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Chromosome Localization of the Loci GOT1, PP, NP, SOD1, PEPA and PEPC in the American Mink (*Mustela vison*)

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Summary. Twenty eight American mink × Chinese hamster somatic cell hybrids were analysed for the expression of mink enzymes and chromosome segregation. This analysis made it possible to assign the genes for glutamate-oxaloacetate transaminase-1 (soluble) (EC 2.6.1.1), inorganic pyrophosphatase (EC 3.6.1.1), purine nucleoside phosphorylase (EC 2.4.2.1) to mink chromosome 2, superoxide dismutase-1 (soluble) (EC 1.11.1.1) to chromosome 5, peptidase A (EC 3.4.11 or 3.4.13) to chromosome 4, and peptidase C (EC 3.4.11 or 3.4.13) to chromosome 13. It is suggested that the synthenic gene group GOT1-PP-NP is located on the short arm of mink chromosome 2.

Key words: American mink – Chinese hamster – Gene mapping – Somatic cell hybrids

Introduction

Progress in comparative gene mapping is limited by the small number of mammals hitherto studied. Gene maps have been built mainly for primates and rodents (Pearson et al. 1979). In this laboratory, genes of the American mink (*Mustela vison*) have been assigned by somatic cell hybridization. For this purpose, a 28 American mink × Chinese hamster clone panel has been set up. Chromosome localization of 14 genes has been achieved already in this species (Rubtsov et al. 1981 a, b; 1982). This report presents the data of gene assignment for glutamate-oxaloacetate transaminase-1 (soluble) (EC 2.6.1.1), inorganic pyrophosphatase (EC 3.6.1.1), purine nucleoside phosphorylase (EC 2.4.2.1), superoxide dismutase-1 (soluble) (EC 1.11.1.1), peptidases A and C (EC 3.4.11 or 3.4.13) to specific chromosomes of American mink (*Mustela vison*).

Materials and Methods

Established lines of Chinese hamster and American mink fibroblasts and 28 clones of independently derived American mink × Chinese hamster somatic cell hybrids were used (Rubtsov et al. 1981 a). We described previously the production and characterization of the hybrid clones (Rubtsov et al. 1981 a). Each cell clone was karyological studied and harvested for marker enzyme assays on the same passage.

Cell extracts were prepared by the standard technique (Rubtsov et al. 1981 a) and the cell lysates were sonicated. Electrophoresis of the cell extracts was carried out in 14% starch gel containing 10% sucrose.

Electrophoresis of superoxide dismutase (SOD) was performed in Tris-EDTA-borate buffer system, pH 8.6, as previously described (Rubtsov et al. 1981 a). The gels were stained for SOD activity according to Harris and Hopkinson (1976).

Electrophoresis of peptidases A and C (PEPA and PEPC) was done in a discontinuous buffer system (Serov 1973). The composition of the electrode buffer was 0.3 M boric acid, 0.05 M NaOH, pH 7.9, and that of the gel buffer was 0.025 M Tris, 0.001 M EDTA : Na_2, 0.004 M boric acid, pH 7.6, and 1% Triton X-100. The cell lysates underwent electrophoresis at a voltage gradient of 15 v/cm for 14–16 h. The gels were stained for PEP activity according to Lewis and Harris (1967) with minor modifications: 15 mg of dipeptide (glycine-leucine), 4 mg of snake venom (*Vipera lebentina*), 0.2 ml of 3 M $MgCl_2$, 1 mg of horse radish peroxidase (500 U/mg), 4 mg of o-dianizidine (in 0.5 ml of 0.5 N HCl) were dissolved in 8 ml of 0.5 M Tris-HCl buffer, pH 7.5, and were mixed with 9 ml of 2% agarose at 42 °C. The mixture was poured out on the gels and incubated at 37 °C for 1–2 h.

Electrophoresis of glutamate-oxaloacetate transaminase (GOT) was carried out by the method of Shows and Ruddle (1968). The GOT isozymes were located after electrophoresis using a staining mixture. To obtain this mixture, 15 mg of aspartic acid, 8 mg of 2-oxoglutaric acid, 8 mg of NADH, 0.05 ml of malate dehydrogenase (1350 U/ml) were dissolved in 10 ml of 0.5 M Tris-HCl buffer, pH 7.5, and mixed with 10 ml of 2% agarose at 42 °C. The staining mixture was applied to the gels and incubated at 37 °C for 30–60 min.

Electrophoresis of inorganic pyrophosphatase (PP) was performed in a discontinuous buffer system (Serov 1973; described above), and 0.003 M of beta-mercaptoethanol was added to the gel buffer. The staining method of Fisher et al. (1974) was used to visualize this enzyme.

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Electrophoresis of purine nucleoside phosphorylase (NP) was done according to Rubtsov et al. (1981a) using a Tris-EDTA-borate buffer system. The gels were stained for NP activity by the method of Harris and Hopkinson (1976).

Results and Discussion

Electrophoregrams of mink, Chinese hamster, and hybrid cell extracts followed by staining for GOT, PP, NP, and SOD are shown in Figs. 1–4. The parental forms of the enzymes differ in electrophoretic mobility, and the hybrid clones, which are positive for the mink enzymes, contain heteropolymeric isozymes with mobility intermediate with respect to the parental forms. The expression of mink enzymes in 28 independent hybrid clones is given in Table 1. Clones MA10, M1110 and L14, which retained all mink chromosomes except the Y, are positive for GOT1, PP, NP and SOD1 derived from the mink.

Table 2 presents the results of the segregation analysis of mink chromosomes and mink GOT1, PP, NP and SOD1 in the remaining 25 hybrid clones. It is easy to see that the genes for GOT1, PP and NP are synthenic and that they are most likely located on mink chromosome 2; there was no association of the loci GOT1, PP and NP with any other mink chromosome (Table 2). The presence of whole mink chromosome 2 and the expression of PP were concordant in all the clones studied (Table 2). The segregation of the expression of mink NP and mink chromosome 2 was discordant in two clones (Table 2). It is possible that these cases of discordant segregation in clones L26 and F6B are due to chromosomal rearrangements. The limitations of the G-technique precluded the identification of these putative rearrangements of mink chromosome 2 in the discordant clones.

The expression of mink GOT1 segregates concordantly in the presence of mink chromosome 2 in all the clones, except L15. Although karyological analysis failed to identify any chromosomal rearrangements in chromosome 2 of clone L15, nevertheless a deletion of a small region of this chromosome cannot be excluded.

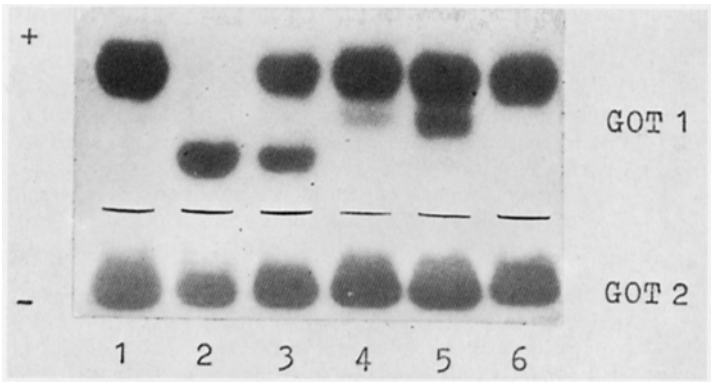

Fig. 1

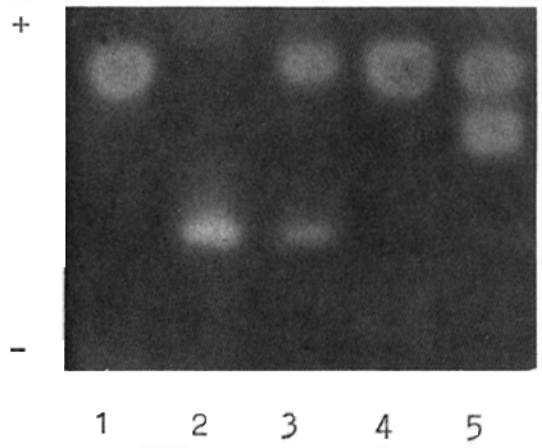

Fig. 2

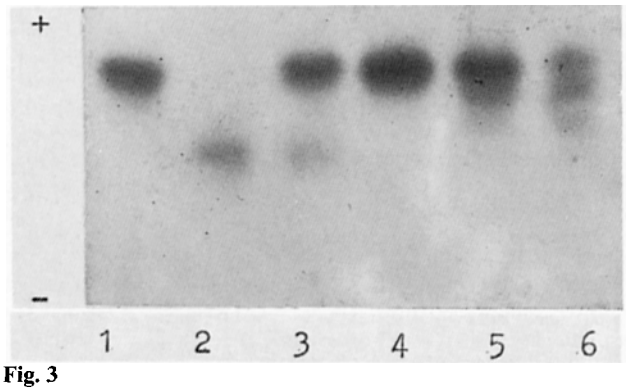

Fig. 3

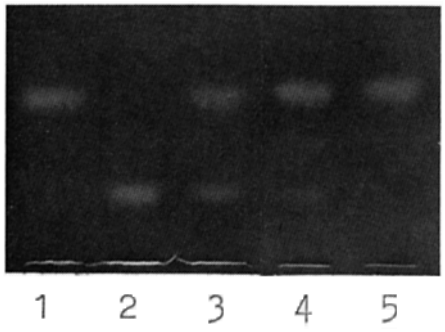

Fig. 4

Figs. 1–4. **1** Electrophoretic GOT patterns of Chinese hamster (1), American mink (2), 1 : 1 mixture of extracts of parental species (3), and hybrid clones: L14 (4), R09 (5) and KO2 (6). GOT1 and GOT2 are soluble and mitochondrial forms of GOT, respectively. **2** An electrophoretic PP patterns of Chinese hamster (1), American mink (2), 1 : 1 mixture of extracts of parental species (3), and hybrid clones: KO4 (4) and L14 (5). **3** An electrophoretic NP patterns of Chinese hamster (1), American mink (2), 1 : 1 mixture of extracts of parental species (3), and hybrid clones: KO4 (4), L14 (5) and RO9 (6). **4** An electrophoretic SOD patterns of Chinese hamster (1), American mink (2), 1 : 1 mixture of extracts of parental species (3), and hybrid clones: L22 (4) and L15 (5). Extracts of Chinese hamster and American mink cells contain the soluble form of SOD only

Table 1. Mink enzyme distribution in 28 American mink x Chinese hamster clones

Number of clone	American mink enzyme							
	GOT1	PP	NP	SOD1	PEPA	PEPC	PGD/ PGM1[a]	IDH1[b]
MA10	+	+	+	+	+	+	+	+
M1110	+	+	+	+	+	+	+	+
L14	+	+	+	+	+	+	+	+
CO113	–	–	–	+	+	–	+	+
M1123	–	–	–	+	–	–	–	–
KO2	–	–	–	–	+	+	–	+
KO4	–	–	–	–	–	+	–	–
KO5	–	–	–	–	+	–	–	+
K11	–	–	–	+	+	–	–	+
K12	–	–	–	+	–	–	–	–
K14	–	–	–	–	–	+	–	+
K15	–	–	–	–	+	+	–	–
L12	–	–	–	–	–	–	–	–
L15	–	+	+	–	+	+	+	+
L18	–	–	–	–	+	+	–	+
L22	–	–	–	+	+	+	–	+
L25	–	–	–	+	–	–	–	–
L26	–	–	+	+	+	+	–	+
RO1	+	+	+	–	+	+	+	+
RO9	+	+	+	–	–	–	+	–
R13	–	–	–	–	–	–	–	–
R14	–	–	–	+	–	+	–	–
D7B	–	–	–	+	+	+	–	+
D11B	–	–	–	–	–	–	–	–
FD16B	–	–	–	+	–	–	–	–
F6B	+	+	–	+	–	+	+	–
F10B	+	+	+	–	–	–	+	–
F12B	+	+	+	+	+	–	+	+

[a] PGD/PGM1 are markers of chromosome 2 (Rubtsov et al. 1981 b)
[b] IDH1 is a marker of chromosome 4 (Rubtsov et al. 1982)

Clone L15 expresses all the other enzyme markers of mink chromosome 2 (PP, NP, PGM1 (phosphoglucomutase-1), and PGD (6-phosphogluconate dehydrogenase) (Table 1); however, the low level of these mink enzymes indicated that a small proportion of L15 cells contained mink chromosome 2. In fact, according to karyological estimates, mink chromosome 2 was present in about 16% of L15 cells. Consequently, it is possible that the expression of mink GOT1 in clone L15 escaped detection.

As to clone CO113, it should be noted that it contains the long arm of mink chromosome 2 (Fig. 5), (Rubtsov et al. 1981 a, b) and that it expresses mink PGM1 and PGD whose genes are located on chromosome 2 (Rubtsov et al. 1981 b). The retention of the long arm of chromosome 2 in clone CO113 did not give rise to expression of mink GOT1, PP and NP (Table 1). It may be suggested that the genes for PGM1 and PGD are located on the long arm of chromosome 2, while those for GOT1, PP and NP are located on its short arm.

Judging by the results of the Table 2, the most likely candidate for SOD1 assignment is chromosome 5. SOD1 is the first biochemical marker established for mink chromosome 5. Discordant segregation in clones R14 and F16B (SOD1 "+"/chromosome 5 "–") is presumably the result of rearrangements, but karyological analysis did not allow us to identify these rearrangements in chromosome 5.

The peptidase patterns obtained from American mink, Chinese hamster and their hybrid cells are shown in Fig. 6. The peptidase patterns of American mink and Chinese hamster were both of two-band type. As seen in Fig. 6, peptidases of the two species differ in electrophoretic mobility. It is suggested that the faster enzyme of mink and Chinese hamster, designated as PEPC, is homologous to human PEPC and mouse PEP3, while the slower one, designated as PEPA, is homologous to human PEPA and mouse PEP1. The results of various investigations favor this suggestion: the dipeptide used is easily hydrolysed by PEPA and PEPC (Harris and Hopkinson 1976); human and mouse PEPA have a

Table 2. Segregation of mink chromosomes and mink GOT1, PP, NP, SOD1, PEPA and PEPC in 25 American mink × Chinese hamster clones

Chromosome		Mink enzyme											
		GOT1		PP		NP		PEPA		PEPC		SOD1	
		+	−	+	−	+	−	+	−	+	−	+	−
1	+	2	8	2	8	2	8	6	4	7	3	6	4
	−	3	12	4	11	4	11	6	9	5	10	6	9
2	+	5	1	6	0	5	1	3	3	3	3	2	4
	−	0	19	0	19	1	18	9	10	9	10	10	9
3	+	3	7	3	7	3	7	6	4	5	5	5	5
	−	2	13	3	12	3	12	6	9	7	8	7	8
4	+	2	9	3	8	4	7	10	1	8	3	6	5
	−	3	11	3	11	2	12	2	12	4	10	6	8
5	+	2	8	2	8	2	8	6	4	4	6	10	0
	−	3	12	4	11	4	11	6	9	8	7	2	13
6	+	3	12	3	12	3	12	6	9	7	8	8	7
	−	2	8	3	7	3	7	6	4	5	5	4	6
7	+	1	5	2	4	2	4	3	3	2	4	3	3
	−	4	15	4	15	4	15	9	10	10	9	9	10
8	+	2	5	3	4	2	5	4	3	4	3	4	3
	−	3	15	3	15	4	14	8	10	8	10	8	10
9	+	5	12	5	12	5	12	11	6	8	9	8	9
	−	0	8	1	7	1	7	1	7	4	4	4	4
10	+	4	13	5	12	6	11	9	8	8	9	9	8
	−	1	7	1	7	0	8	3	5	4	4	3	5
11	+	2	7	3	6	3	6	7	2	6	3	6	3
		3	13	3	13	3	13	5	11	6	10	6	10
12	+	3	10	3	10	2	11	7	6	6	7	9	4
		2	10	3	9	4	8	5	7	6	6	3	9
13	+	2	12	3	11	3	11	9	5	12	2	6	8
		3	8	3	8	3	8	3	8	0	11	6	5
14	+	5	12	6	11	3	11	9	8	9	8	7	10
	−	0	8	0	8	0	8	3	5	3	5	5	3
X	+	5	18	6	17	6	17	11	12	11	12	11	12
	−	0	2	0	2	0	2	1	1	1	1	1	1

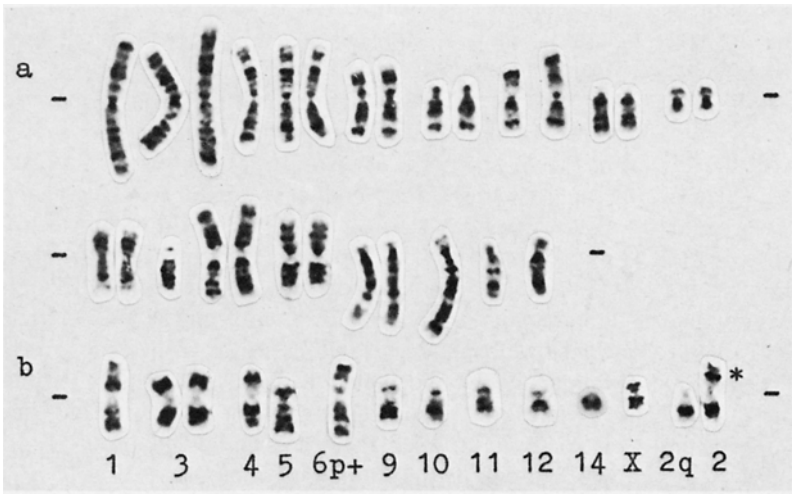

Fig. 5a and b. G-banded karyotype of hybrid clone CO113. **a** Chinese hamster chromosomes; **b** American mink chromosomes; 6p+ of mink chromosome – t(6;?) (p13;?); 2q of mink chromosome – del (2) (:cen qter). * = it was compared with normal chromosome 2 from a mink bone marrow cell illustrating possible derivation of 2q

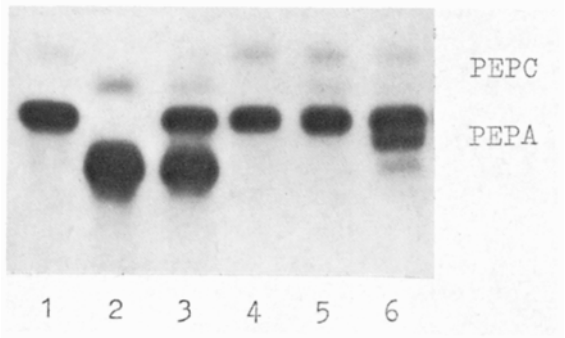

Fig. 6. An electrophoretic PEP patterns of Chinese hamster (1), American mink (2), 1:1 mixture of extracts of parental species (3), and hybrid clones: R13 (4), R14 (5) and KO2 (6).

dimeric structure (Harris and Hopkinson 1976; Lewis and Truslov 1969) and PEPC has a monomeric one (Harris and Hopkinson 1976; Womack and Cramer 1980). The presence of the heteropolymeric isozyme in hybrid clones positive for PEPA is clear-cut evidence for the dimeric structure of mink PEPA (Fig. 6). The absence of heteropolymeric isozymes of PEPC in clones positive for mink PEPC provide evidence that its structure may possibly be monomeric.

Table 1 presents the data for the distribution of mink PEPA and PEPC in hybrid clones. The data indicate that the marker enzymes segregate independently with respect to each other.

Judging by the results of segregation of mink chromosomes and mink PEPA and PEPC, the most likely candidates for PEPA and PEPC assignments are chromosomes 4 and 13, respectively.

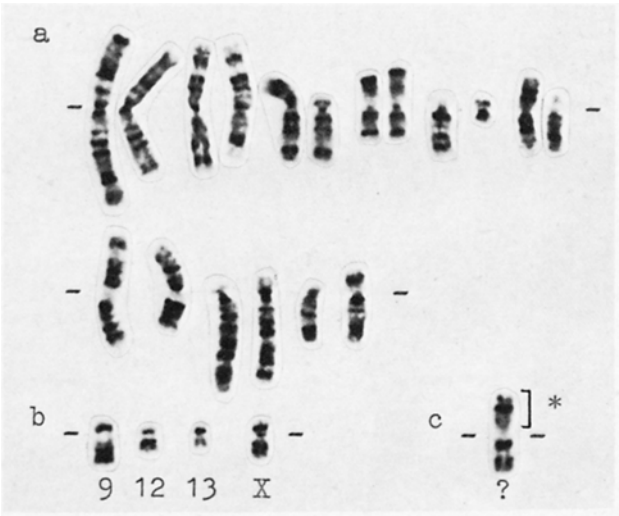

Fig. 7 a – c. G-banded karyotype of hybrid clone KO5. **a** Chinese hamster chromosomes; **b** mink chromosomes; **c** chromosome of unknown derivation.* = a chromosome region presumably including some genetic material of mink chromosome 4

PEPC is the first marker located for mink chromosome 13. However, chromosome localization of this marker is tentative, because there was a discordant segregation PEP "–"/chromosome 13 "+" in clones KO5 and L25 (Table 2). This discordance might have been due to a small deletion of chromosome 13 which we possibly overlooked. It may be also assumed that chromosomal rearrangements gave rise to a chromosome outwardly resembling another, chromosome 13 in the case envisaged.

PEPA is the second marker for chromosome 4. In a previous paper, evidence was presented for the assignment of the gene for the isocitrate dehydrogenase-1 (soluble) (IDH1) to chromosome 4 (Rubtsov et al. 1982). Discordance in clone K14, PEPA "–"/chromosome 4 "+"; IDH1 "+", is presumably the result of chromosomal rearrangements. Absence of PEPA in this clone suggests that chromosome 4 is deleted. We were unable to identify this deletion.

It is worthy to note that each of the KO5 (PEPA "+"; IDH1 "+"/chromosome 4 "–") and K15 (PEPA "+"/chromosome 4 "–"; IDH1 "–") discordant clones had a chromosome of unclear origin. Precise identification of these chromosomes was beyond the resolving capacity of the G-staining technique. Nevertheless, the G-banding patterns of the chromosomes in clone KO5 make it plausible that the unidentified chromosome comprises a fragment of, at least, the short arm of chromosome 4 (Fig. 7). Consequently, this supports the assignment of the gene for PEPA to mink chromosome 4.

Thus, the results obtained, as well as previous our data (Rubtsov et al. 1981a, b; 1982), allowed us to locate now 20 genes coding for biochemical traits on the 11 specific chromosomes of the American mink.

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