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Assignment of human α_1-antitrypsin to chromosome 14 by somatic cell hybrid analysis

(gene mapping/differentiated cell function)

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ABSTRACT **Human α_1-antitrypsin (α-1-AT;Pi) production was analyzed in 11 primary mouse hepatoma–human lymphoid cell hybrids and in 14 secondary rat hepatoma–human fetal liver fibroblast hybrids. The presence of human α-1-AT was determined by Laurell immunoelectrophoresis of concentrated and isotopically labeled supernatant medium. Human α-1-AT production segregated in the mouse–human hybrids concordantly with human purine nucleoside phosphorylase and with chromosome 14. All rat–human hybrids that were α-1-AT positive were also positive for human purine nucleoside phosphorylase and chromosome 14. Our study demonstrated the usefulness of rodent hepatoma cell hybrids for mapping human liver-specific genes because differentiated functions are expressed despite the fact that the human parental cells did not express these functions. Our study also showed that human α-1-AT gene product can be processed for secretion in the rodent hepatoma cellular environment. The mouse–human hybrids showed that no other human chromosome carries genes necessary for processing or secretion of human α-1-AT in the hybrid cell milieu.**

α_1-Antitrypsin (α-1-AT) is a protease inhibitor synthesized and secreted by the liver. The protein is a monomer with a M_r of ≈54,000 and has four carbohydrate side chains of two types that contain *N*-acetylglucosamine, mannose, galactose, and sialic acid (1). Considerable genetic variation exists at this locus, the Pi locus (for protease inhibitor), with 26 allelic variants identified (2). The most common form of α-1-AT is the M type with an allele frequency of 0.87 (3). The Z form of α-1-AT is a variant that results in low levels of the protein in serum (10–15% of normal) of homozygous individuals. Childhood cirrhosis, neonatal jaundice, and early onset of emphysema are associated with the ZZ genotype. A single amino acid substitution distinguishes the Z form of α-1-AT from the M form (1, 4). The accumulation of immunologically reactive globules of α-1-AT in the hepatocytes of ZZ individuals has been observed (5, 6). It is possible that the ZZ protein is improperly processed as a result of the alteration in amino acid sequence.

Using both rat and mouse hepatoma cell lines to generate rodent–human hybrids expressing human α-1-AT, we found that the α-1-AT gene is located on chromosome 14 of man. Gedde-Dahl *et al.* (7, 8) have shown, by pedigree analysis, linkage between α-1-AT and Gm, the polymorphic marker for the heavy chain of human immunoglobulin. The genes for human immunoglobulin heavy chains have been assigned to chromosome 14 by somatic cell genetic techniques (9, 10). The assignment of the Pi locus thus confirms the position of the immunoglobulin heavy chain locus.

Furthermore, this study demonstrates the potential of somatic cell hybrids for mapping specialized or tissue-specific functions. The majority of genes mapped by the technique of somatic cell hybridization have been those expressed by the human parental cell line. The human lymphoblastoid line used in these studies did not produce α-1-AT in the unfused state. However, the mouse hepatoma–human lymphoblastoid hybrid cells expressed human α-1-AT, a liver-specific product. These "activated" hybrids permitted the assignment of a gene normally expressed only in hepatocytes.

MATERIALS AND METHODS

The mouse hepatoma–human lymphoblastoid cell hybrids were established in the laboratory of G.J.D. and the rat hepatoma–human liver fibroblast hybrids were produced by K.H.A. and M.S.

Cell Lines and Hybrid Cell Formation. The mouse hepatoma cell line, HH, is a derivative of Hepa 1 (11) that lacks hypoxanthine phosphoribosyltransferase and has a modal chromosome number of 85. The human partner in the mouse hepatoma cross was an established lymphoblastoid line obtained from a male with mucopolysaccharidosis type II. These cells did not produce human α-1-AT. For fusion, 4×10^6 cells of each type were incubated in 1000 hemagglutinating units of Sendai virus (B-propiolactone inactivated) for 15 min at 4°C and then for 40 min at 37°C. The cell and virus mixture was diluted and plated in Falcon flasks (25 mm^2). The cells were fed twice weekly with Waymouth medium 87/3 (GIBCO)/10% fetal calf serum (Flow Laboratories)/1% penicillin-streptomycin (GIBCO)/2 mM aminopterin (Sigma). Hybrids grew as attached colonies and were isolated with cloning rings ≈3 weeks after fusion.

The rat hepatoma, FU5AH (hypoxanthine phosphoribosyltransferase lacking), was initially established by Mary Weiss (12, 13) and supplied by Marcello Siniscalco. The rat hepatoma was fused with human fibroblasts from a fetal liver. The human cells were not examined for α-1-AT production. The cells were fused by incubating them together in serum-free Dulbecco's modified Eagle's minimal essential medium (GIBCO)/47% polyethylene glycol 6000/15% dimethyl sulfoxide for 2 min (14). Additional serum-free medium was added and the cells were washed twice before being plated in the same Dulbecco's medium/10% fetal calf serum/hypoxanthine/aminopterin/thymidine/5 μM ouabain. A single colony was observed after several weeks in the selective medium. This colony was isolated, expanded, and subcloned by placing 100 cells in a Falcon flask (25 mm^2) and pick-

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Abbreviations: α-1-AT, α_1-antitrypsin; NP, purine nucleoside phosphorylase.

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ing individual colonies with a cloning ring. Usually, only a single clone was picked from a flask. Several clones were further subcloned by the same technique.

Karyotypic Analysis. Karyotypic analysis of the mouse hepatoma hybrids was carried out by using the protocol of Bobrow *et al.* (15) for Giemsa-11 staining. G-banding analysis was done by using the protocol of Klinger (16).

In the rat–human hybrids, the human chromosomes were identified first by staining with Giemsa-11 (17). The slides were then destained and restained with quinacrine hydrochloride for Q banding (18).

Isozyme Analysis. The following enzymes were assayed in one or both sets of hybrids using either cellogel or starch gel electrophoresis: phosphoglucomutase 1 and 2 (PGM1 and -2; EC 2.7.5.1), malate dehydrogenase (MDH; EC 1.1.1.37), aminoacylase 1 (ACY1; EC 3.5.1.14), hexosaminidase A and B (HexA and -B; EC 3.2.1.30), malic enzyme (ME; EC 1.1.1.40), β-glucuronidase (GUSB; EC 3.2.1.31), glutathione reductase (GSR; EC 1.6.4.2), adenylate kinase (AK1, -2, and -3; EC 2.7.4.3), glutamate oxaloacetate transaminase (soluble) (GOT; EC 2.6.1.1), esterase A4 and D (ESA4 and -D; EC 3.1.1.1), peptidase A, B, C, and D (PEPA, -B, -C, and -D; EC 3.4.11), purine nucleoside phosphorylase (NP; EC 2.4.2.1), mannose phosphate isomerase (MPI; EC 5.3.1.8), phosphoglycollate phosphatase (PGP; EC 3.1.3.18), glucose phosphate isomerase (GPI; EC 5.3.1.9), adenosine deaminase (ADA; EC 3.5.4.4), superoxide dismutase (soluble and mitochondrial) (SOD1 and -2; EC 1.15.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), lactate dehydrogenase A and B (LDHA and -B; EC 1.1.1.27), triose phosphate isomerase (TPI; EC 5.3.1.1), phosphoglycerate kinase (PGK; EC 2.7.2.3), aconitase (soluble and mitochondrial) (ACONS and ACONM; EC 4.2.1.3).

In the rat–human hybrids, aminoacylase 1, glutathione reductase, and both aconitases were analyzed by using published methods (19–21). The rest of the marker enzymes were analyzed by using the methods described by Harris and Hopkinson (22) except for the following, which were separated on starch gels using different buffer systems to achieve better separation of rat and human isozymes: for malate dehydrogenase and malic enzyme, the buffer regularly used with phosphoglucomutase was used; for hexosaminadase B, the glutamate oxaloacetate transaminase buffer was used; for esterases A4 and D and mannose phosphate isomerase, the phosphoglycollate phosphatase buffer was used; for glucose phosphate isomerase, the peptidase buffer was used; and for peptidases A and B, the diaphorase buffer was used. All buffers are described by Harris and Hopkinson (22).

Nucleoside Phosphorylase. Purine nucleoside phosphorylase (NP) in the mouse–human hybrids was analyzed by cellogel electrophoresis (20). NP in the rat–human hybrids was analyzed by starch gel electrophoresis using a modification of the buffer system of Swallow and Harris (23). The bridge buffer was 0.15 M trisodium citrate/0.024 M sodium dihydrogen phosphate adjusted to pH 7.0 with sodium hydroxide. The gel buffer was a 1:40 dilution of the bridge buffer and its pH was adjusted to 7.0 with 0.2 M citric acid. The gel was run overnight on cooling plates at 100 V across the gel.

Isozyme analysis of the mouse–human hybrids was kindly done by E. Nichols by published procedures (24). Twenty-four isozymes with known assignments to 16 different human chromosomes were examined.

Serum Protein Analysis. Anti-human α-1-AT was purchased from Kallestadt (Chaska, MN). Supernatant medium was collected, concentrated, and analyzed as described by Rankin and Darlington (25). Isotopically labeled secreted proteins were analyzed by Laurell immunoelectrophoresis (11). Neither the rat hepatoma nor the mouse hepatoma cells produced a protein that crossreacted with the antiserum to human α-1-AT.

RESULTS

Mouse–Human Hybrids. Eleven independently isolated mouse hepatoma–human lymphoblastoid cell line hybrids were examined for their human chromosomal complement by Giemsa-11 staining and G-banding, for the production of human α-1-AT, and for 23 human isozymes, including the chromosome 14 marker NP (26).

Six of these hybrids produced human α-1-AT. Table 1 shows an absolute correlation between chromosome 14 or human NP and the expression of human α-1-AT. It is apparent that no other human chromosome showed concordant segregation with α-1-AT in this group of independently isolated hybrid clones. These hybrids showed rapid and preferential segregation of the human complement and, as a consequence, many human chromosomes were not represented in the karyotypes of this set of hybrids.

One hybrid from this set was grown in 6-thioguanine (30 μg/ml) to select for loss of the human X chromosome. The original clone contained the short arms and the proximal two-thirds of the long arm of chromosome 4, chromosome 14, and the X chromosome and produced α-1-AT. The back-selected clone had the same karyotype except that it lacked the X chromosome and continued to produce α-1-AT.

Rat–Human Hybrids. Eight clones isolated from the original hybrid colony were examined for the production of human α-1-AT and for their human chromosomal content. All but two of the hybrids expressed human α-1-AT. Analysis of their karyotypes showed that the hybrids retained a representative of almost every pair of human chromosomes.

Table 1. Segregation of human α-1-AT and human chromosomes in mouse–human hybrids

Chromosome	Enzyme(s)	α-1-AT/chromosome: Concordant +/+	Concordant −/−	Discordant +/−	Discordant −/+	Total: Concordant	Total: Discordant
1	PEPC, AK2, PGM1	0	5	6	0	5	6
2	MDH1	0	4	6	1	4	7
3		0	5	6	0	5	6
4		1	5	5	0	6	5
5	HEXB	1	5	5	0	6	5
6	ME1/SOD2	0	5	6	0	5	6
7		1	5	5	0	6	5
8	GSR	0	5	6	0	5	6
9	AK1	0	5	6	0	5	6
10	GOT1	0	5	6	0	5	6
11	LDHA	0	5	6	0	5	6
12	LDHB, PEPB, TPI	0	5	6	0	5	6
13	ESD	0	5	6	0	5	6
14	NP	6	5	0	0	11	0
15	MPI, HEXA	0	5	6	0	5	6
16		0	5	6	0	5	6
17		1	4	5	1	5	6
18	PEPA	0	5	6	0	5	6
19	GPI, PEPD	1	5	5	0	6	5
20	ADA	1	4	5	1	5	6
21	SOD1	3	3	3	2	6	5
22		1	4	5	1	5	6
X	G6PD, PGK	2	3	4	2	5	6
Y		0	5	6	0	5	6

Human chromosomes were identified by karyotyping or enzyme markers or both. Enzyme abbreviations are as given in *Materials and Methods*.

Table 2. Segregation of human α-1-AT and human chromosomes in secondary rat–human hybrids

Chromosome	Enzyme(s)	α-1-AT/chromosome				Total	
		Concordant		Discordant			
		+/+	−/−	+/−	−/+	Concordant	Discordant
1	PGM1	3	4	7	0	7	7
2	MDH1	4	3	6	1	7	7
3	ACY1	6	3	4	1	9	5
4	PGM2	10	0	0	4	10	4
5	HEXB	6	2	3	1	8	4
6	ME1	10	0	0	4	10	4
7	GUS	3	4	6	0	7	6
8	GSR	7	0	2	4	7	6
9	AK/ACONS	1	4	9	0	5	9
10	GOT1	10	0	0	4	10	4
11	ESA4	8	0	2	4	8	6
12	PEPB	9	0	0	4	9	4
13	ESD	8	0	0	3	8	3
14	NP	10	3	0	1	13	1
15	MPI	9	0	0	4	9	4
16	PGP	5	1	4	3	6	7
17	—	4	3	6	1	7	7
18	PEPA	4	3	6	1	7	7
19	GPI	9	1	1	2	10	3
20	ADA	9	0	1	2	9	3
21	SOD1	7	0	2	4	7	6
22	ACONM	1	1	4	0	2	4
X	G6PD	7	0	0	4	7	4

Human chromosomes were identified by karyotyping or enzyme markers or both. Enzyme abbreviations are as given in *Materials and Methods*.

Two of the primary clones were subcloned. Eleven of the 14 secondary clones examined were positive for human α-1-AT. The segregation of human α-1-AT and human chromosomes in these 14 hybrids as assayed by enzyme analysis or karyotyping (or both) is shown in Table 2. The only human chromosome that showed a high degree of concordant segregation with the expression of human α-1-AT was chromosome 14. Only one clone of the 14 examined was found to be discordant. This clone did not produce α-1-AT but expressed low levels of human NP. Karyotypic examination showed that only 2 out of 10 cells had human chromosome 14.

NP Isozymes in Mouse–Human and Rat–Human Hybrids. Human NP activity in both mouse–human and rat–human hybrids is shown in Fig. 1 The isozyme pattern in the mouse–human hybrids shows the typical four bands reported by Ricciuti and Ruddle (25) whereas the rat–human hybrids have only a two-band pattern and do not show heterotrimers.

α-1-AT Analysis. To show that the hybrids were synthesizing human α-1-AT, cells were incubated with [^{35}S]methionine in serum-free medium. The medium was then examined by Laurell immunoelectrophoresis. An autoradiograph of supernate from rat hepatoma–human fibroblast hybrids is shown in Fig. 2. Three hybrids secreted isotopically labeled α-1-AT while a fourth was negative. Furthermore, human α-1-AT secreted by 17A, a mouse–human hybrid, was shown to have immunologic identity by Ouchterlony double diffusion with a protein in human serum (Fig. 3).

DISCUSSION

α-1-AT has been assigned by techniques of somatic cell hybridization to chromosome 14 of man. This assignment is consistent with the assignment of the immunoglobulin heavy chain locus to chromosome 14 (9, 10).

The mouse hepatoma–human lymphoid hybrids used in this analysis expressed human α-1-AT although the human parental line was not of hepatic origin. Expression of human α-1-AT in the lymphoblastoid hybrids showed a direct correlation with the presence of chromosome 14 suggesting that, in this set of clones, the human gene was activated whenever it was present. The mechanism of activation is unknown. However, the murine synthetic, processing, and secretory mechanisms of the hepatoma cells must be sufficiently similar to those of man to permit the expression of the human α-1-AT gene transcript. No other human chromosomes were consistently present in the hybrid cells and, thus, it appears that no human processing genes are required for expression of the protein in this hybrid cell system.

Although our data show that no other unlinked human gene is required for the expression of human α-1-AT in the mouse–human hybrids, a second locus cannot be excluded in the rat hybrids. Examination of the rat–human hybrids shows that chromosomes 4, 6, 10, 12, 13, 15, and the X are all candidates for carrying a second locus governing α-1-AT expression because no hybrid produced α-1-AT in the absence of these chromosomes. Additional subclones would need to be studied to confirm or deny the role of genes on any or all of these chromosomes in the expression of human α-1-AT.

It is interesting that the isozyme pattern of NP in the rat–human hybrids shows no heteropolymers. One possible explanation of this observation is that the rat enzyme may be a monomer that does not interact with the human subunits.

It is theoretically possible that chromosome 14 carries a gene that is responsible for glycosylation of α-1-AT rather than the structural locus. In this case, the protein would be of rodent origin with a human glycosyl or sialic acid pattern. Under this hypothesis, the antiserum would recognize only the carbohydrate residues. The antiserum used recognizes the ZZ variant

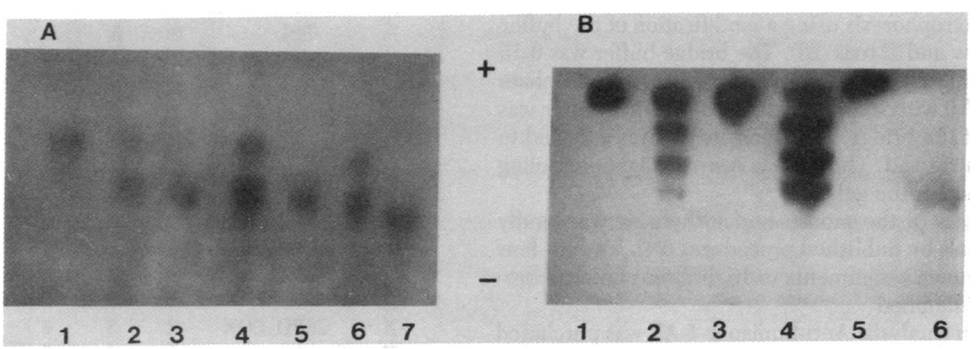

FIG. 1. NP in rat–human (*A*) and mouse–human (*B*) hybrids. (*A*) NP was analyzed by starch gel electrophoresis. Lanes: 1, human control; 7, rat control; 2, 4, and 6, rat–human hybrids positive for expression of human NP; 3 and 5, hybrids negative for human NP. (*B*) NP was analyzed by cellogel electrophoresis. Lanes: 6, human control; 1, mouse control; 2 and 4, mouse–human hybrids positive for expression of human NP; 3 and 5, hybrids negative for human NP.

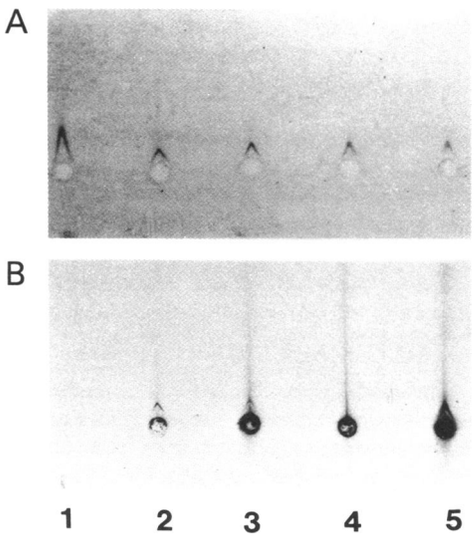

FIG. 2. (*A*) Laurell immunoelectrophoresis gel stained with Coomassie blue. Wells: 1, 1:128 dilution of human serum; 2, 3, and 5, 1:256 dilution of human serum as carrier protein and [^{35}S]methionine-labeled supernatant medium from rat–human hybrids that produced α-1-AT; 4, 1:256 dilution of human serum and supernatant medium from a nonproducing clone. (*B*) Autoradiogram of the gel in *A*. Labeled human α-1-AT is present in the medium from hybrids in wells 2, 3, and 5. No peak is seen in well 4, showing the absence of human α-1-AT in this hybrid.

form of α-1-AT. The ZZ protein is reported to have aberrant addition of sialic acid residues, suggesting that this heterologous serum may have specificity for the protein portion of the molecule. The fact that the protein from 17A, a mouse–human hybrid, has no partial reaction and shows identity with human α-1-AT in serum shows that the protein portion of the α-1-AT secreted by the hybrid cell is not of mouse origin.

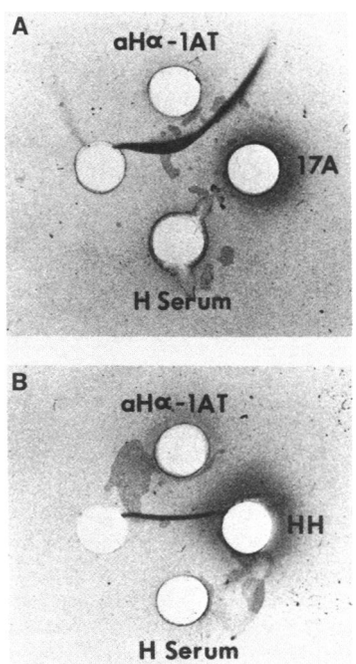

FIG. 3. Ouchterlony double-diffusion tests. (*A*) The right-hand well contains concentrated (80-fold) serum-free supernatant medium from mouse–human hybrid 17A; the bottom well contains human (H) serum (1:480 dilution), and the top well contains anti-human α-1-AT (aHα-1-AT) (1:8 dilution). The precipitation line shows complete identity of a protein in the two wells. (*B*) An identical Ouchterlony plate with the exception that the right-hand well contains concentrated (100-fold) supernatant medium from the mouse parent line HH. No cross-reaction of mouse and human α-1-AT was found.

The retention of α-1-AT in the hepatocytes of patients who have the ZZ phenotype has been described by others (5, 6). One explanation for the accumulation of the ZZ α-1-AT protein is that it is improperly processed and fails to be secreted. It would be of interest to determine whether the ZZ gene transcript would be aberrantly processed in the mouse–human hybrid cells.

In conclusion, our data place α-1-AT on chromosome 14. This assignment was made by using somatic cell hybrids between rodent hepatoma cells and human lines, one of which was of nonhepatic origin. The expression of human α-1-AT in the hybrids, despite the fact that the human lymphoid cells did not produce this protein, demonstrates the usefulness of activated hybrid cells for mapping human liver-specific genes.

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