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EXPERIMENTAL GENETICS

Expression of the Human α-1-Antitrypsin Gene in Heterologous Mammalian Cells

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A genetic-engineering construction is developed containing the full-size cDNA of human α-1-antitrypsin, controlled by the promotor and enhancer elements from cytomegalovirus. It is shown that, after transfection with this recombinant DNA, it is properly expressed in heterologous animal cells.

Key Words: *α-1-antitrypsin; cell cultures; gene transformation*

Alpha-1-antitrypsin (AAT) is a protein inhibitor of serine proteases, the main function of which is the controlled suppression of elastase released by neutrophilic leukocytes in inflammatory foci [1]. A hereditary deficiency of AAT, which is one of the most widespread autosomal recessive diseases in man, most frequently results from mutations in the AAT gene, leading to disrupted secretion of this protein into the circulation and to its accumulation in the parenchymatous cells of the liver [1,2]. Specific methods of treatment of this disease should be based on substitutive introduction of normal AAT into the organism or on the radical genetic correction of the primary defect of the AAT gene. Either approach requires the development of genetic-engineering constructions providing for proper and effective expression of the AAT gene in heterologous cells. In this connection we constructed a recombinant DNA containing the full-size cDNA of AAT, controlled by the promotor

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and enhancer elements of cytomegalovirus, studied its expression in heterologous animal cells (hamster oocytes; strain CHOLA3), and demonstrated that AAT synthesized and secreted by this system corresponds in molecular weight to the full-size glycosylated human AAT.

MATERIALS AND METHODS

Isolation, cloning, and restriction analysis of recombinant DNA were performed after Maniatis *et al.* [3]. CHOLA3 cells were cultured in HAMS F12 medium with 10% fetal calf serum. Transformation was performed by precipitation with calcium phosphate [4]. The cells were then transferred to a serum-free medium. Forty-eight hours after transformation, temporal expression was assayed by immunoblotting on Amersham membranes [5] with rabbit polyclonal antibodies to human AAT, followed by treatment with antibodies against rabbit peroxidase-conjugated immunoglobulins. The protein bands were identified using diaminobenzidine as the substrate. Electrophoresis of proteins derived from transformed cells and from the serum-free culture medium was carried out after Laemmli [6] in 10% polyacrylamide gel (4 h, 50 mA). Untransformed CHOLA3 cells were used as the negative control, and purified human AAT as the positive control.

RESULTS

We isolated the EcoRI-HindIII fragment (1.3 kilobase pairs, kbp) of polAT3 plasmid containing the full-size cDNA of AAT, to obtain a pCMVAT gene construction. This fragment was ligated along the two corresponding sites with the EcoRI-HindIII fragment of pCMVcat plasmid containing the promotor and enhancer sequences of cytomegalovirus (Fig. 1).

By means of transformation of CHOLA3 cells, performed thereafter, and by immunoblotting of the protein products, we demonstrated the presence of a protein band with a molecular weight of about 53 kD, which corresponds to the molecular weight

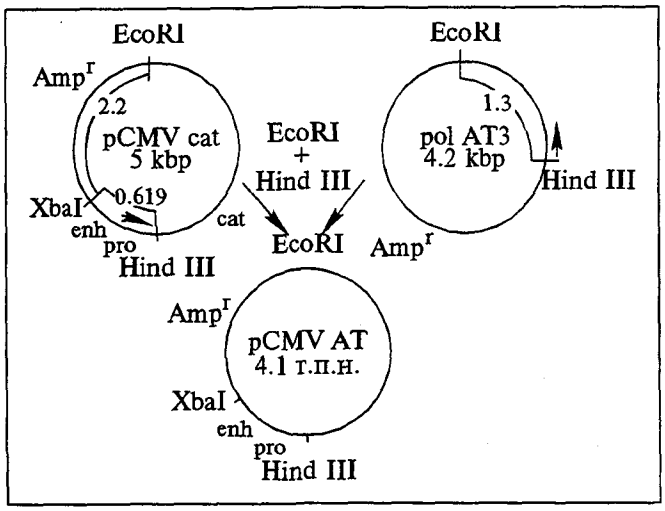

Fig. 1. Schematic of pCMVAT gene construction.

of native glycosylated AAT, in the transformed cells and in the serum-free culture medium. This attests to temporal expression of human AAT in transformed heterologous cells. At the same time, the CHOLA3 cells were able to synthesize, posttranslationally glycosylate, and secrete recombinant AAT into the culture medium.

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