Chromosomal Assignments of Human Genes for Serine Proteases Trypsin, Chymotrypsin B, and Elastase

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Abstract—The genes for the serine proteases trypsin, chymotrypsin B, and elastase were chromosomally assigned in man using cDNA probes that have been isolated from a rat pancreatic cDNA library. DNA from human × rodent somatic cell hybrids was cleaved with BamHI or EcoRI and analyzed by Southern filter hybridization methods for the segregation of the genes for trypsin-1 (TRY1), chymotrypsin B (CTR), and elastase-1 (ELA1). TRY1 was assigned to human chromosome 7q22→qter, CTR to chromosome 16, and ELA1 to chromosome 12. Although the three genes are members of the same gene family, they are dispersed over different chromosomes.

INTRODUCTION

The serine proteases are a group of extensively studied enzymes that include the pancreatic endopeptidases, enzymes involved in blood clotting, and enzymes involved in the complement system (3–9). The serine proteases have related structures and functions and have a reactive serine residue at the active site (10). These enzymes are closely related and are suggested to have evolved from a common ancestral gene (11, 12).

Trypsin (EC 3.4.11.4), chymotrypsin B (EC 3.4.11.6), and elastase (EC 3.4.11.7) are pancreatic endopeptidases involved in digestion (13–15). They are synthesized as inactive zymogens (trypsinogen, chymotrypsinogen B, and proelastase) which are activated by the cleavage of a NH$_2$-terminal activation peptide fragment. Each enzyme has a different and characteristic substrate specificity: trypsin cleaves peptides at lysine and arginine residues, chymotrypsin B cleaves at aromatic residues, and elastase cleaves at neutral residues, with a preference for residues with small side chains.

The serine proteases comprise a gene family with related function, and the study of the genetic organization of trypsin, chymotrypsin B, and elastase will increase our understanding of the evolution and chromosomal organization of this gene family. Trypsin-
ogen-1, chymotrypsinogen B, and proelastase-1 cDNA probes have been isolated from a rat pancreatic cDNA library (16, 17; Bell et al., manuscript in preparation), and these probes were used to assign the corresponding human genes to their chromosomes using human × mouse somatic cell hybrids. We report that the gene coding for trypsin-1 (TRY1) is on the q22—qter region of chromosome 7, the gene for chymotrypsin B (CTRB) is on chromosome 16, and the gene for elastase-1 (ELAI) is on chromosome 12.

MATERIALS AND METHODS

Somatic Cell Hybrids. Human × mouse somatic cell hybrids were generated by using polyethylene glycol (18) or Sendai virus (19) to fuse mouse cell lines (LM/TK−, A9, or RAG) to various human fibroblasts. Hybrid clones were isolated from the fusions using the hypoxanthine, aminopterin, thymidine selection system (20). The cell hybrids isolated were from the independent hybrid set DUA and ICL (21), XTR (22, 24), ATR (23), TSL (24), EXR and XER (25), WIL (26), REW (27), ACR and JWR (28), JSR (29), NSL (30), DUM, MAR, RAS and REX (31). The JSR hybrids were derived from a fusion between RAG and the human cell line JoSt [46,XY;17p(q22);p24] (29).

Chromosome Composition of Cell Hybrids. The human chromosomal contents of the hybrid cell lines were determined by direct karyotyping (32) and by assaying enzyme markers previously assigned to each of the 22 human autosomes and the X chromosome (33, 34).

Proelastase, Trypsinogen, and Chymotrypsinogen B cDNA Probes. The probes for the three genes were obtained from a rat pancreatic cDNA library. The proelastase-1 probe (pXP13) contains a 920-bp insert (17), the trypsinogen-1 probe (pXP4-78) contains an 850-bp insert (16), while the chymotrypsinogen B probe (pXP33) has a 370-bp insert (Bell et al., manuscript in preparation).

DNA Isolation and Southern Blot Hybridization. DNA was isolated from hybrid cell cultures at the same passage for which chromosome studies and homogenates for enzyme marker studies were prepared (35, 36). Samples of DNA (10 μg) were cleaved with restriction endonucleases using buffers suggested by the manufacturers. DNA fragments were separated by electrophoresis through an 0.8% agarose gel and transferred to nitrocellulose filters (Schleicher and Schuell) by the method of Southern (35, 37). The cDNA probes were labeled with 32P nick translation (38) and hybridized to the nitrocellulose filters at 42°C as described (39, 40), except that 40% formamide was used. The filters were washed at 50°C and exposed to XAR film at −70°C as described (39).

RESULTS

Chromosomal Assignment of Trypsin Gene. The trypsinogen-1 cDNA probe hybridized to human BamHI-digested DNA fragments of lengths ~28 kb, 18.3 kb, 13.3 kb, 8.1 kb, 6.2 kb, and 3.8 kb (Fig. 1). The most intensely hybridizing DNA fragment (3.8 kb) could be distinguished from the mouse DNA fragments (which ranged from 28 kb to 5.0 kb) and was therefore used to follow the segregation of human trypsin-1 (TRY1) gene sequences in human × mouse somatic cell hybrids. Twenty-one independent hybrid cell lines previously characterized for their human chromosome contents by karyotyping were analyzed for human TRY1 (Table 1). The analysis of an additional 32 cell hybrids characterized by enzyme markers is summarized in the “% discordant” section of Table 1. The 3.8-kb BamHI fragment segregated with chromosome 7 and the chromosome 7 markers β-glucuronidase (GUSB) and phosphoserine phosphatase (PSP) in the cell hybrids with only a single discordancy. That is, TRY1 was present when chromosome 7 was present, and absent when chromosome 7 was absent. All other markers showed ≥29% discordancy.
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Fig. 1. Hybridization of the trypsinogen-1 cDNA probe to human (lane 6), mouse (lane 5), and cell hybrid (lanes 1–4) DNA cleaved with BamHI. Cell hybrid DNAs in lanes 2 and 3 are positive for the human 3.8-kb band, and the DNAs in lanes 1 and 4 are negative.

The subclones JSR-17G and JSR-17S both had a q22→qter deletion in chromosome 7 (29), and TRY1 was not detected in either subclone. TRY1 was therefore localized to the region 7q22→qter. Since TRY1 is located in the terminal region of chromosome 7, it is possible that this region has been deleted in the discordant cell hybrid (NSL-16). Other chromosomal breaks have been observed in the NSL hybrids (unpublished data).

Chromosomal Assignment of Chymotrypsin B Gene. The chymotrypsinogen B cDNA probe hybridized to human EcoRI-digested DNA fragments of lengths 12 kb and 8.5 kb (Fig. 2). Mouse RAG bands of lengths 13.6 kb, 6.1 kb, and 5.4 kb, and mouse LM/TK- bands of lengths 13.6 kb and 5.4 kb were detected, thus demonstrating a DNA polymorphism for these two cell lines. The 8.5-kb human band could be distinguished from the mouse bands and was used to follow the segregation of chymotrypsin B (CTRB) gene sequences in 51 karyotyped and nonkaryotyped cell hybrids (Table 1). CTRB segregated concordantly with chromosome 16 and the chromosome 16 marker, adenine phosphoribosyltransferase (APRT), with only one discordancy, while all other markers showed ≥28% discordancy. The discordant cell hybrid had a weak human APRT, indicating that chromosome 16 was present in only a small proportion of cells. Because of the low hybridization intensity of the rat CTRB cDNA probe to the human gene, it was not possible to detect the weak signal in this cell hybrid.

Chromosomal Assignment of Elastase Gene. The proelastase cDNA probe hybridized to human BamHI-digested DNA fragments of lengths 15.9 kb, 8.4 kb, 6.0 kb, and 3.6 kb and to mouse DNA fragments of lengths 10.0 kb and 4.2 kb (Fig. 3). The segregation of the most intensely hybridizing human DNA fragment (15.9 kb), containing elastase-1 (ELAI) gene sequences, could be followed in 41 cell hybrids, while the other bands were too weak to be reliably detected in cell hybrids (Table 1). ELAI segregated concordantly only with chromosome 12 and the chromosome 12 markers lactate dehydrogenase B (LDHB) and peptidase B (PEPB), with all other markers showing ≥33% discordancy. The 15.9-kb human band was relatively weak in the cell hybrids, as human chromosome 12 was not selected for and was retained in these hybrids at low percentages.

DISCUSSION

The trypsinogen-1 and proelastase-1 cDNA probes detected complex patterns of human BamHI-digested DNA fragments. The most intensely hybridizing DNA bands (3.8 kb for trypsinogen-1 and 15.9 kb for proelastase-1) probably had the greatest homologies with the probes, and the gene sequences thus identified were designated as trypsin-1 (TRY1) and elastase-1 (ELAI), respectively. We assigned TRY1 to chromosome 7q22→qter and ELAI to chromosome 12. The less intensely hybridizing bands could not be mapped either because they could not be distinguished from mouse bands (trypsin) or because they were too weak to be detected reliably in cell hybrids (elastase). It is not presently known whether these DNA fragments contain part of the TRY1 or ELAI gene sequences or whether some of them may rep-
| Try-1 | Ctrb | Era-1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X |
| DUA-1CsAzF | + | □ | □ | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| DUA-1CsAzH | + | □ | □ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| REW-5 | □ | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + |
| ICL-15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| WIL-6 | - | - | - | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | - | - | - | - | - | - | - |
| WIL-8X | + | - | - | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | - | - | - | - | - | - | - |
| XTR-22 | - | - | + | - | + | + | + | - | + | + | - | - | + | + | + | + | + | + | - | - | - | - | - | - | - |
| TSL-2 | - | - | + | - | - | - | - | - | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - |
| NSL-9 | - | + | + | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| JWR-26C | + | + | □ | - | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | - | - | - | - | - | - | - |
| XER-7 | + | - | □ | - | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| XER-11 | + | + | □ | - | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - |
| NSL-15 | + | - | □ | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| NSL-16 | - | □ | - | - | + | + | + | + | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ATR-13 | + | □ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| WIL-14 | - | □ | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| DUM-13 | + | □ | □ | - | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NSL-5 | - | □ | - | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| REW-7 | + | □ | □ | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| REW-11 | □ | + | □ | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| EXR-3 | □ | + | □ | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| EXR-9 | □ | + | □ | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + |

Table I. Segregation of TRY1, CTRB, and ELA1 with Human Chromosomes in Somat Cell Hybrids

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| Cell Line | q12 | q11 | q10 | q9 | q8 | q7 | p1 | p2 | p1 | X | 11 | 12 | Total % | Discordancy |
|-----------|-----|-----|-----|----|----|----|----|----|----|----|----|----|---------|--|-------------|
| XER-9     | D   |     |     | +  |     |     | +  |     |     |     | +  |     |     | 11/X    |             |
| MAR-2     | D   |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| DUAS1     |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| REX-12    | D   |     |     |     |     |     | +  |     |     |     | +  |     |     |         |             |
| ALR-18    | D   |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| WIL-2     |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| WIL-13    |     |     |     |     |     |     | +  |     |     |     | +  |     |     |         |             |
| JSR-17G   |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| JSR-17S   |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
| Total %   |     |     |     | +  |     |     | +  |     |     |     | +  |     |     | 22/X*   |             |
| Discordancy |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |
|            |     |     |     | +  |     |     | +  |     |     |     | +  |     |     |         |             |

*17/3 (3qter→3q21::Xq28→Xpter), 17/3 (3qter→3p21::17p13→17pter) and 3/17 (3qter→3p21::17p13→17pter).
11/X (Xpter→Xq11::11p11→11qter) and X/11 (Xpter→Xq11::11p11→11qter).
17/9 (17pter→17p11::9p11→9pter) and 9/17 (17pter→17p11::9p11→9pter).
X/5 (Xpter→Xq22::5q35→5qter) and 5/X (Xpter→Xq22::5q35→5qter).
X/15 (15qter→15q11::Xpter→Xp11) and 15/X (15pter→15q11::Xp11→Xpter).
22/X (22pter→22q13::Xq22→Xpter).
7q*: del (7q22→qter).

Percent discordant segregation between human chromosome and serine protease gene. The total discordances for karyotyped cell hybrids and cell hybrids analyzed for enzyme markers only (data not shown) are included here; 32 nonkaryotyped cell hybrids were analyzed for the segregation of TRY1, 31 for the segmentation of **CTR**, and 28 for the segmentation of **ELA**.

* DNA not tested with this probe.
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Fig. 2. Hybridization of the chymotrypsinogen B cDNA probe to human (lane 7), mouse (lanes 1, 6), and cell hybrid (lanes 2–5) DNAs cleaved with EcoRI. Lane 1 contains DNA isolated from LM/TK- and lane 6 contains DNA isolated from RAG. Cell hybrid DNAs in lanes 2 and 4 are positive for human CTRB, and the DNAs in lanes 3 and 5 are negative.

A number of gene families have been identified, and their constituent genes may be dispersed over several chromosomes or closely linked on a single chromosome (for examples see refs. 33, 41–45). Dispersed gene families may reflect the antiquity of the time of duplication of the ancestral genes and divergence of structure and function. On the other hand, members of gene families that are on the same chromosome may suggest that the ancestral gene duplications occurred relatively recently or that there are functional constraints requiring that the genes remain closely linked.

The serine proteases are located on a number of different chromosomes. Chymotrypsin B (chromosome 16) has an overall 38% identity in amino acid sequence with both trypsin (chromosome 7) and elastase (chromosome 12) in mammals, while trypsin and elastase have a 35% identity (13). This homology suggests that they evolved from a common ancestral gene that duplicated and diverged early in vertebrate history (11). Other serine proteases that have previously been mapped onto different chromosomes are the genes for plasminogen (chromosome 4), plasminogen activator (chromosome 6), complement component BF (chromosome 6), coagulation factor X (chromosome 13), complement component C3 (chromosome 19), and coagulation factor IX (X chromosome) (33, 46, 47). Haptoglobin is not a serine protease, but has 19% homology with chymotrypsin B and is thought to have evolved from a common ancestral gene (43, 48). The gene for haptoglobin is also on chromosome 16 (49). The presence of the serine proteases on different chromosomes implies that there are no functional or regulatory constraints requiring linkage.

It would now be of interest to determine whether closely related serine proteases resulting from recent duplications, such as trypsin-1 and trypsin-2 (with 89% homology in rat), chymotrypsin A and chymotrypsin B

Fig. 3. Hybridization of the proelastase-1 cDNA probe to human (lane 6), mouse (lane 5), and cell hybrid (lanes 1–4) DNAs cleaved with BamHI. Cell hybrid DNAs in lanes 1 and 2 are positive for human ELAI, and the DNAs in lanes 3 and 4 are negative.
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(with 79% homology in cow), and elastase-1 and elastase-2 (with 58% homology in rat) are on the same or different chromosomes.

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LITERATURE CITED