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Chinese Hamster × American Mink Somatic Cell Hybrids: Characterization of a Clone Panel and Assignment of the Mink Genes for Malate Dehydrogenase, NADP-1 and Malate Dehydrogenase, NAD-1

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Summary. Chinese hamster × American mink somatic cell hybrids were obtained and examined for chromosome content and expression of mink malate dehydrogenase, NADP (MOD-1; EC 1.1.1.40), malate dehydrogenase, NAD (MOR-1; EC 1.1.1.37), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) and hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8). All the hybrid clones examined were found to segregate mink chromosomes. A clone panel containing 25 clones was set up. The possibilities and limitations of this panel for mink gene mapping are analysed. Using this panel, it is feasible to rapidly map genes located on chromosomes 1-13 and to provisionally assign genes located on chromosome 14 and the X. Based on the data obtained, the genes for MOD-1 and MOR-1 were firmly assigned to mink chromosomes 1 and 11, respectively, and the genes for G6PD and HPRT were provisionally assigned to the X.

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

Introduction

Interspecific somatic cell hybrids, which preferentially segregate chromosomes of one species, permit the assignment of genes to chromosomes of this species. The gene maps built so far have concerned primates and rodents (Pearson et al. 1979). With respect to other mammalian species, only a small number of genes have been mapped utilizing this approach (Pearson et al. 1979). However, data on gene location in other mammalian species would help to clarify problems concerning chromosome organization and evolution.

Herein we report our first experimental results with Chinese hamster × American mink somatic cell hybrids. Further studies are in progress in this laboratory with the aim of mapping mink genes.

Materials and Methods

Cells and Media

The following Chinese hamster lines were used: M-15-1 and 237_I (Petrova et al. 1967; Zakharov et al. 1964) derived from a normal Chinese hamster cell line, Blld-ii-FAF-28, which was established by Yerganian and Leonard (1961). 237_I cells were grown in Eagle's medium (E medium) supplemented with 10% bovine serum (E-BS medium). M-15-1 cells, deficient in hypoxanthine phosphoribosyltransferase ($HPRT^-$), were cultivated in E-BS medium containing 30 μg/ml of 8-azaguanine ('Sigma', U.S.A.). The mink cell line used was MV, an embryonic fibroblast line (Reznik et al. 1981).

In addition to these cell lines, mink white blood cells (WBC) and bone marrow cells (BMC) were utilized in the fusion experiments. WBC were obtained from five adult males. Heart blood was withdrawn into a syringe containing heparin (500 units in 0.5 ml for each 10 ml of whole blood). BMC were prepared from the bone marrow of the femurs of two adult mink males.

Production of Somatic Cell Hybrids

To produce somatic cell hybrids, M-15-1 cells ($HPRT^-$) and American mink cells ($HPRT^+$) were fused either by means of inactivated Sendai virus or polyethylene glycol (PEG). Hybrids were selected in E medium to which 20% fetal calf serum ('Serva', FRG), hypoxanthine, aminopterine and thymidine were added (E-HAT) (Littlefield 1964).

The method of Weiss and Ephrussi (1966) was employed to isolate hybrid cells between M-15-1 cells and MV cells (Expts. 1-3). MV cells were co-cultivated with M-15-1 cells at ratios of 1:20 (Expts. 1 and 3) and 1:70 (Expt. 2). Cell fusion was induced by UV-inactivated Sendai virus (Giles and Ruddle 1973). After incubation at 37°C for 18 hrs, the cells were collected by trypsinization and plated in 60 mm dishes (2×10^4 cells per dish) in E-HAT medium. The cells were grown in E-HAT medium in a 5% CO_2 atmosphere. The selective medium was renewed every 2-3 days. Colonies developing in the selective medium were isolated 21 days after fusion. Hybrid clones were grown in nonselective growth medium. Secondary clones, CO113 and M1123, were obtained from clones CO110 and M1120, respectively.

The procedures used to obtain somatic cell hybrids between M-15-1 and BMC of mink (Expt. 4), and also those between M-15-1 cells and mink WBC (Expts. 5 and 6) have been described elsewhere (Hu et al. 1977).

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In Experiments 7-9, cell fusion was induced by PEG (molecular weight 950-1050). BMC were suspended in E-medium containing either 250 μg/ml PHA ('Welcome', U.S.A.) (Expts. 8 and 9) or no supplement (Expt. 7). 60-mm dishes containing a nonconfluent monolayer of M-15-1 cells were inoculated with BMC suspensions. The ratio of M-15-1 cells to BMC was 1:30. The cells were incubated for 1.5 hr at 37°C. The medium was then removed by aspiration and 5 ml of PEG solution (41.7% weight/weight) in E-medium either with (Expts. 7 and 8) or without 10% dimethylsulfoxide (Expt. 9) was added. One minute later, the PEG was removed, the cells were rinsed 5 times with E-medium, and E-BS medium was added to the dishes. All the procedures were carried out at 37°C. The cultivation conditions were the same as in Experiments 1-6. The colonies which appeared in the selective medium were isolated 14 days after fusion. The cells of all the clones were cultivated in E-HAT medium. To be sure that the hybrid lines were of independent origin, only one colony was taken from each dish.

Chromosome Analysis

The cells were treated with colcemid (0.05 μg/ml) for 2 hrs prior to harvest. All slides were prepared with the air-dried method. M-15-1, MV and hybrid clones were karyotyped with trypsin-Giemsa banding as described in a previous paper (Radjabli and Krukova 1973). 25-30 metaphase spreads were photographed and analysed per hybrid clone.

Electrophoresis

The cells were lysed essentially according to the method of Nichols and Ruddle (1979). The extraction buffer was of the following composition: 10 mM Tris-HCl, pH 7.6, 1 mM $MgCl_2$, 20 mM KCl, 10% glycerol, 0.1 mM DTT, 0.5% Triton X-100 and 0.5% mink serum albumin. The electrophoretically pure mink albumin was isolated from the serum (Serov et al. 1976). The cell extracts were analysed by vertical starch gel electrophoresis employing 14% Electrostarch for the expression of glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), malate dehydrogenase, NADP (soluble)(MOD-1; EC 1.1.1.40) and malate dehydrogenase, NAD (soluble) (MOR-1; EC 1.1.1.37). Electrophoresis for G6PD was performed for 12 hrs at 10-12 v/cm at 4°C, using a Tris-EDTA-Borate, pH 8.6, buffer system. The stock buffer consisted of 0.9 M Tris-0.02 M EDTA-0.5 M borate. It was diluted 1:20 for the gel buffer; 1:7 and 1:5 for the cathode and anode electrode buffers, respectively (Porter et al. 1964). G6PD was visualized by specific staining as previously described (Serov et al. 1978).

Mink and Chinese hamster forms of MOD-1 and MOR-1 were distinguished by electrophoresis using a Tris-Citrate buffer system. The electrode buffer consisted of 0.1 M Tris-Citrate buffer, pH 7.35. This buffer was diluted 1:5 in gel. Electrophoresis was run for 14 hrs at 10-12 v/cm at 4°C. MOR-1 was stained by the standard method (Serov et al. 1979). The staining mixture for MOD contained 25 ml 0.1 M Tris-HCl buffer, pH 8.0, 20 mg NADP, 2 ml 1 M sodium malate, 1 ml 3 M $MgCl_2$, 10 mg tetranitrotetrazolium blue, 1 mg phenazinmethosulfate, 50 mg sodium pyruvate and water to 100 ml. Optimal staining was achieved in about 3-6 hrs at 37°C. HPRT of mink and Chinese hamster was assayed by agarose gel electrophoresis (Bakay et al. 1978).

Results and Discussion

Enzyme Assays

Mink, Chinese hamster, and hybrid cell G6PD, MOD-1, and MOR electrophoretic phenotypes are shown in Figs. 1-3. The mink form of each enzyme was clearly distinguished from the Chinese hamster homologous enzymes.

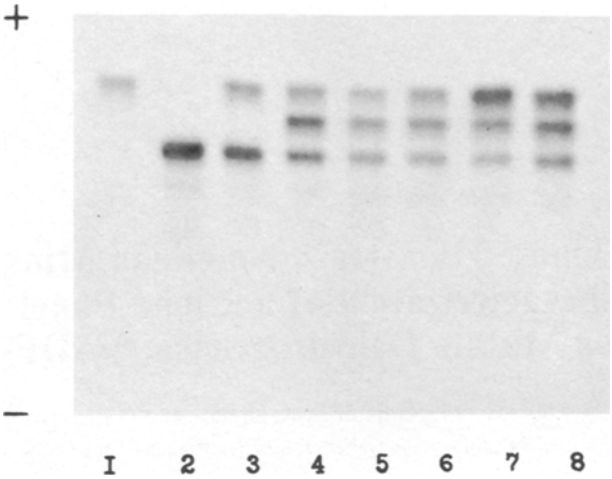

Fig. 1. Electrophoretic pattern of G6PD in parental and hybrid cells. Channel *1* = Chinese hamster fibroblasts, M-15-1 cells; channel *2* = mink fibroblasts, MV cells; channel *3* = mixture (1:1) of mink fibroblasts and Chinese hamster fibroblasts; channel *4* = clone KO 2; channel *5* = clone K 11; channel *6* = clone K 12; channel *7* = clone K 14; channel *8* = clone KO 4

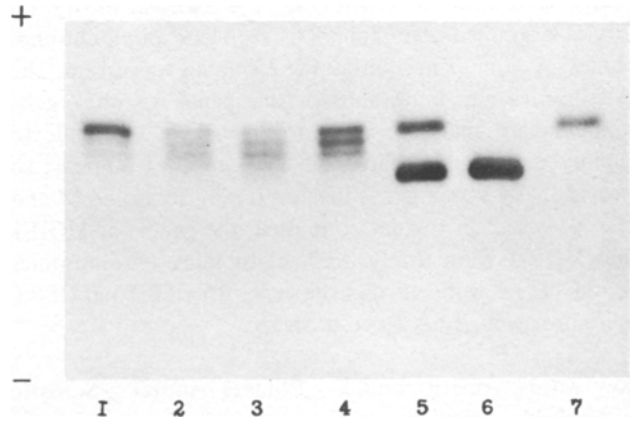

Fig. 2. Electrophoretic pattern of MOD-1 (soluble) in parental and hybrid cells. Channel *1* = clone K 2; channel *2* = clone K 11; channel *3* = clone K 14; channel *4* = clone K 4; channel *5* = mixture (1:1) of mink fibroblasts and Chinese hamster fibroblasts; channel *6* = mink fibroblasts, MV cells; channel *7* = Chinese hamster fibroblasts, M-15-1 cells

The cell hybrids positive or negative for mink G6PD could be easily scored. The positive hybrids showed three bands of which the slow-migrating band was mink, the fast-migrating band was Chinese hamster, and the intermediate band was a heteropolymeric isozyme probably consisting of mink and Chinese hamster subunits (Fig. 1).

Mink MOD-1 derived from fibroblasts (MV cells) migrated as a single isozyme to the anode. The Chinese

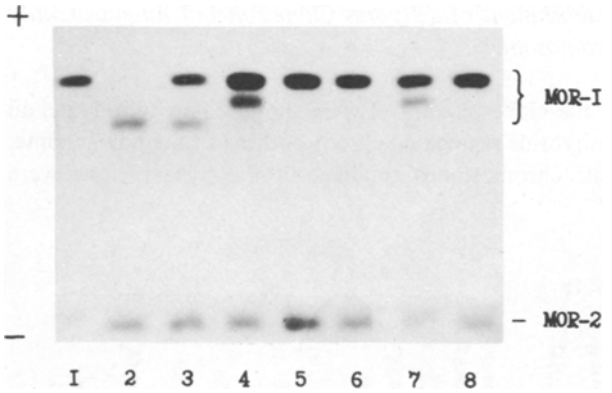

Fig. 3. Electrophoretic pattern of MOR in parental and hybrid cells. Channel *1* = Chinese hamster fibroblasts, M-15-1 cells; channel *2* = mink fibroblasts, MV cells; channel *3* = mixture (1:1) of mink fibroblasts and Chinese hamster fibroblasts; channel *4* = clone F6B; channel *5* = clone F10B; channel *6* = clone F12B; channel *7* = clone D7B; channel *8* = clone D11B. MOR-1 soluble form of MOR; MOR-2 mitochondrial form of MOR

hamster enzyme moved faster than that of the mink. MOD-1 is a tetramer (Shows and Ruddle 1968; Baker and Mintz 1969). The cell hybrids exhibiting mink MOD-1 activity had five bands: two bands with parental electrophoretic mobilities and three intermediate bands (Fig. 2).

Chinese hamster MOR-1 migrated as a single band of faster activity than mink MOR-1. Hybrids, which expressed mink MOR-1, had a heteropolymeric isozyme whose mobility was intermediate to those of the Chinese hamster and mink cells (Fig. 3). Hybrids lacking mink MOR-1 had only the Chinese hamster enzyme (Fig. 3). On these gels, malate dehydrogenase, NAD (mitochondrial) MOR-2 could also be detected. However, in the electrophoretic system utilized, mink and Chinese hamster MOR-2 had the same electrophoretic mobility (Fig. 3). The results of the G6PD, MOD-1, and MOR-1 assays are given in Table 1.

Mink HPRT from MV cells displayed two electrophoretic bands corresponding to two isozyme forms with higher electrophoretic mobility than their Chinese hamster

Table 1. Mink chromosome constitution of hybrid clones and expression of mink forms of G6PD, HPRT, MOD-1 and MOR-1

Experiment	Number of hybrid clone	Number of M-15-1 genomes cell	Mink chromosome																G6PD	HPRT	MOD-1	MOR-1
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	X	Y				
1	CO113	2	+	–	+	+	+	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+
2	M1110	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
	M1120	2																	+			
	M1123	2	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
3	MA10	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
4	KO2	2	+	–	+	+	–	+	–	–	+	+	–	+	+	+	+	+	+	+	+	–
	KO4	2	+	–	+	–	–	–	–	–	–	–	–	–	+	+	+	–	+	+	+	–
	KO5	1	–	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	+	+	–	–
	K11	2	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	–	+	+	+	+
	K12	2	+	–	+	–	+	+	+	–	–	+	+	+	–	+	+	–	+	+	+	+
	K14	2	+	–	+	+	–	+	–	+	–	+	–	+	+	+	+	–	+	+	+	–
	K15	1	–	–	–	–	–	–	–	–	+	+	+	–	+	+	–	+	+	+	–	+
5	L12	2	–	–	–	–	–	+	–	+	–	+	–	–	–	–	+	–	+	+	–	–
	L14	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
	L15	2	–	+	–	+	–	–	+	+	–	+	+	–	+	+	+	–	+	+	–	+
	L18	1	–	–	–	+	–	–	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	L22	2	+	–	–	+	+	+	+	–	+	–	–	+	+	–	+	–	+	+	+	–
	L25	1	–	–	–	–	+	+	–	–	–	+	+	–	+	–	+	–	+	+	–	–
	L26	2	+	–	–	+	+	+	–	–	+	+	+	–	+	+	+	–	+	+	+	+
6	R01	2	+	+	+	+	–	+	–	–	+	+	+	–	+	+	+	–	+	+	+	+
	R09	1	–	+	–	–	–	+	–	–	+	+	–	–	–	+	+	+	+	+	–	–
	R13	1	–	–	–	–	–	+	–	–	+	–	–	–	–	–	+	+	+	+	–	–
	R14	1	–	–	–	–	–	+	–	–	–	+	–	+	+	–	+	–	+	+	–	–
7	D7B	2	–	–	+	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	–	+
	D11B	2	–	–	–	–	–	+	+	–	+	–	–	–	–	+	+	–	+	+	–	–
8	FD16B	2	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–
9	F6B	2	+	+	–	–	+	+	–	+	+	–	+	+	+	+	+	–	+	+	+	+
	F10B	2	–	+	+	–	–	–	+	–	+	+	–	+	–	+	+	–	+	+	–	–
	F12B	2	–	+	+	+	+	–	–	+	+	+	–	+	–	+	+	–	+	+	–	–

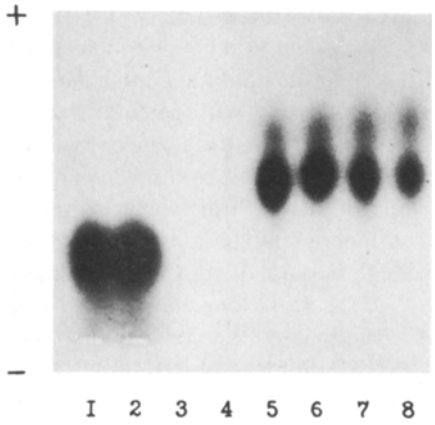

Fig. 4. Electrophoretic pattern of HPRT in parental and hybrid cells. Channels *1*, *2* = Chinese hamster fibroblasts, 237_I cells ($HPRT^+$); channel *3* = Chinese hamster fibroblasts, M-15-1 cells ($HPRT^-$); channel *4* = clone M1123; channel *5* = clone KO 2; channel *6* = clone L 12; channel *7* = clone RO9; channel *8* = mink fibroblasts, MV cells

counterpart from clone 237_I cells, which showed a single band (Fig. 4). M-15-1 cells and hybrid cells were negative for Chinese hamster HPRT. All the hybrid clones, except the secondary clone M1123, showed mink HPRT activity (Table 1).

Chromosome Content of Hybrid Clones

The trypsin-Giemsa banding method was utilized to identify all the chromosomes of the Chinese hamster and American mink in the parental and hybrid cells. A karyotype of the M-15-1 is shown in Figure 5. A typical karyotype of the line MV is presented in Figure 6, using the nomenclature of Mandahl and Fredga (1975) for mink chromosomes. The karyotype of a cell from the hybrid clone RO9 is shown in Fig. 7.

Establishment of a Hybrid Clone Panel of American Mink Chromosomes

All the clones examined were shown to be hybrid, and all the hybrids segregated chromosomes of the mink genome. Mink chromosomes retained in the hybrid clone were

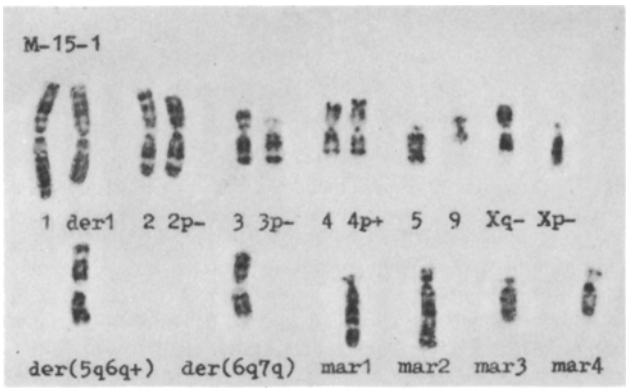

Fig. 5. Karyotype of M-15-1 cell line following trypsin-Giemsa banding

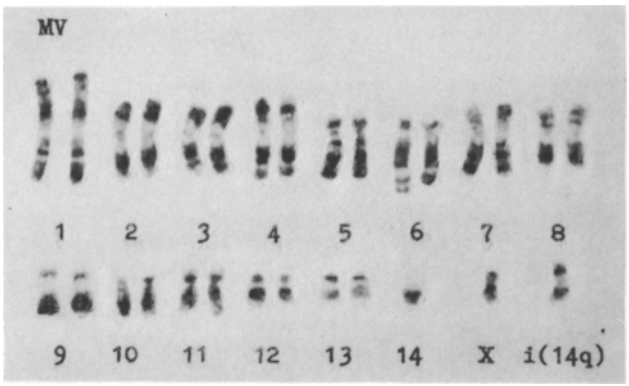

Fig. 6. Karyotype of MV cell line following trypsin-Giemsa banding

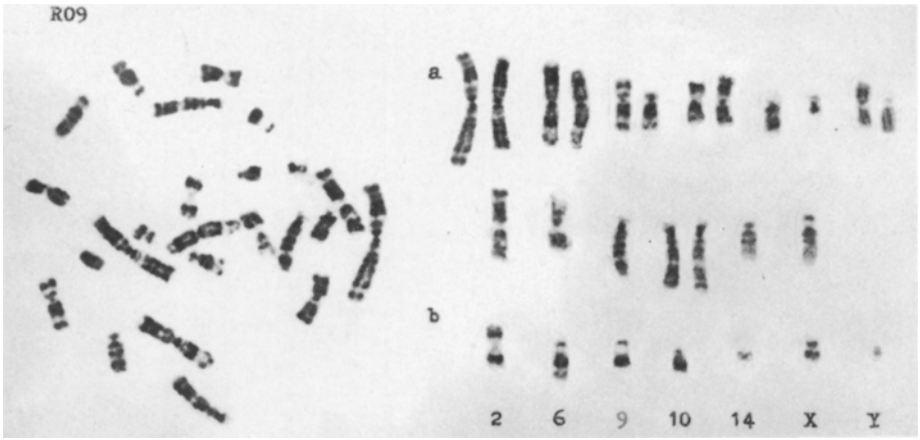

Fig. 7. Metaphase spread from a Chinese hamster × American mink hybrid clone RO9 (left); right – Chinese hamster (*a*) and American mink (*b*) chromosomes are karyotyped

present in one or two copies per cell in at least 15% of cells. The results of the chromosome analysis are summarized in Table 1. MA10, M1110 and L14 cells retained all the chromosomes from the mink, except the Y. The remaining 25 clones lost variable numbers of mink chromosomes. The identified rearrangements of mink chromosomes were as follows: t(6) (p13) and del (2)(:cen → qter) in clone CO113, and t(10) (p13) in clone M1123.

Utilization of the Hybrid Clone Panel for Mink Gene Mapping: Possibilities and Limitations

Gene assignment is based on a series of hypotheses which were established for each chromosome individually (Wijnen et al. 1977; Cowmeadow and Ruddle 1978). The criterion of Wijnen et al. (1977) excludes the assignment of the gene to the chromosome in question. We used this criterion to identify the mink chromosomes carrying genes amenable to assignment by means of the 25 clone panel. The rationale was as follows. The specific chromosome for which we checked the hypothesis for assignment of the gene for the marker (M) to the chromosome was designated A. The chromosome on which this gene is located in reality was designated B. We proceeded from the following assumptions: 1. The method used for detecting the presence of M gives positive results when the gene for M is present in at least 15% of the cells of the hybrid clone. 2. There are no chromosome rearrangements involving chromosome B. Under these conditions, only the clones in which chromosome B is identified (B^+) have detectable gene expression (M^+). This permits one to calculate from the data of Table 1 the number of clones of type A^+M^+ for each particular chromosome B.

Table 2. Number of A^+M^- type hybrid clones

Chromosome	Chromosome B														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	X
1	0	8	3	3	4	1	7	7	3	3	4	3	3	1	0
2	4	0	3	3	4	3	4	3	1	1	3	3	3	0	0
3	3	7	0	3	5	4	7	6	3	1	5	2	5	1	0
4	5	9	5	0	6	5	9	7	2	2	5	5	3	2	1
5	4	8	5	4	0	3	7	6	2	2	4	2	5	4	1
6	6	12	9	8	8	0	11	11	5	4	9	6	7	5	0
7	3	4	3	3	3	2	0	5	2	2	3	2	4	1	0
8	4	4	3	2	3	3	6	0	3	1	3	2	3	2	0
9	10	12	10	7	9	7	13	13	0	6	10	8	8	5	2
10	10	12	8	7	9	6	13	11	6	0	9	7	8	5	2
11	3	6	4	2	3	3	6	5	2	1	0	4	3	1	1
12	6	10	5	6	5	4	9	8	4	3	8	0	5	5	0
13	7	11	9	5	9	6	12	10	5	5	8	6	0	5	1
14	8	11	8	7	11	7	12	12	5	5	9	9	8	0	1
X	13	17	13	12	14	8	17	16	8	8	15	10	10	7	0

In Table 2, all cases in which chromosome B is either the mink X chromosome or one of the autosomes are included.

The presence of more than one independent hybrid clone of A^+M^- type excludes the assignment of the gene for M to the chromosome A (criterion of Wijnen et al. 1977). With respect to genes located on the chromosomes 1-5, 7, 8 and 11-13, the application of this criterion can result in an assignment limited to one autosome (Table 2).

A more detailed analysis of the clone panel shows that the genes located on chromosomes 6,9, and 10 can be assigned to a specific chromosome (Table 3). The assignment of the genes located on chromosome 14 and the X are provisional (Table 3).

Assignment of the Genes for MOD-1 and MOR-1

The results of the assignment analysis for MOD-1 and MOR-1 are summarized in Tables 4 and 5. In these analyses, we used Wijnen's criterion, the chi square measure of dependence, the phi coefficient, the OR statistical calculated according to Cowmeadow and Ruddle (1978). As seen in the Tables, the gene for MOD-1 can be assigned to mink chromosome 1, and the gene for MOR-1 can be assigned to mink chromosome 11.

Assignment of the Genes for G6PD and HPRT

As shown in Table 6, the X is the most likely candidate for the assignment of genes for G6PD and HPRT. However, the assignment of these genes to the X performed by means of this clone panel is provisional, because there is no clone of type $A^-G6PD^+HPRT^+$ in the panel (A being one of mink autosomes). Rearrangements and selective retention of the genetic material of chromosome A can produce a large number of $A^-G6PD^+HPRT^+$ clones in the case when genes for G6PD and HPRT are located on this chromosome. Consequently, the other chromosomes cannot be discarded as likely candidates for gene assignment. This clone panel does not permit us to make final decisions regarding the assignment of genes located on the X.

This report is the first result of the utilization of Chinese hamster x American mink somatic cell hybrids for gene mapping studies. The results presented suggest that the clone panel obtained in this laboratory may be favorable system for mink gene mapping.

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Table 3. Assignment analysis for genes located on mink chromosomes 6, 9, 10 and 14, and the X

Chromosome B	Chromosome A	Hybrid clones A^+M^+ / A^+M^-	Hybrid clones A^-M^+ / A^-M^-	Phi	Shi sq.	OR
6	1	9 1	6 9	0.39	3.86	5.46
9	2	5 1	12 7	0.23	1.37	5.81
10	2	5 1	12 7	0.23	1.37	5.25
	3	9 1	8 7	0.33	2.73	7.00
	8	6 1	11 7	0.26	1.68	5.85
	11	8 1	9 7	0.30	2.33	5.95
14	1	9 1	8 7	0.30	2.73	6.83
	2	6 0	11 8	0.30	2.27	6.83
	3	9 1	8 7	0.30	2.73	6.83
	7	5 1	12 7	0.23	1.37	5.52
	11	8 1	9 7	0.30	2.33	6.34
X	1 – 14			0.37	3.27	9.75

Table 4. Assignment of the gene for MOD-1 to chromosome 1

Chromosome	MOD-1 plus		MOD-1 minus		Phi	Chi sq.	OR
	Chrom. plus	Chrom. minus	Chrom. plus	Chrom. minus			
1	10	0	0	15	1.00	25.00	11.27
2	2	8	4	11	−0.08	0.17	5.27
3	7	3	3	12	0.50	6.25	7.89
4	7	3	4	11	0.42	4.57	7.04
5	6	4	4	11	0.33	2.78	6.92
6	9	1	6	9	0.50	6.25	8.08
7	3	7	3	12	0.11	0.33	6.57
8	3	7	4	11	0.04	0.03	5.49
9	7	3	10	5	0.03	0.03	4.82
10	7	3	10	5	0.03	0.03	4.43
11	6	4	3	12	0.41	4.17	7.36
12	7	3	6	9	0.37	3.29	6.32
13	7	3	7	8	0.23	1.33	6.73
14	9	1	8	7	0.38	3.71	6.83
X	10	0	13	2	0.24	1.39	4.97

Table 5. Assignment of the gene for MOR-1 to chromosome 11

Chromosome	MOR-1 plus		MOR-1 minus		Phi	Chi sq.	OR
	Chrom. plus	Chrom. minus	Chrom. plus	Chrom. minus			
1	6	3	4	12	0.41	4.17	7.36
2	3	6	3	13	0.16	0.67	7.18
3	5	4	5	11	0.24	1.42	6.29
4	6	3	5	11	0.34	2.93	6.64
5	6	3	4	12	0.41	4.17	7.81
6	6	3	9	7	0.10	0.26	5.37
7	3	6	3	13	0.16	0.67	7.37
8	4	5	3	13	0.27	1.89	7.41
9	7	2	10	6	0.16	0.62	5.71
10	8	1	9	7	0.33	2.82	6.35
11	9	0	0	16	1.00	25.00	11.67
12	5	4	8	8	0.05	0.07	4.81
13	6	3	8	8	0.16	0.65	6.33
14	8	1	9	7	0.33	2.82	6.34
X	8	1	15	1	0.09	0.18	3.37

Table 6. G6PD and HPRT by chromosome 2 X 2 tables

Chromosome	G6PD, HPRT plus		G6PD, HPRT minus		Phi	Chi sq.	OR
	Chrom. plus	Chrom. minus	Chrom. plus	Chrom. minus			
1	10	14	0	1	0.16	0.69	4.57
2	6	18	0	1	0.11	0.33	3.07
3	10	14	0	1	0.16	0.69	4.26
4	11	13	0	1	0.18	0.88	5.05
5	9	15	1	0	−0.25	1.56	3.74
6	15	9	0	1	0.25	1.56	9.44
7	6	18	0	1	0.11	0.33	3.26
8	7	17	0	1	0.13	0.41	3.67
9	16	8	1	0	−0.14	0.49	9.34
10	16	8	1	0	−0.14	0.49	8.31
11	9	15	0	1	0.15	0.59	4.17
12	13	11	0	1	0.21	1.13	6.77
13	14	10	0	1	0.23	1.32	8.42
14	17	7	0	1	0.30	2.21	10.53
X	23	1	0	1	0.69	11.97	15.38

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