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Mapping of 53 Loci in American Mink (*Mustela vison*)

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Abstract

Fifty-three genes were mapped in the American mink genome using polymerase chain reaction (PCR)-based analysis of a Chinese hamster–American mink somatic cell hybrid panel. Heterologous primers designed for cat gene mapping were used in this study. Forty-nine of these loci were localized into expected chromosome regions according to Zoo-FISH data, whereas four loci—*ALPL*, *CDC20*, *ERF-2*, and *Fc(Mv)23617*—were mapped out of expected conserved regions. PCR products amplified with primers corresponding to these four markers were partly sequenced and verified using BLAST. The results showed the homology to be more than 90% between mink and human or cat counterparts. At present, the gene map of American mink has expanded to 127 loci.

The American mink (*Mustela vison*) is a representative of the large family *Mustelidae* belonging to the suborder *Caniformia* in the order *Carnivora*. Carnivores include hundreds of species living in a broad range of geographic zones from the Arctic to Antarctica, and they are of great interest in comparative gene mapping because they have complex evolution and phylogeny. Moreover, gene mapping in carnivore species contributes to our understanding of mammalian evolution in general.

The American mink has been bred on fur farms for a long time, and mink breeders have focused their efforts on the identification of genes affecting coat color. It is difficult to identify and localize such genes (Robinson 1975), but there is the possibility of finding the link between coat color genes and genes we can easy identify and localize by modern methods. Gene mapping in mammals has traditionally been inferred using two main approaches: cytogenetic mapping and genetic linkage or physical mapping. Previous gene localizations in American mink were made mainly with the use of two panels of somatic cell hybrid clones: mink-Chinese hamster (Rubtsov et al. 1981) and mink-mouse (Pack et al. 1992). Enzyme electrophoresis and Southern blotting were used substantially in analysis of these panels. Segregation analysis of mink chromosomes and markers in the hybrid cells made it possible to assign about 74 mink genes to specific chromosomes or chromosome regions (Serov 1998). Progress in developing the gene map of mammalian species has recently been advanced by use of polymerase chain reaction (PCR) analysis (Lyons et al. 1997). This study is concerned with chromosomal localization of 53 loci in American mink made by PCR analysis of mink-Chinese hamster hybrid clones, using primers designed for the feline genome project (Murphy et al. 2000).

Materials and Methods

Hybrid Cell Clones

The Chinese hamster–American mink somatic cell hybrid panel, consisting of 14 clones (Matveeva et al. 1987), was used in this study. Distribution of mink chromosomes among Chinese hamster–American mink hybrid clones is presented in Table 1. Assignment of a gene to a specific chromosome requires both (1) a high level of concordant segregation of a marker and a specific chromosome and (2) a sufficiently high level of discordant segregation of the marker and other chromosomes (Cowmeadow and Ruddle 1978; Rubtsov et al. 1981; Wijnen et al. 1977). Pair-compared analysis of mink chromosomes in this panel showed that the percentage of discordant clones for any chromosome pair was not less than 28%—that is, not less than 4 among 14 hybrid clones, although in most cases the estimation is much higher than 40%. This degree of discordance rendered the panel reliable for gene mapping.

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Table 1. Distribution of mink chromosomes among Chinese hamster-American mink hybrid clones

Name of clone	Mink chromosomes															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	X	Y
D7B			+	+	+	+		+	+	+		+	+		+	+
D13M	+			+	+						+	+		+	+	
L15		+	+		+		+	+		+			+	+	+	
FD9M	+	+			+			+	+	+		+		+	+	
D11B							+				+	+		+	+	
D12M	+		+		+				+		+	+	+	+	+	
K02	+		+	+					+	+	+	+	+	+	+	+
F12B		+	+	+	+	+			+			+	+	+	+	
R01	+	+	+	+				+		+	+	+	+	+	+	
F3M	+			+		+	+			+	+	+		+	+	
L25						+			+	+	+		+		+	
L22	+		+			+	+		+	+	+	+			+	
D3M	+			+			+		+				+		+	+
R14									+	+	+		+		+	

Chromosome Nomenclature

The majority of previous gene localizations were made with the use of chromosome nomenclature proposed by Mandahl and Fredga (1975). However, Christensen et al. (1996) later suggested a new nomenclature for mink chromosomes. In this study we used the latter nomenclature because it was already used in the Zoo-FISH painting study of mink (Hameister et al. 1997).

PCR Analysis

We used PCR primers designed for mapping in the cat radiation hybrid panel (Murphy et al. 2000). DNA of hybrid clones and parental cells was extracted with DNAzol (Life Technologies, USA) according to the manufacturer's recommendations, and Standard Life Technologies or Roche kits were used throughout. The composition of the 25 μl reaction mixture was as follows: 0.5–1 mg of DNA of parental cells or hybrid cells, 1 pM of forward or reverse primers, 0.5 U *Taq* polymerase, 200 mM Tris-HCl buffer, pH 8.4, containing 500 mM KCl, and 0.2 mM of each deoxynucleotide (dATP, dCTP, dGTP, and dTTP). PCR was performed with a Biometra UNO2 thermal cycler. Each amplification reaction underwent an initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 55–57°C for 15 s, and extension at 72°C for 30 s. The resulting amplified products were separated by electrophoresis in a 3–4% agarose gel in a Tris-EDTA-borate buffer. If there was a discrepancy between expected (according to Zoo-FISH painting) and observed chromosome localizations of tested markers, the PCR products were sequenced and the sequences were compared with published data for human and cat counterparts (Murphy et al. 2000).

Results

Two hundred forty primer pairs were prescreened in mink DNA samples to identify which of the initial set of sequence tagged sites (STSs) would produce a specific PCR product. From 79 primer pairs which passed this first screen, 53 (67%) produced a single mink PCR product either without the hamster PCR counterpart or with a different mobility from hamster product. These 53 primer pairs were used in further gene mapping in mink.

Table 2 presents the data on chromosome localizations of 53 loci. From the data of Table 2, we can see that 33 gene localizations were based on 100% of concordance between a marker and a specific mink chromosome; 14 localizations were determined with 93% of concordance, and 6 localizations were based on 86% of concordance. Thus chromosome localizations for 47 mink loci were established with high reliability, whereas localizations of the *SPARC*, *CSNK2A1*, *RPS11*, *RPS26*, *H123*, and *Mv.101282* loci should be considered as provisional (Table 2 and Figure 1). However, it should be emphasized that consideration of other chromosomes as candidates for localization of these mink loci produced a value of discordance of more than 35%.

The designations of mink genes in Table 2 are the same as those used by Murphy et al. (2000). Some human loci are designated by their expressed sequence tag cluster identifiers (Unigene expressed sequence tag [EST] clusters, NCBI; e.g., *Hs.148528*, *Hs.58885*). Following the convention used for the cat ESTs (Fc [*Felis catus*], followed by the human Unigene cluster identifier), these loci were renamed with Mv. (*Mustela vison*) as the prefix.

Cross-species chromosome painting (Zoo-FISH) allows visualization of chromosomal regions homologous across mammalian orders (Scherthan et al. 1994; Weinberg et al. 1990, 1997). According to the data of Hameister et al. (1997) and Graphodatsky et al. (2002), fluorescence in situ hybridization (FISH)-labeled human chromosome-specific probes cross-hybridized with 32 large regions of mink chromosomes. These researchers observed that many chromosomal DNA probes hybridized to only one mink chromosome region—for example, the human chromosome 9 probe hybridized only to the mink chromosome 9, and the probe for human chromosome 10 to only the long arm of mink chromosome 2 (Figure 1). In other cases, the probes derived from two or three different human chromosomes have painted single mink chromosomes (Figure 1). In general, the Zoo-FISH painting study between human and mink has revealed large conserved regions common to both species (Graphodatsky et al. 2000; Hameister et al. 1997). The inference is in good accordance with the data on gene mapping in mink (Serov 1998; Serov et al. 1987).

It is not surprising that the majority of mink loci were mapped on expected chromosome regions predicted from Zoo-FISH painting data (Table 2 and Figure 1), with the exception of the genes for *Fc(Mv).23617*, *ERF-2*, *ALPL*, and *CDC20* (Table 2 and Figure 1). According to predictions based on Zoo-FISH painting, we may expect the following localizations: *Fc(Mv).23617* to mink chromosome 2 or 10, but not chromosome 1 as we found; *ERF-2* to mink chromosome 3 or 8, rather than 1; *ALPL* to mink chromosome 2 or 10, rather than 11; and *CDC20* to mink chromosome 9, but not 2 (Table 2 and Figure 1). To verify that the feline primers

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Table 2. Chromosome localizations of 53 loci in the American mink

Name of locus	Localization on human chromosome	Expected[a] localization on mink chromosome	Observed localization on mink chromosome	Concordance[b] (%)
SPARC	5	1	1	86
Mv.148528	nd[c]		1	100
Mv.58885	5	1	1	100
CAMK4	5	1	1	100
IL12B	5	1	1	100
PPAP2A	5	1	1	100
IL6	7	4 or 14	4	93
KIAA0892	19	6 or 7	6	100
RAD23A	19	6 or 7	6	100
Fc.9960	nd		4	93
CSNK2A1	20	8	8	86
BC10	20	8	8	93
HS.182238	nd		13	93
PPP1CB	2	3 or 8	8	93
MLR	4	11	11	100
PRP4	6	1	1	100
RDS	6	1	1	100
Mv.5741	6	1	1	100
COL9A1	6	1	1	100
CYP19	15	13	13	93
Mv.7771	nd		12	93
RPS26	12	3 or 12	3	86
RPS11	nd		1	86
FGR	1	2 or 10	10	93
ERF-2	2	3 or 8	1	100
ALPL	1	2 or 10	11	100
H123	5 or 14	1 or 13/1 or 10	13	86
Mv.23617	1	2 or 10	1	100
CDC20	9	9	2	100
CDH2	18	3	3	100
GAD1	2	3 or 8	3	100
NRAS	1	2 or 10	2	100
KNSL6	1	2 or 10	2	93
Mv.8980	nd		2	100
CAP	1	2 or 10	2	93
Mv.5324	2	3 or 8	3	86
Mv.101282	2	3 or 8	3	100
HRB	2	3 or 8	3	93
RARB	3	6	6	100
Mv.37443	nd		6	100
PCCB	nd		6	100
KCNQ1	11	7	7	100
HBB	11	7	7	100
Mv.12293	11	7	7	100
ALDH2	12	3	3	100
H3F3B	17	5	5	100
Mv.3447	17	5	5	100
Mv.8179	17	5	5	100
HIC-1	17	5	5	100
STAT5B	17	5	5	93
KIAA0623	17	5	5	93
MYL4	17	5	5	100
TRADD	16	7 or 14	7	93

[a] Expected localization on mink chromosome judging the Zoo-FISH painting data (Hameister et al. 1997).

[b] 93%, one discordant clone; 86%, two discordant clones.

[c] No data.

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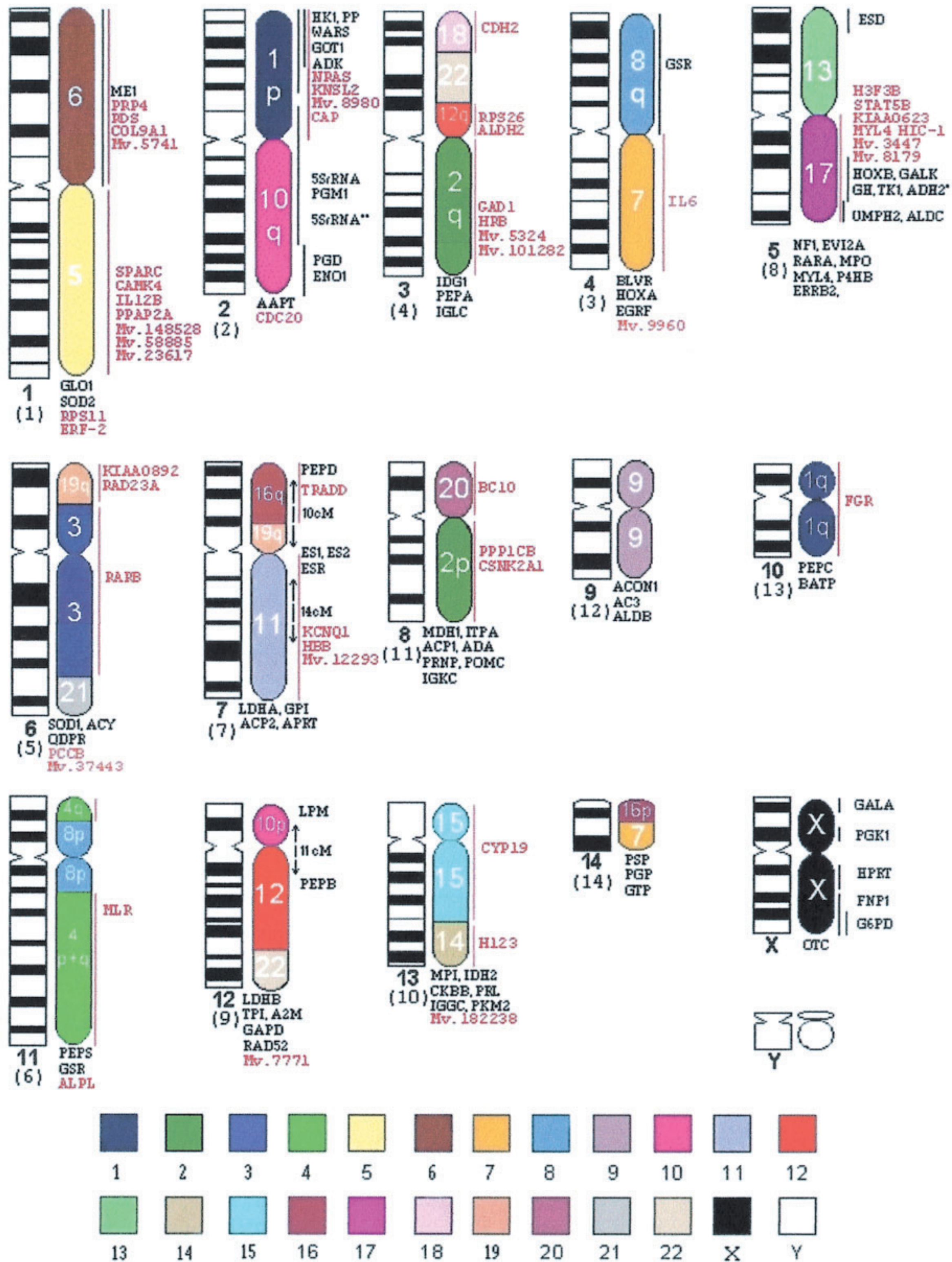

Figure 1. Gene map of American mink. Mink chromosomes are designated (below) according to new nomenclature (Christensen et al. 1996), and according to the former nomenclature in parentheses (Mandahl and Fredga 1975). Color designation of regions homologous to human is given according to Graphodatsky et al. (2000). Previous gene localizations are given in black color, new localizations in red.

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Table 3. Comparison of partial sequenced mink genes with human or cat counterparts

Mink loci	Homologous gene	Similarity (%)
CDC20	*Homo sapiens*, CDC20 cell division cycle 20 homologues (*S. cerevisiae*) Accession no. BC024257.1	95
Mv23617	*Homo sapiens*, similar to hypothetical protein FLJ20531 Accession no. BC002948.1	92
ALPL	*Felis catus* alkaline phosphatase (Alpl) mRNA, complete cds Accession no. U31569.1	92
ERF-2	*Homo sapiens*, ERF-2 mRNA complete sequence Accession no. X78992.1	93

provided a correct amplification of the homologous mink loci, the mink PCR products were isolated from gels, partially sequenced (~100 bp), and then compared with human or cat counterparts using BLAST. The results showed that the mink sequences had a homology greater than 92% with human or cat counterparts (Table 3). The data suggest that the PCR products derived from mink DNA have an origin from genes homologous to the human or cat.

Discussion

Application of the PCR with the use of primers designed for the feline genome project (Murphy et al. 2000) allowed us to establish chromosome localization for 53 mink loci. It should be noted that 60% of the gene localizations were determined with 100% concordance between a marker and a specific chromosome, 26% of the localizations were established at a level of 92% of concordance (1 discordant clone among 14), and 10% of the localizations were established at a level of 86% of discordance (2 discordant clones out 14). Of interest is that there were no discordant clones observed in previous gene mapping studies when enzyme electrophoresis and Southern blotting were used as a method for detection of markers (Serov 1998). Revelation of the discordant clones in this study suggests that some hybrid clones contain cells with chromosome rearrangements overlooked in cytogenetic analysis. Discovery of the discordant hybrid clones was possible due to application of a PCR method that had a higher sensitivity than enzyme electrophoresis or Southern blotting. However, in general, a level of discordance during localization of 53 mink loci was quite low, about 3.5%. Thus the data suggest that PCR analysis with the use of primers designed for the feline genome can be successfully used for gene mapping in mink. Moreover, the approach allowed us to expand the gene map of American mink to 127 loci.

At present, comparative chromosome painting has gained ground in comparative gene mapping in mammals (Murphy et al. 2001; O'Brien et al. 1999). In most cases, data from this method have been largely confirmed by other gene mapping approaches, though small rearrangements are often not detected by chromosome painting. Both approaches revealed large conserved regions common to many mammalian species. The nature of the conservation of large syntenic gene associations during the evolution of mammals is unknown, and perhaps large-block conservation is a more widespread phenomenon than previously thought. Comparison of mink with human genetic maps supports the idea. Indeed, 49 gene localizations for mink genes could be predicted from human gene mapping; only four mink loci were found and those are located outside expected conserved regions (Table 2 and Figure 1). It is the first evidence that in mink, evolution could take place with small rearrangements involving few genes. Moreover, some of them could be involved in formation of new syntenic groups. For instance, in a previous study, a syntenic group was found that is specific to mink (Khlebodarova et al. 1995). This group, including the genes for GPT, PGP, and PSP located on human chromosomes 8, 16, and 7, respectively, is located on mink chromosome 14 (Figure 1), which is the smallest in the mink karyotype and is stained by probes from human chromosomes 7 and 16 (Graphodatsky et al. 2002; Hameister et al. 1997). All of these genes are located on distinct chromosomes in human, and possibly this gene association has arisen de novo in *Mustelidae* due to small rearrangements. In fact, comparative analysis of the GTG-banding patterns of the chromosomes of more than 20 species representing six genera of the *Mustelidae* family revealed that all of them possessed a chromosome similar to mink chromosome 14 (Graphodatsky et al. 1989). The data were supported by recent results obtained by Zoo-FISH painting with a DNA probe from mink chromosome 14 to other *Mustelidae* species (Graphodatsky et al. 2002).

Based on the existence of conserved regions of syntenic genes in phylogenetically distant mammalian species, comparative mapping data may be used to search for the important genes in fur-bearing animals. It is possible to use the gene maps like a periodical system in genetics. In searching for the location of a gene in the fur animals, one should first determine whether this particular gene belongs to a syntenic group in other species. Development of microsatellites, widely used in the last decade to map economic trait loci in farm animals and pets, can eventually be applied to search for the location of genes of phenotypic interest in mink.

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