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Assignment of the α_1-Antitrypsin Gene and a Sequence-Related Gene to Human Chromosome 14 by Molecular Hybridization

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SUMMARY

α_1-Antitrypsin is a major plasma protease inhibitor synthesized in the liver. Genetic deficiency of this protein predisposes the affected individuals to development of infantile liver cirrhosis or chronic obstructive pulmonary emphysema. The human chromosomal α_1-antitrypsin gene has been cloned and shown to contain three introns in the peptide-coding region. When the cloned α_1-antitrypsin gene was used as a hybridization probe to analyze *Eco* RI-digested genomic DNA from different individuals, two distinct bands of 9.6 kilobases (kb) and 8.5 kb in length were observed in every case. Further analysis using only labeled intronic DNA as the hybridization probe has indicated that the authentic α_1-antitrypsin gene resides within the 9.6-kb fragment. Thus the 8.5-kb fragment must contain another gene that is closely related in sequence to the α_1-antitrypsin gene. Using a series of human-Chinese hamster somatic cell hybrids containing unique combinations of human chromosomes, the α_1-antitrypsin gene as well as the sequence-related gene have been assigned to human chromosome 14 by Southern hybridization and synteny analysis.

INTRODUCTION

α_1-Antitrypsin deficiency is a human genetic disorder that has been associated with development of pulmonary emphysema and liver cirrhosis [1–10]. α_1-Antitrypsin is a complex molecule containing multiple carbohydrate side chains, including

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N-acetylglucosamine, galactose, mannose, and sialic acid [11]. Familial studies had previously established a genetic linkage between α_1-antitrypsin and the polymorphic marker GM for the production of immunoglobulin heavy chain [12, 13]. The group of genes for human immunoglobulin heavy chain was assigned to human chromosome 14 using somatic cell hybrids and synteny analysis [14, 15]. The assignment has subsequently been confirmed by the use of molecular probes to immunoglobulin heavy chain genes for detection of human genomic sequences in the somatic cell hybrids [16]. Thus, α_1-antitrypsin can also be assigned indirectly to human chromosome 14 by virtue of its linkage to GM. The chromosome localization of the human α_1-antitrypsin gene has also been recently identified by analysis of protein production in somatic cell hybrids [17] and by cytogenetic mapping [18]. We recently reported the cloning and structural characterization of the human chromosomal α_1-antitrypsin gene [19]. Here we describe the confirmation of assignment of the α_1-antitrypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization techniques using cloned gene probes.

MATERIALS AND METHODS

Hybrid Cell Formation and Maintenance

Human/Chinese hamster CHO-Kl cell hybrids were prepared from several independent fusions involving various CHO-Kl auxotrophic mutants [20] and human cells like normal fibroblasts and lymphocytes. Hybrids were isolated under selective conditions [21] in accordance with each specific auxotrophic mutant used. From over 40 independent primary hybrids, seven were chosen to form a clone panel for their unique combinations of human chromosomes that can discriminate all 22 autosomes and the X chromosome [22]. The human chromosome content in the hybrids was determined by isozyme markers for each specific human chromosome. Cytogenetic analysis with chromosome banding [23] was also used to confirm the isozyme data in some but not all hybrids.

The hybrid cells were routinely cultivated in the selective medium F12D [24], a condition which we found could confer more stability to the human chromosomes retained in the hybrids. Most of the hybrids that were chosen for mapping studies were 2s hybrids containing two sets of the CHO genome plus various numbers of human chromosomes. We found that 2s hybrids were more stable than 1s hybrids (containing only one set of CHO genome) in maintaining human chromosomes for long periods in culture. The procedures for preparing DNA from the cultured hybrid cells were the same as previously described [22].

Preparation of Hybridization Probes

pAT9.6 is a chimeric plasmid containing a 9.6-kb human DNA inserted into the *Eco* RI site of pBR322 (fig. 1). Digestion of pAT9.6 DNA with *Bam* HI generated a 6.5-kb fragment (AT6.5) that contained the α_1-antitrypsin gene sequences. The digested DNA was sized by electrophoresis in a 1% agarose gel. After ethidium bromide staining, the gel segment containing the 6.5-kb DNA fragment was excised. DNA was eluted from the gel into a buffer containing 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA (TE) in a dialysis bag by electrophoresis. The DNA was then extracted with saturated phenol and precipitated by 2 vol of ethanol. The DNA was resuspended and stored in TE buffer for use as a hybridization probe.

Digestion of pAT9.6 DNA with *Xho* I and *Xba* I produced two small intronic fragments of 0.35 and 0.5 kb in length (fig. 1). These two fragments were purified by exhaustively digesting the chimeric plasmid, subjecting the DNA to electrophoresis and eluting them

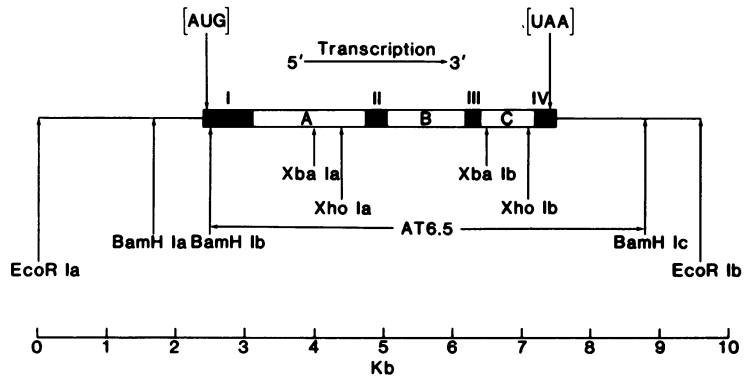

FIG. 1.—Molecular structure of the cloned human α_1-antitrypsin gene. pAT9.6 is a chimeric plasmid containing a 9.6-kb human DNA inserted into the *Eco* RI site of pBR322. (■), α_1-Antitrypsin structural sequences; (□), intervening sequences.

from the gel as described above. DNA probes were labeled with [^{32}P] by nick translation (specific activity: 6 × 10^8 cpm per μg DNA) as described [25].

"Southern" Blotting and Hybridization

Fifteen μg of DNA from individual hybrid cell lines were digested with restriction endonucleases (purchased from Bethesda Research, Gaithersburg, Md.) according to recommended assay conditions. The digested DNA was heated at 68° for 5 min and applied to a 1% horizontal agarose slab gel.

Electrophoresis was carried out in a Tris-acetate buffer as described [26]. DNA in the agarose slab gel was visualized on a uv illuminator after ethidium bromide staining. The gel was then treated, in succession, with 0.25 N HCl for 10 min, 0.5 N NaOH + 1.5 M NaCl for 30 min, and 1 M Tris-HCl, pH 7.4, 0.6 M NaCl for 60 min. The gel was then rinsed well with deionized water and soaked in 20 × SSC for 5 min. DNA in the treated agarose gel was transferred onto nitrocellulose filters (BA-85, S & S) by the method of Southern [27]. After the transfer, the filter was baked at 68°C for 3 hrs and then pretreated in a 6 × SSC solution containing 0.04% each of ficoll, polyoinylpyrrolidone, and bovine serum albumin at 68°C for 5 hrs. The filter was then hybridized with [^{32}P]-labeled DNA probes in the same solution containing 0.5% SDS and 1 mM EDTA at 68°C for 16 hrs. It was washed with a 1 × SSC + 0.5% SDS solution at 68°C for a total of 6 hrs and exposed to a Kodak X-ray film (XAR-5) in the presence of a Dupont Cronex intensifying screen at −20°C for 3 days.

RESULTS

A restriction endonuclease map of a cloned 9.6-kb *Eco* RI DNA fragment containing the human chromosomal α_1-antitrypsin gene is presented in figure 1. *Bam* HI cleaves this DNA fragment to generate a 6.5-kb fragment (AT6.5) that contains four exons and three introns of the gene, and is devoid of any repetitive DNA sequences. When AT6.5 DNA was labeled with [^{32}P] and hybridized with *Eco* RI-digested human placenta DNA by Southern blotting, two distinct bands at positions 9.6 kb and 8.5 kb were observed (fig. 2*A*, lane 1). The intensity of the 9.6-kb band was found to be equivalent to the signal generated by a unique sequence DNA in the human genome (fig. 2*A*, lanes 2–4). The detection of the 8.5-kb *Eco* RI DNA fragment by this analysis was unexpected since the hybrid-

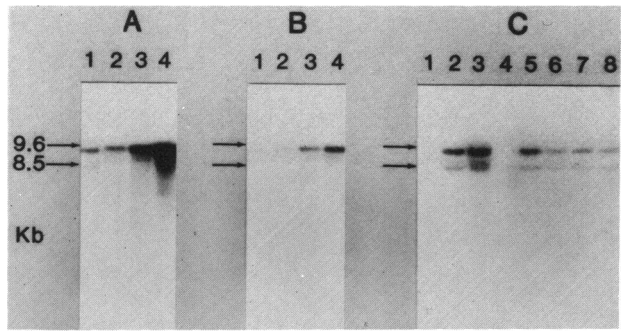

FIG. 2.—Autoradiographs of Southern hybridization analysis of human DNA and clonal cell hybrid DNAs using [^{32}P]-labeled α_1-antitrypsin gene sequences as the probe. In *panels A* and *B*, *lanes 1* contained 15 μg of *Eco* RI-digested human placenta DNA; *lanes 2*, *3*, and *4* contained *Eco* RI-digested pAT9.6 DNA in quantities equivalent to one, three, and six copies of the α_1-antitrypsin gene in 15 μg of genomic human DNA, respectively. In *panel C*, DNAs applied to the gel were from the parental CHO-K1 line (*lane 1*); CP27 (*lane 2*); CP29 (*lane 3*); CP28 (*lane 4*); CP15 (*lane 5*); CP18 (*lane 6*); CP26 (*lane 7*), and CP16 (*lane 8*). The [^{32}P]-labeled probes used were AT6.5 DNA in *panels A* and *C*, and the two small *Xba*I/*Xho*I fragments containing only intronic sequences in *panel B*.

ization probe is a unique sequence and is located within the 9.6-kb *Eco* RI DNA fragment. To distinguish the two bands, two intron-specific probes were isolated from the plasmid pAT9.6 DNA by double digestion with *Xba* I and *Xho* I (fig. 1). When these probes were used to hybridize with human placenta DNA digested with *Eco* RI, preferential hybridization with the 9.6-kb band is evident (fig. 2*B*, lane 1). These results confirm that the human α_1-antitrypsin gene resides within a 9.6-kb *Eco* RI fragment, and that a sequence-related gene must exist within a distinct 8.5-kb *Eco* RI DNA fragment in the human genome. We recently cloned this 8.5-kb *Eco* RI DNA fragment and found that its restriction map is distinctly different from the cloned 9.6-kb *Eco* RI DNA fragment (V. J. Kidd, unpublished results).

To assign the α_1-antitrypsin gene and the sequence-related gene to specific human chromosomes, we analyzed DNA isolated from a series of human/Chinese hamster somatic cell hybrids by Southern blotting. Total DNA from the seven hybrid clones that constitute a clonal panel containing combinations of various human chromosomes that could be used to discriminate all autosomes and the X chromosome were digested with *Eco* RI and analyzed by Southern hybridization using as the probe [^{32}P]-labeled AT6.5 fragment. Hybridization signals at 9.6 and 8.5 kb were observed in DNA from all the hybrid clones except CP28 and the parental Kl line (fig. 2*C*). These results indicated that the presence or absence of the α_1-antitrypsin gene in the hybrid clones correlated only with human chromosome 14 (table 1, upper section). Analysis of 16 additional hybrid clones has substantiated this tentative assignment (table 1, lower section). Definitive confirmation was obtained by analysis of hybrid CP43, which contains chromosome 14 as the only human genetic material (fig. 3*A*). When DNA from this hybrid was allowed to hybridize with the α_1-antitrypsin gene probe, the presence of the expected 9.6-kb band was evident (fig. 3*B*, lane a). The segregant CP43-1, which

TABLE 1

HUMAN/CHO-K1 CELL HYBRIDS FOR HUMAN CHROMOSOMAL ASSIGNMENT OF THE α_1-ANTITRYPSIN GENE

CELL HYBRIDS	HUMAN CHROMOSOMES*																						α_1-ANTITRYPSIN GENE†
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
CP27	−	+	+	+	+	+	+	−	−	−	−	+	+	+	−	+	−	+	−	−	+	−	+	+
CP28	−	−	+	+	+	+	−	−	+	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−
CP29	−	+	+	+	−	−	−	+	−	−	+	+	−	+	+	−	−	+	+	+	+	+	+	+
CP15	−	+	+	+	+	−	−	−	+	−	+	+	−	+	−	−	+	−	+	−	−	−	−	+
CP18	+	−	+	−	−	−	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+
CP26	+	−	−	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−	+
CP16	−	−	+	−	+	−	+	−	−	−	+	−	−	+	−	+	+	−	+	+	+	−	+	+
CP12	−	+	−	+	−	−	−	+	+	−	+	+	+	+	−	+	−	+	−	−	−	+	+	+
CP3	+	+	+	+	+	−	+	+	+	−	+	+	−	+	−	+	−	+	+	+	+	−	+	+
CP4	−	+	+	+	+	−	+	+	−	−	+	+	−	+	+	−	+	−	+	−	−	−	+	+
CP5	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	+	−	−	+	+	+	+
CP6	−	−	+	+	−	+	+		+	+	−	+	−	+	−	−	+	+	+	+	+	+	−	+
CP11	+	−	+	+	+	+		+	+	−	−	−		+	−			+	+	−	+		−	+
CP14	+	+	−	+	+	−	−	−	+	−	+	+	−	+	−	−	+	+	+	+	+	+	+	+
CP17	+	−	−	+	+	−	−	−	+	−	−	+	−	+	+	−	−	+	+	−	+	+	−	+
CP20	−	+	+	+	+	−	−	+	+	−	+	−	−	+	−	−	+	−	+	−	+	−	−	+
CP32	+	+	−	−	−	−		−	−	−	+	−	−	−	−		+	+	+	−	−	−	−	−
CP39	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
CP42	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
CP38	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
CP24	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
CP43	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
CP43-1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Concordant hybrids	11	13	16	18	16	10	10	14	14	9	15	17	9	23	12	9	14	15	16	11	16	11	13	...
Discordant hybrids	12	10	7	5	7	13	11	8	9	14	8	6	13	0	11	12	8	8	7	12	7	11	10	...
Concordant frequency	48	57	70	73	70	44	48	64	41	39	65	74	41	100	52	43	64	65	70	48	70	50	5	...

* The following isozyme markers were assayed for determining human chromosome content in the hybrids. Blanks indicate that assays were not done. PGD (phosphogluconate dehydrogenase) (#1), MDH1 (malate dehydrogenase, NAD, soluble) (#2), GLB1 (β-galactosidase-1) (#3), PGM2 (phosphoglucomutase-2) (#4), ARSB (arylsulfatase-B) (#5), SOD2 (superoxide dismutase, mitochondrial) (#6), GUSB (β-glucuronidase) (#7), GSR (glutathione reductase) (#8), AK1 (adenylate kinase-1) (#9), GOT1 (glutamic-oxaloacetic transaminase, soluble) (#10), LDHA (lactate dehydrogenase-A) (#11), LDHB (lactate dehydrogenase-B) (#12), ESD (esterase-D) (#13), NP (nucleoside phosphorylase) (#14), HEXA (hexosaminidase-A) (#15), PGP (phosphoglycollate phosphatase) (#16), GALK (galactokinase) (#17), PEPA (peptidase-A) (#18), GPI (glucose phosphate isomerase) (#19), IT (inosine triphosphatase) (#20), SOD1 (superoxide dismutase, soluble) (#21), ACO2 (aconitase, mitochondria) (#22), G6PD (glycose-6-phosphate dehydrogenase) (X). Nos. in parentheses refer to the human chromosome no.

† Molecular hybridization results of the cell hybrids using the α_1-antitrypsin probe are shown as presence (+) or absence (−) of hybridization bands at positions 9.6 and 8.5 kb corresponding to the α_1-antitrypsin probe used.

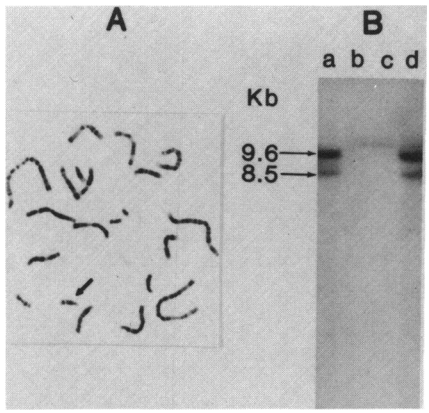

FIG. 3.—*Panel A*, photomicrograph showing a metaphase cell of the cell hybrid CP43 containing a single human chromosome 14 (*arrow*). Giemsa 11 differential staining was also performed on this hybrid and proved that chromosome 14 was the only human chromosomal material present in this hybrid. *Panel B*, definitive assignment of the human α_1-antitrypsin gene to chromosome 14 by *Eco* RI digestion and Southern hybridization using [^{32}P]-labeled AT6.5 DNA as the probe. DNA applied to the gel was prepared from the hybrid clone CP43 (*lane a*), the segregant CP43-1 (*lane b*), the parental hamster cell K1 (*lane c*), and human placenta (*lane d*), respectively.

is a subclone from CP43 and has lost the single human chromosome 14, has also lost the hybridization signal (fig. 3*B*, lane b).

The 8.5-kb *Eco* RI fragment is also present in clone CP43 and absent in segregant CP43-1 (fig. 3*B*, lanes a and b). Since this 8.5-kb sequence segregated with the α_1-antitrypsin gene, it must also be located on human chromosome 14. Whether the two genes are physically linked cannot be established by the present data.

DISCUSSION

Using the cloned α_1-antitrypsin gene as the hybridization probe, our results complement and confirm recent reports on the chromosome localization of this gene [18–20]. A unique feature of using cloned DNA probes for mapping genes in somatic cell hybrids is that no specific cell types are required to generate hybrids in order for the genes to be expressed into assayable proteins. This approach will also facilitate regional mapping of syntenic genes on a particular chromosome by establishing a series of deletion hybrids from any cell types, each of which is carrying a different terminal deletion of that chromosome. A deletion hybrid series of chromosome 14, similar to the one established for human chromosome 11 [28] that has been successfully used for regional mapping of the β-globin gene [29], will be very useful in the regional mapping of the α_1-antitrypsin gene as well as the sequence-related gene on chromosome 14.

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AMERICAN BOARD OF MEDICAL GENETICS, INC.: EXAMINATION AND TRAINING ANNOUNCEMENT. *Certification examinations* for clinical geneticists, Ph.D. medical geneticists, genetic counselors, clinical laboratory cytogeneticists, and biochemical geneticists will be given on June 27 and 28, 1984. Deadline for applications is September 15, 1983; a late fee will be assessed thereafter. No applications will be accepted after December 31, 1983. Individuals who plan to complete their fellowships by June 1984 and have otherwise fulfilled the requirements of the Board will be eligible to apply for this set of examinations. *Accreditation of clinical genetics training programs* will be undertaken by the Board this year. Information and application forms for both certification and accreditation can be obtained by writing: David L. Rimoin, M.D., Ph.D., President, American Board of Medical Genetics, Inc., Harbor-UCLA Medical Center, 1000 W. Carson St., E-4, Torrance, CA 90509.