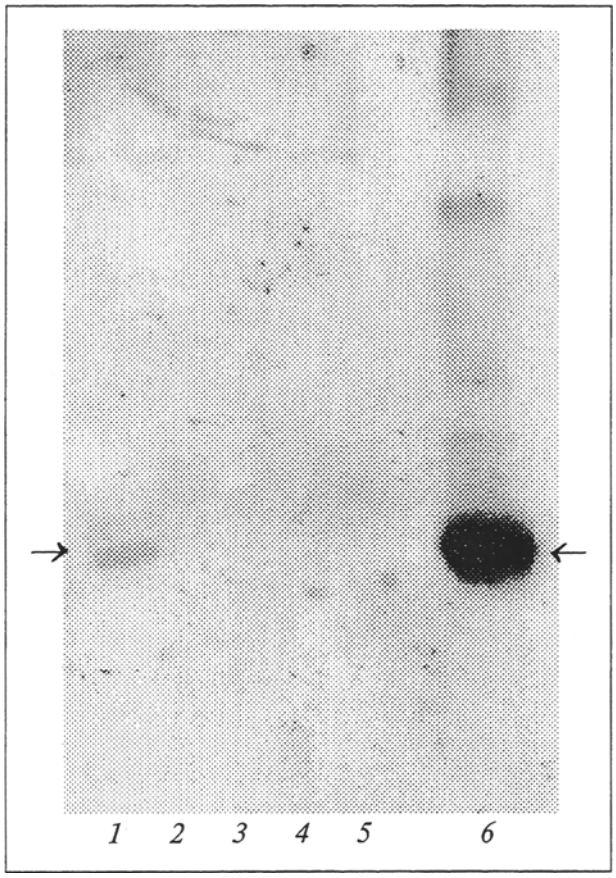

Fig. 2. Detection of human AAT in serum of transgenic rats by the immunoblotting method. *1*) negative control (serum of nontransgenic rat diluted 1:50); *2–5*) serum of transformed rats (1:50); *6*) positive control (human serum diluted 1:100).

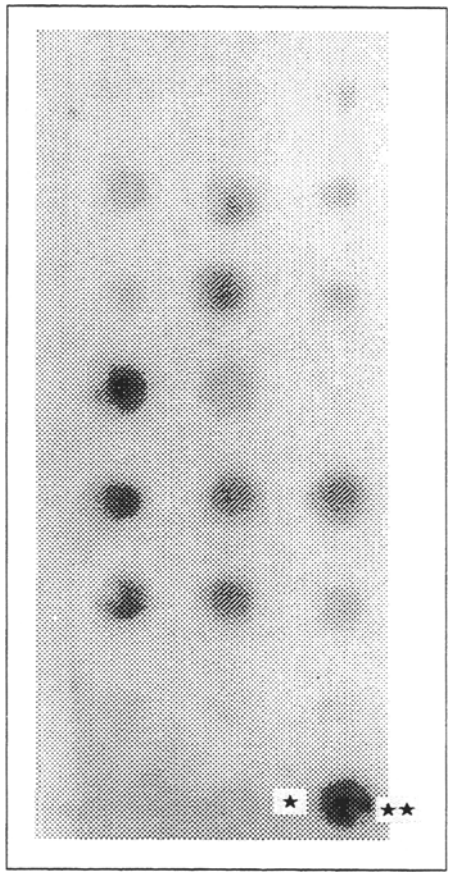

Fig. 3. Dot–hybridization of chromosomal DNA from offspring of transgenic rats of the second generation with human ^{32}P–AAT–cDNA. DNA was loaded at a rate of 10 μg per spot. *negative control (chromosomal DNA of nontransformed rat), **positive control (chromosomal DNA of nontransformed rat + 100 pg DNA of pAT–MT plasmid).

may underlie the insignificant differences in electrophoretic mobility between the AAT that was the product of transgene expression and the authentic AAT from human serum under denaturing conditions. This is quite likely, since the promoter of the metallothionein gene used in our experiments possesses functional activity in different organs of the animals. The results obtained indicate that the genetic-engineering construction provides for the expression of human AAT-cDNA in organs of transgenic rats.

Offspring (22 animals) were obtained by further crossings using a transgenic male. Preparations of chromosomal DNA from transgenic rats of the second generation were analyzed by dot-hybridization to reveal the integrated sequences of human AAT-cDNA. The results of these determinations (Fig. 3) show that the genomic DNA contains the sequences encoding human AAT in 9 of the 22 studied offspring from the transgenic male. These animals may be used subsequently to study the mechanisms of regulation of the human AAT gene controlled by the MT-promoter as well as to obtain rats of inbred strains homozygous for the human AAT transgene.

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