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Localization of the α_2-macroglobulin gene and *Lpm* gene family on mink chromosome 9

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Summary. Using cloned cDNA for human α_2-macroglobulin (*A2M*) as a probe, mink-Chinese hamster hybrid cells were analysed. The results allowed us to assign a gene for *A2M* to mink chromosome 9. Breeding tests demonstrated that the *Lpm*-locus coding for other related α-macroglobulin protein and the gene for peptidase B (*PEPB*) are linked 11 ± 3 cm apart. The *PEPB* gene is located on mink chromosome 9, and hence, the *Lpm*-locus is on the same mink chromosome. The relationship of the genetic systems controlling the isotypically different α-macroglobulins in mink serum are discussed.

Key words: α-Macroglobulin genes – Chromosome mapping – Mink

Introduction

α_2-Macroglobulin is a serum glycoprotein with two functions. One function is to inactivate the endopeptidases of various classes, the other is to modulate the influences exerted upon the immune system (Sottrup-Jensen et al. 1984). There is increasing evidence indicating that serum α-macroglobulins are represented by a group of proteins. They have the same molecular weight and common antigenic determinants. The structural genes for these proteins might have derived from a common precursor gene (Weström et al. 1983; Carlsson et al. 1985; Sand et al. 1985). Of the proteins in this group, human α_2-macroglobulin (*A2M*) appears to be the one most studied. The complete amino acid sequence of its polypeptide chain has been determined (Sottrup-Jensen et al. 1984), the encoding gene *A2M* has been located on chromosome 12, and the primary structure of its cDNA is known (Kan et al. 1985; Bell et al. 1985). The genes for the other proteins of the α-macroglobulin group have not, so far, been investigated.

The mink is advantageous for analysis of the α-macroglobulin protein group. In the serum from adult minks, at least two isotypically different variants of α-macroglobulins can be identified (Yermolaev and Baranov 1980), as in dog, pig (Weström et al. 1983), and other mammalian species, with the exception of man (Sottrup-Jensen et al. 1984; Sand et al. 1985). One of the α-macroglobulin variants is homologous to human *A2M* in general antigen structure. It will henceforth be designated as mink *A2M*. A salient feature of another α-macroglobulin variant, tentatively termed *Lpm* (Lipo-Protein of Mink), is its alloantigenic polymorphism. Immunogenetic studies demonstrated the presence of closely linked genes at the complex *Lpm*-locus in mink. These genes show a high level of homology and form a multigene family (Baranov 1976, 1988; Baranov et al. 1976, 1978, 1984).

This paper is concerned with the localization of *Lpm* and *A2M* genes on mink chromosome 9, which presumably has a conserved region similar to human chromosome 12.

Materials and methods

To map the *A2M* gene, clones of the originally described somatic cell hybrids between mink and Chinese hamster were used (Serov et al. 1987). All the clones of the panel were examined cytogenetically. Clones with no visible chromosomal rearrangements were chosen for further analysis. There were two exceptional clones: D7B-1 in which we identified a chromosome similar in G-banding pattern to mink chromosome 13, and L25-1 containing its fragment. The distribution of mink chromosomes among the hybrid clones of the panel were described elsewhere (Matveeva et al. 1987; Khlebodarova et al. 1988).

The procedures of DNA extraction from cell hybrids and from the parental cell lines, the conditions for digestion of DNA

with restrictase EcoR1, electrophoresis in 0,8% agarose gel, and transfer to nitrocellulose were all described (Matveeva et al. 1987).

A ^{32}P-labeled cDNA fragment of the human *A2M* gene (664 and 1,425 bp) was used as a probe in the localization of mink *A2M* gene. The fragment was derived from the hybrid plasmid pkT 218, kindly provided by G. I. Bell (Bell et al. 1985). The DNA probes were prepared by nick translation to a specific activity of $3-4 \times 10^8$ cpm/mg of DNA. All prehybridizations and hybridizations were performed standardly (Matveeva et al. 1987).

Allotyping of the *Lpms* in mink serum was carried out using the previously raised alloantisera by double immunodiffusion in agar gel (Baranov et al. 1978, 1984). Figure 1 shows how *Lpm1* was allotyped. The immune precipitate formed between the well with antiserum and the tested sample was evidence that it contained the corresponding immunogenetic markers of *Lpm*. The set of *Lpm*-allotypes identified in a mink make up its *Lpm*-phenotype. The *Lpm*-allotypes are inherited as an allogroup encoded by the respective *Lpm*-haplotypes (Baranov et al. 1978, 1984; Baranov 1988).

The enzyme peptidase B occurs in minks as two allelic variants. The method utilized for its detection has been described elsewhere. The *PEPB* gene resides on mink chromosome 9 (Mullakandov et al. 1986).

The minks were from a herd bred at the experimental farm of this Institute. Three groups of females from this herd were homozygotes for *Lpm*-haplotypes, namely $Lpm^{1,2,6,7}/Lpm^{1,2,6,7}$, $Lpm^{3,4,6,8}/Lpm^{3,4,6,8}$, and $Lpm^{4,6,8}/Lpm^{4,6,8}$. They were under continuous immunogenetic control of the *Lpm*-system for years (Baranov et al. 1976, 1978, 1984). All the females were homozygous for the $PEPB^a$ allele too.

Three males were from the freely mating part of the herd. They were heterozygotes for both the *Lpm*-locus ($Lpm^{1,2,6,7}/Lpm^{3,4,6,8}$, $Lpm^{1,2,6,7}/Lpm^4$, and $Lpm^{3,4,6,8}/Lpm^{2,4,5,7}$) and the peptidase B gene ($PEPB^a/PEPB^b$). The mating scheme is given in Table 2.

The significance of the data was treated by Student's t-test.

Results and discussion

Figure 2 presents the results of hybridization of ^{32}P-labeled cDNA for human *A2M* to a Southern blot of EcoR1-digested DNA from Chinese hamster, mink, and several hybrid clones. The EcoR1 fragments of mink DNA of 5 kb, 3 kb, and 1 kb are species-specific, and they were identified in 10 out of the 15 studied clones. The presence of the mink EcoR1 fragments was found to be consistently concordant with chromosome 9 (Table 1). This allowed us to assign the *A2M* gene to mink chromosome 9.

The breeding data summarized in Table 2 show the distribution of the phenotypes controlled by the genes at the *Lpm*-locus and *PEPB*. There were two phenotypic classes of *Lpm* in an almost 1:1 ratio (23:20, 24:24, and 9:11) in each cross. This was in agreement with the results for the *Lpm*-system obtained in our previous studies. The ratio expected for the distribution of the $PEPB^a$ and $PEPB^b$ alleles (21:22, 23:25, and 12:8) and of the progeny sex (61:50) was close to this ratio too. Thus, segregation in Mendelian fashion was observed for each set of traits analysed one by one.

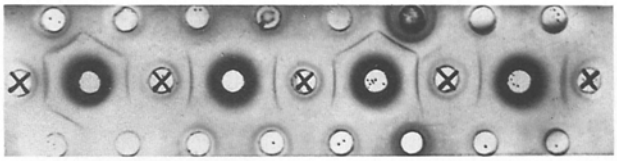

Fig. 1. Allotyping of *Lpm1* in mink sera by Ouchterlony double diffusion. x – a well with control mink serum; the other wells in the middle row contain antiserum against allotype *Lpm1*; the bottom and top wells are with the tested serum sample

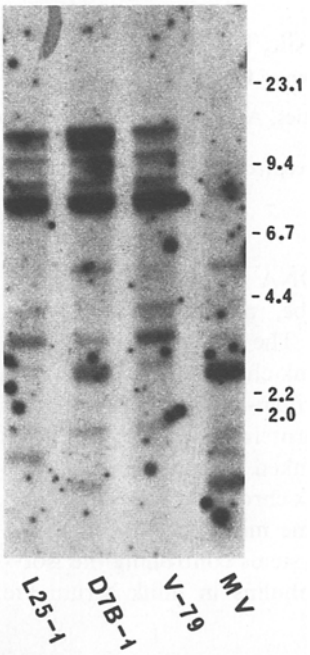

Fig. 2. Hybridization of the *A2M* cDNA probe to mink (MV), Chinese hamster (V-79), and cell hybrid (D7B-1 and L25-1) DNA digested with EcoR1. Cell hybrid DNA positive for the mink 5, 3 and 1 kb bands (D7B-1), the DNA is negative for it (L25-1). The sizes of the hybridizing DNA fragment are indicated in kb (right)

The distribution of the set of traits controlled at the *Lpm* and *PEPB* loci conformed to another pattern. All the four phenotypic classes of progeny that were expected in the test crosses did appear. However, two classes were major in each cross, comprising the majority of progeny, and the other two included its minority. In the case of independent inheritance, the ratio of the identified classes would approach 1:1:1:1. The observed ratio are much different, thereby indicating that the genes for the *Lpm*-allotypes and allelic variants of peptidase B are linked.

The recombination frequencies between the *Lpm*-locus and *PEPB* gene are given in Table 2. The frequencies were estimated for each cross separately and the three crosses taken together. The difference between the recombination coefficient for cross 2 and that for crosses 1 and 3 is insignificant. Taken together, the distance be-

Table 1. Segregation of mink chromosomes and *A2M* gene in mink-Chinese hamster somatic cell hybrids

Cell hybrid	*A2M*	Chromosome														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	X
F3M	+	+	−	+	+	+	+	+	−	+	−	−	−	+	+	+
F12B-1	+	−	+	+	+	+	−	−	+	+	+	−	+	−	+	+
FD9M	+	+	+	−	−	−	−	−	+	+	−	+	+	+	+	+
K02-1	+	+	−	+	+	−	+	−	−	+	+	−	+	+	+	+
L22-1	+	+	−	−	+	+	+	−	−	+	−	−	+	+	−	+
R01-1	+	+	+	+	+	−	+	−	−	+	+	+	−	+	+	+
D7B-1	+	−	−	+	+	+	−	−	+	+	+	+	+	−	−	+
D11B-1	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	+
D12M	+	+	−	−	+	−	+	−	+	+	+	−	+	−	−	+
D13M	+	+	−	+	−	−	+	−	+	+	−	−	−	−	+	+
K12-1	−	+	−	+	−	+	+	+	−	−	+	+	+	−	+	+
L15-1	−	−	−	−	+	−	−	−	+	−	+	+	−	+	+	+
L25-1	−	−	−	−	−	+	+	−	−	−	+	−	+	−	−	+
R14-1	−	−	−	−	−	−	+	−	−	−	+	−	+	+	−	+
D3M	−	+	−	+	−	−	−	+	−	−	+	−	+	−	−	+
Discordant (%)		33	46	40	26	53	40	66	40	0	66	60	53	46	33	33

Table 2. Analysis of the joint inheritance of *Lpm*-allotypes and allelic *PEPB* variants in mink

NN	Genotype* female	Genotype male	Litters	Progeny	Phenotypic classes of offspring and its number				Rf
					Nonrecombinant		Recombinant		
1	1, 2, 6, 7 b / 1, 2, 6, 7 b	1, 2, 6, 7 b** / 3, 4, 6, 8 a	7	43	1, 2, 3, 4, 6, 7, 8 ab 19	1, 2, 6, 7 bb 18	1, 2, 3, 4, 6, 7, 8 bb 4	1, 2, 6, 7 ba 2	11
2	3, 4, 6, 8 b / 3, 4, 6, 8 b	1, 2, 6, 7 b / 4 a	10	48	1, 2, 3, 4, 6, 7, 8 bb 23	3, 4, 6, 8 ba 22	1, 2, 3, 4, 6, 7, 8 ba 1	3, 4, 6, 8 bb 2	6
3	4, 6, 8 b / 4, 6, 8 b	2, 4, 5, 7 b / 3, 4, 6, 8 a	5	20	2, 4, 5, 6, 7, 8 bb 7	3, 4, 6, 8 ba 10	2, 4, 5, 6, 7, 8 ba 2	3, 4, 6, 8 bb 1	15
Total			22	111	99		12		11±3

[a] The *Lpm*-genotypes and *Lpm*-phenotypes are abbreviated (Baranov et al. 1984); the allelic variants of $PEPB^a$ and $PEPB^b$ are assigned symbols "a" and "b", respectively

[b] The linkage of $PEPB^a$ to haplotype $Lpm^{3,4,6,8}$ and Lpm^4 in males is based on the date obtained

tween the *Lpm*-locus and *PEPB* gene is 11±3 cm. In previous experiments, we have estimated that the gene for *PEPB* is located on mink chromosome 9 (Mullakandov et al. 1986) and, hence, *Lpm*-locus resides on the same mink chromosome.

Thus, chromosomal localization of the mink *A2M* gene and *Lpm*-gene family was achieved by two different mapping procedures. Mink *A2M* gene was mapped by mink-Chinese hamster cell hybrids tested with a cloned cDNA for the human *A2M*. The alloantigen polymorphism of *Lpm* (Baranov 1988) allows breeding tests to be applied in analysis of the linkage of this gene family to the polymorphic *PEPB* enzyme whose gene has been previously mapped (Mullakandov et al. 1986).

It should be noted here that the symbol *Lpm* was assigned to a group of the strongest serum alloantigenes of mink. Their immuno-precipitates interact weakly with Sudan black and show esterase activities. The genetic system controlling these allotypes was assigned the same symbol (Baranov et al. 1976; Baranov 1988).

The total number of complex *Lpm*-allotypes identified in mink is now 14. The *Lpm*-allotypes are inherited as allogroups, i.e., as 11 joining to form a fixed combination. For example, 1,2,6,7,10,11,13,14, 3,4,6,8,9,10,11,13,14, 2,4,5,7,9,10,11,12,13,14, 4,9,11,12, 4,6,8,9,10,11,12,13,14, are those occurring in mink involved in the crosses done to localize the *Lpm*-locus. Like all the allogroups, the listed ones are controlled by a complex haploid set of

linked structural genes known as *Lpm*-haplotypes. Thus, at the *Lpm*-locus there is a family of distinct structural genes that show close linkage and allelic relationships (Baranov et al. 1976, 1978, 1984; Baranov 1988).

The studies of the other (Baranov et al. 1978) properties of molecules marked with *Lpm* allodeterminants demonstrated that the mink *Lpm*-protein is similar to the human *A2M* in molecular weight and electrophoretic mobility. This allowed us to refer it to alpha-macroglobulin (Yermolaev and Baranov 1980). Thus, of the two mink α-macroglobulins, the protein with "high" mobility was found to correspond to *Lpm* and the "slower" one to *A2M*. Several genetic and phylogenetic relationships of the mink *Lpm*-system and system *A2M* have been established (Baranov 1988; Yermolaev et al. 1988).

There are increasing data indicating that certain variants of α-macroglobulin are related proteins, although different in more than one respect, and that their controlling genes might have derived from a common precursor (Sottrup-Jensen et al. 1984; Sand et al. 1985; Carlsson et al. 1985; Weström et al. 1983). It thus becomes apparent that the α-macroglobulin group comprises a set of inter- and intraspecific homologs that may be, with good reason regarded as members of the same family.

The most thoroughly characterized member of the α-macroglobulin family is the *A2M* protein and its corresponding unique human *A2M* gene (Sottrup-Jensen et al. 1984; Kan et al. 1985; Bell et al. 1985). Because a part of the human *A2M* gene (Bell et al. 1985) was involved in the experiments, there is reason to suggest that we have located a gene homologous to human *A2M* gene.

Taking advantage of intraspecific polymorphism of mink, we succeeded in localizing the genes for *Lpm*, another mink genetic system of α-macroglobulin. This provided us with direct evidence indicating that two related genetic systems of α-macroglobulins reside on mink chromosome 9.

It should be noted that mink chromosome 9 has a gene common to human chromosome 12 (Serov et al. 1987; Lalley and McKusick 1985).

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