Chromosomal Assignments of Genes for Trypsin, Chymotrypsin B, and Elastase in Mouse


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Abstract—The mouse genes for the serine proteases trypsin (Try-1), chymotrypsin B (Ctbrb), and elastase (Ela-1) were chromosomally assigned using Southern blot hybridization of mouse × Chinese hamster cell hybrid DNA. cDNA probes for the three genes were hybridized to cell hybrid DNA cleaved by BamHI or HindIII and the segregation of Try-1, Ctbrb, and Ela-1 was correlated with the segregation of mouse chromosomes. Try-1 is located on chromosome 6, Ctbrb is on chromosome 8, and Ela-1 is on chromosome 15. The three genes fall into three syntenic groups that are conserved in the mouse and human genomes.

INTRODUCTION

Trypsin (EC 3.4.4.4), chymotrypsin B (EC 3.4.4.6), and elastase (EC 3.4.4.7) are members of a group of enzymes known as the serine proteases, which are characterized by their similar proteolytic functions and involvement of a serine residue in their active sites (2–4). Trypsin, chymotrypsin B, and elastase are synthesized as inactive precursors (zymogens) that are activated by the cleavage of NH₂-terminal peptides. They act in the intestinal digestion of proteins, although each has a characteristic substrate specificity, and trypsin has the additional function of zymogen activation. The three enzymes have similar three-dimensional structures and related amino acid sequences, suggesting that they have evolved from a common ancestral gene and are members of a gene family (5, 6).

As an increasing number of genes are being mapped in both man and mouse, more conserved linkage groups are being identified (7). In each of these conserved linkage groups, two or more genes that are syntenic in man are also found to be syntenic in mouse. Such observed linkage groups imply that certain regions of chromosomes may have been maintained without undergoing major rearrangements following the divergence of the human and mouse ancestors. Some syntenic groups have now been found to be conserved over a number of different species; for example, phosphogluconate dehydrogenase (PGD) and
enolase-1 (EN01) are linked in 17 different mammalian species (7). As our knowledge of linkage groups that have been conserved in man and mouse (and in other mammals) increases, the pattern of chromosomal changes that has taken place since the divergence of their ancestors will be better understood.

cDNA probes for the zymogens trypsinogen-1, chymotrypsinogen B, and proelastase-1 have been isolated from a rat pancreatic cDNA library (8, 9, Bell et al., manuscript in preparation). We used these probes to map the genes for the corresponding enzymes in mouse using mouse × Chinese hamster cell hybrids. We assigned the gene for trypsin-1 (Try-1) to chromosome 6, chymotrypsin B (Ctbrb) to chromosome 8, and elastase-1 (Ela-1) to chromosome 15.

We have chromosomally assigned the equivalent human genes for trypsin-1, chymotrypsin B, and elastase-1 (10), and they fall into three syntenic groups that are conserved in man and mouse. The comparison of the chromosomal locations of these three genes in man and mouse increases our knowledge of the comparative gene map and should aid our understanding of the evolution and genetic organization of the serine protease gene family.

MATERIALS AND METHODS

Somatic Cell Hybrids. Mouse × Chinese hamster somatic cell hybrids were generated by using polyethylene glycol (11) to fuse Chinese hamster cells (clone E36 derived from V-79, or Dona3) with mouse cells [BALB/c spleen cells or an F1 (Peru × B10129)]. The EBS series of cell hybrids was derived from E36 and BALB/c (12, 13), while the PBH cell hybrid was derived from Dona3 and the F1 (Peru × B10129) (unpublished data). The hybrids were characterized for their mouse chromosome content by karyotyping and analysis of enzyme markers of known chromosomal location (13). The cell hybrids EBS-5Cs, EBS-9Cs, EBS-13Cs, and PBH-8 were analyzed by enzyme markers only, and so mouse chromosomes 3, 13, and 15 could not be scored in these cases.

Trypsinogen, Chymotrypsinogen B, and Proelastase cDNA Probes. Rat pancreatic probes for trypsinogen-1 [pcXP4-78, with an 850-bp insert (8)], chymotrypsinogen B [pcXP33, with a 370-bp insert (Bell et al., manuscript in preparation)], and proelastase-1 [pcXP13, with a 920-bp insert (9)] were obtained from rat pancreatic cDNA library. These probes were used to detect the genes for the corresponding enzymes trypsin-1 (Try-1), chymotrypsin B (Ctbrb), and elastase-1 (Ela-1).

DNA Isolation and Southern Blot Hybridization. DNA was isolated (14) from hybrid cell cultures at the same passage for which chromosome studies and homogenates for enzyme studies were prepared. The DNA was cleaved with restriction endonucleases, separated by agarose electrophoresis, and transferred to nitrocellulose by the method of Southern (15) as previously described (16). The nitrocellulose filters were hybridized to the cDNA probes labeled with 32P by nick translation (17–19). After two days' hybridization, the filters were washed and exposed to Kodak XAR film at −70°C, as previously described (17).

RESULTS

Chromosomal Assignment of Trypsin-1. The trypsinogen-1 cDNA probe hybridized to mouse BamHI-cleaved DNA fragments of lengths 24 kb, 18 kb, 12.8 kb, 9.2 kb, and 6.5 kb, and to Chinese hamster DNA fragments of lengths 24 kb, 18 kb, and 8.4 kb (Fig. 1A). The segregation of the most intensely hybridizing mouse bands (12.8 kb and 6.5 kb) and of the 9.2-kb band was followed in 20 mouse × Chinese hamster hybrids (Table 1). These bands, containing mouse trypsin-1 (Try-1) gene sequences, segregated only with chromosome 6 and the enzyme marker for chromosome 6, trioseposo-
Fig. 1A. Hybridization of the trypsinogen-1 cDNA probe to mouse (M), Chinese hamster (CH), and cell hybrid DNAs cleaved with BamHI. Cell hybrid DNAs in the lanes marked + are positive for mouse Try-1, and the DNAs in the lanes marked – are negative.

Fig. 1B. Hybridization of the trypsinogen-1 cDNA probe to mouse (M), Chinese hamster (CH), and cell hybrid DNAs cleaved with EcoRI. Cell hybrid DNAs in the lanes marked + are positive for mouse Try-1, and the DNAs in the lanes marked – are negative.
Table 1. Segregation of Try-1, CIRb, and ELa-1 with Mouse Chromosomes in Hybrids

| Try-1 | CIRb | ELa-1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | X |
| EBS-1 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-3 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-4 | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| EBS-5 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-5C* | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-9 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-9C* | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-10 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-11 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-12 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-13C* | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-15 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-17 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-18 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-51 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-63 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-71 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| EBS-74 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| PH-8* | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |

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*These cell hybrids were analyzed by enzyme markers only, and not by karyotyping.

*NS Not scored.

<10% of cells have chromosome 6.

*EBS-12 was not analyzed with the proelastase-1-cDNA probe.

*Broken chromosome 15.

phate isomerase-1 (*Tpi-1*). The trypsinogen-1 probe hybridized to nine mouse EcoRI DNA fragments (18.3 kb, 14 kb, 13.2 kb, 10.6 kb, a double band at 8.7 kb, 7.3 kb, 3.1 kb, and 2.9 kb) (Fig. 1B), and the segregation of seven of these bands could readily be followed in mouse × Chinese hamster cell hybrids. All seven bands segregated with chromosome 6.

**Chromosomal Assignment of Chymotrypsin B.** The chymotrypsinogen B cDNA probe hybridized to mouse HindIII-cleaved DNA fragments of lengths 5.3 kb and 3.1 kb, which were distinguishable from the Chinese hamster DNA fragments (8.8 kb, ~5 kb, and 2.1 kb) (Fig. 2). Twenty mouse × Chinese hamster cell hybrids were analyzed for the presence of the mouse DNA fragments containing mouse chymotrypsin B (*CIRb*) gene sequences (Table 1). *CIRb* segregated only with chromosome 8 and the enzyme marker for chromosome 8, glutathione reductase (Gr1).

**Chromosomal Assignment of Elastase-1.** The proelastase-1 cDNA probe hybridized to mouse BamHI-cleaved DNA fragments of lengths 10.0 kb, 8.9 kb, and 4.2 kb, which were easily distinguished from the Chinese hamster DNA fragments (15.9 kb and 6.1 kb) (Fig. 3). Sixteen hybrids were analyzed for the presence of the 10.0 kb and 4.2 kb bands (Table 1); however, the 8.9-kb band was weak in mouse parental DNA and generally too weak to score hybrids. The elastase-1 (*ELa-1*) gene sequences segregated only with chromosome 15. The hybrid EBS-18 contained a mouse chromosome 15 with a deletion of the terminus in the band F3. Mouse *ELa-1* was detected in EBS-18, however, suggesting that the gene is located in the region proximal to the breakpoint in F3.
different chromosomes. In contrast, a group of 25–30 highly homologous (~75%) kallikrein genes that form a subgroup of the serine protease gene family are clustered, probably to a single locus, on mouse chromosome 7 (20, 21). These observations indicate that at least some closely related serine protease genes may be linked, but the more divergent members of the gene family are likely not to be linked.

There are a number of trypsin, chymotrypsin, and elastase genes within the serine protease family. Three trypsin activities have been reported in mouse, with one of them coded by the Prt-1 locus (22). Prt-3 is a closely linked locus that controls the levels of activity of the Prt-1 gene product (23). It is not known whether Try-1 is equivalent to Prt-1 or represents a gene coding for one of the other trypsin activities. There may be a number of trypsin-like genes or pseudogenes in rat (unpublished data), and one has been identified that is closely related to trypsin-1 and cross-hybrids with it (8). However, as at least three of the five BamHI and seven of the nine EcoRI DNA fragments detected by the trypsinogen-1 probe cosegregate in cell hybrids (see results), any trypsin genes thus detected in mouse in addition to Try-1 are probably also on chromosome 6.

A single chymotrypsin activity has been reported in mouse, which is coded by the Prt-2 locus on chromosome 8 (22, 24). However, at least two chymotrypsins [A and B, with 78% homology (2)] have been characterized in other mammals, and both genes may similarly be present in mouse. We therefore cannot be certain whether Prt-2 corresponds to Ctrb or to some other chymotrypsin gene on chromosome 8.

cDNA probes for two rat elastase genes (elastase-1 and elastase-2, with 58% amino acid homology) have been isolated (9). However, these probes did not cross-hybide and, if there are similarly two equivalent mouse elastase genes, it is not likely that we detected the elastase-2 gene.
When homologous genes are mapped in man and mouse, conserved linkage groups are often observed, in which two or more genes that are syntenic in man are syntenic in mouse. We have mapped the human genes equivalent to Try-1 (TRY1) on human chromosome HSA (HSA refers to the Homo sapiens chromosome) 7q22→qter, Cirb (CTRB on HSA 16), and Ella-1 (ELA1 on HSA 12) using the same DNA probes (10). When the chromosomal assignments of the three genes were compared in man and mouse, three conserved linkage groups were identified. Mouse carboxypeptidase A (Cpa) is on mouse chromosome MMU (MMU refers to the Mus musculus chromosome) 6 and human CPA is on HSA 7q22→qter (1, 27) and therefore forms a syntenic group with trypsin-1. The gene for elastase-1 forms a syntenic group with the putative mammalian tumor oncogene int-1 on MMU 15 and INTI on HSA 12 (28, 29). The gene for chymotrypsin B forms part of a syntenic group with mouse glutamate oxaloacetate transaminase-2 (Got-2) and adenine phosphoribosyltransferase (Aprt) on MMU 8 and human G0T2 and APRT on HSA 16 (25, 26, 30). These and other conserved linkage groups identified between particular mouse and human chromosomes illustrate the chromosomal rearrangements that have taken place during the divergence of man and mouse. As more data are collected on syntenic groups in these and other species, a more thorough knowledge of the chromosomal evolution between species will be developed.

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LITERATURE CITED


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