ASSIGNMENT OF THE RECEPTOR FOR ECOTROPIC MURINE LEUKEMIA VIRUS TO MOUSE CHROMOSOME 5

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Identification of host factors that regulate leukemia virus replication and expression is an important feature in understanding virus-host interaction. One host gene, Fv-1, which regulates ecotropic (mouse-tropic) murine leukemia virus (MuLV)[1] replication, has been assigned to chromosome 4 in the mouse, closely linked to the enzyme glucose-6-phosphate dehydrogenase (1). This factor operates at a postpenetration step (2, 3) and may influence integration of proviral DNA into the host chromosome (4, 5). Additional host control is exerted at the level of differentiation. One example is the ability of bone marrow derived but not thymus-derived lymphocytes to support replication of exogenous MuLV (6). This restraint may be distinct from, or identical to, that which is influenced by the viral receptor. Huang et al. (2) demonstrated that whereas mouse cells absorbed pseudotype virus particles consisting of vesicular stomatitis virus (VSV) cores with ecotropic MuLV coats, Chinese hamster cells could not. Similar conclusions were drawn by DeLarco and Todaro (7) who studied the binding of labeled MuLV glycoprotein (gp 71) to mouse and Chinese hamster cells. This cellular capacity is most likely under the control of a cell structure known as the ecotropic MuLV receptor.

The purpose of the studies reported here was to analyze the genetic control of MuLV replication at the level of the receptor. Gazdar et al. (8), in an important study, have recently implicated chromosome 5 in the regulation of MuLV replication. However, the level of control was not defined in their experiments. In this paper we provide additional evidence that this function is the receptor for ecotropic MuLV. The experimental material consists of somatic cell hybrids between mouse and Chinese hamster cells that segregate murine chromosomes. We have shown an association between the presence of mouse chromosome 5 as determined by karyotype and isozyme analysis, and the capacity to absorb and replicate ecotropic MuLV. Through subcloning experiments, we have shown that the replicative function is syntenic with phosphoglucomutase-1 (PGM-1). We thus assign the gene for the receptor for ecotropic MuLV (Rev) to mouse chromosome 5.

Materials and Methods

Cells. The experimental material consisted of somatic cell hybrids between mouse primary

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[1] *Abbreviations used in this paper: Fv-1, Friend virus-1;* MuLV, murine leukemia virus; PFU, plaque-forming units; *Rev,* receptor for ecotropic MuLV; VSV, vesicular stomatitis virus.

cells and E36, a line of Chinese hamster lung fibroblasts which are deficient in hypoxanthine phosphoribosyltransferase (HPRT) activity. Selection was in HAT (hypoxanthine, aminopterin, thymidine) medium (9) and back selection in azaguanine. The primary mouse parents were derived from two strains of *Mus musculus*, A/HeJ and BALB/c, and from the I strain of *Mus poschiavinus*. Mice of the I strain of *Mus poschiavinus*, the tobacco mouse, were obtained from Doctors M. Davisson and T. H. Roderick of The Jackson Laboratory, Bar Harbor, Maine, who had received them originally from Dr. Alfred Gropp as an F_1 hybrid between NMRI-Swiss mice and *Mus poschiavinus*. The mice were maintained by brother-sister mating at The Jackson Laboratory and were in the F_{12} generation at the time their cells were used to generate the hybrids used here.

The murine origin of individual hybrid clones is indicated in Results and particularly in Tables II and III. MACH (A/HeJ) and PIM (I strain) clones were generated from crosses between murine peritoneal exudate cells and E36 and have been previously described (10). The BE and BEM clones were generated specifically for this study between BALB/c embryo fibroblasts and E36. Data are only included from hybrid clones which were tested and found to be negative for mycoplasma (PPLO). It has been our experience in the past that false negative XC tests are often observed in PPLO infected lines.

Karyotypic Analysis. Hybrid cells were analyzed for mouse chromosomes by the sequential Giemsa-Viokase, Hoechst "33258" technique (11). Briefly, cell monolayers were incubated in 0.05 μg/ml Colcemid for 50 min. followed by swelling of harvested mitotic cells in 0.075 M KCl at 37°C for 30 min and fixation. Slides were baked for 5 days at 60°C and treated with a 0.02% solution of Viokase (Grand Island Biological Co., Grand Island, N. Y.) in 0.05 M Tris, pH 7.5 and stained with Giemsa. An average of 25 spreads was photographed from each clone. Slides were destained and restained with the fluorochrome Hoechst 33258 (American Hoechst Co., Somerville, N. J.) which specifically stains mouse centromeric DNA brightly.

Isozyme Analysis. Each cell line was assayed for a series of enzyme markers from a pellet of 5 × 10⁷ cells. The 19 enzymes have been assigned to 13 mouse linkage groups and can be distinguished from the hamster forms. The isozymes and their analyses are described in Table I. The method of starch gel electrophoresis which was employed is described in detail by Nichols and Ruddle (12, 13). The following isozymes were analyzed: alpha galactosidase (α-GAL); adenosine kinase (ADOK, EC 2.7.1.20); adenine phosphoribosyltransferase (APRT, EC 2.4.2.7); dipeptidase-1 (DIP-1, EC 3.4.-); dipeptidase-2 (DIP-2, EC 3.4.-); dipeptidase-3 (DIP-3, EC 3.4.-); esterase-10 (ES-10); galactokinase (GALK, EC 2.7.1.6); glyoxylase I (GLO I, EC 4.4.1.5); glucose phosphate isomerase (GPI, EC 5.3.1.9); glutathione reductase (GR, EC 1.6.4.2); malate oxidoreductase (MOD-1, EC 1.1.1.40); mannose phosphate isomerase (MPI, EC 5.3.1.8); nucleoside phosphorylase-1 (NPI-1, EC 2.4.2.1); phosphoglucomutase-1 (PGM-1, EC 2.7.5.1); phosphoglucomutase-2 (PGM-2, EC 2.7.5.1); 6-phosphogluconate dehydrogenase (PGD-1, EC 1.1.1.44); triose phosphate isomerase (TPI; EC 5.3.1.1); tripeptidase-1 (TRIP-1, EC 3.4.-).

Virus Replication Assays

XC TESTS. Hybrid clones were analyzed for replication of N and B tropic MuLV by using the direct UV-XC assay developed by Rowe et al. (28) as previously described (29). The virus preparations were WN 1802B (B-tropic MuLV), WN 1802N (N tropic MuLV) and clone 2, (Moloney NB tropic MuLV). The N-tropic and B-tropic viruses were isolated from mice by Doctors Hartley and Rowe, National Institutes of Health, and kindly supplied by them.

Hybrid cells were inoculated in 35-mm tissue culture dishes. 24 h later virus was added to duplicate dishes. Each hybrid was tested in individual duplicate dishes with 2,000 XC plaque-forming units (PFU) and 200 PFU of B and N tropic virus, and was also tested for release of endogenous virus. The PFU had been defined on BALB and NIH Swiss mouse embryo fibroblasts, the standard test cells for B and N tropic virus. BALB and Swiss controls were also run concurrently in each experiment. 7 days after addition of virus, a 24-h supernate was collected, and the cells received 1,500 ergs ultraviolet irradiation and were overlaid with 5 × 10⁵ XC cells. The cells were fixed and stained with Giemsa 3 days later, and the XC plaques counted. Each plaque was defined as a minimum of two giant cells with 10 nuclei. To increase the sensitivity further, the 24-h supernates (1 ml) described above were added to BALB and Swiss mouse embryo fibroblasts. These embryo fibroblasts were irradiated 7 days later, overlaid with XC cells, and fixed and stained as above. Each clone was analyzed in at least two separate tests. Some negative clones were tested as many as five times. Supernates from negative clones were always further tested as above in BALB and NIH Swiss embryo fibroblasts.

VSV (MuLV) PSEUDOTYPE TEST.   A cloned stock of VSV t117 was kindly provided by Doctors J. Zavada and R. Weiss. This mutant was a thermolabile glycoprotein and is easily inactivated at 45°C (30). VSV titration was carried out as described earlier (31). VSV t117 (Mol-MuLV) pseudotypes were prepared as previously described (32).

Infective center assay for VSV (Mol-MuLV) pseudotypes: due to difficulties performing regular VSV assays on hybrid cells the following procedure was adopted. Cells were plated at 5×10^5 cells/5 cm plate. The following day they were inoculated with virus in Dulbecco's modified Eagle's medium containing 8 μg/ml polybrene. After 3 h adsorption at 32°C, the cells were trypsinized for 7 min at 32°C and then collected by centrifugation. The infected cells were resuspended in medium and serially diluted into tubes containing 2 ml of CHO cells (2-4 $\times$ 10^6 cells/ml) in Dulbecco's modified Eagle's medium plus 10% heat inactivated fetal bovine serum. 2 ml of medium containing 0.6% agarose was then added to each tube and 1 ml of this mixture was plated onto 5-cm plates containing 5 ml of bottom agar (medium with 1% agar). After 2 days at 32°C in a humidified CO_2 incubator, plaques could be read without staining the cells with neutral red. NIH/3T3 cells were included in all experiments as positive control. The titer on these cells usually varied only slightly. The ratio of the titers of the virus samples treated without and with MuLV antiserum usually was between 500 and 1,500.

Neutralizations.   Anti-Mol-MuLV serum from tumored Fischer rats was provided by the Virus Cancer Program, National Cancer Institute. Neutralizations with this antiserum were done at a final antibody concentration of 1:40 as described earlier (32).

Statistical Procedures.   Statistical analysis was conducted with the aid of a computer program, ASSIGN, developed for the purpose of gene assignment with material generated by somatic cell hybridization (33). Viral replication and chromosome constitution of individual clones was analyzed and compared. To ascertain the gene assignment for viral replication, three different statistical procedures were used, Chi square, Phi, and OR. Chi square, employed as a method of dependence, rather than a test of significance, was calculated in the ordinary way. The Phi coefficient, which is insensitive to sample size is the ratio of the observed Chi square to the perfect Chi square. It is calculated by dividing the square root of the regular Chi square by the square root of the sample size. When there exists a perfect association between a marker and a chromosome, the value of Phi is +1. Zero indicates no association, and −1, a negative association. The OR statistic was designed for determining gene assignments (33). It is an inverse weighted scoring system which is independent of Chi square. It is calculated by assigning each clone a score of 100 and dividing that value evenly among all chromosomes in concordance with the tested marker (viral replication in the present study). The clones are scored, the scores are added for each chromosome, and then divided by the number of clones. The chromosome with the highest number is considered a candidate for assignment of the gene for viral replication. Statistical analyses which are summarized in Table IV were calculated with a chromosome cut off point of 20%. This level was used because experience has shown that many isozyme markers are not detectable with chromosome frequencies below that.

Results

Mouse Chinese Hamster Hybrids.   Experimental material consisted of clones derived from crosses between Chinese hamster cells (E36) and murine peritoneal exudate cells (A/HeJ and *Mus poschiavinus* strain I) or embryo fibroblasts (BALB/c). Both *Mus musculus* strains were of the Fv-1^{bb} genotype, thus permissive for B-tropic virus replication and relatively restrictive for N-tropic virus. The Fv-1 genotype of *Mus poschiavinus* is unknown. Mouse by Chinese hamster hybrid clones (segregating murine chromosomes) were analyzed for the expression of 19 isozymes which are mapped in the mouse genome to 13 chromosomes (Table I). Karyotypes were also performed on an average of 25 cells for each clone. An example of chromosome banding patterns is presented in Fig. 1. It has been our experience that the karyotype method is a more sensitive indicator of chromosome presence, but that the isozyme method is technically simpler and more useful for screening purposes. We have also

TABLE I

Linkage Assignments of Marker Enzymes in the Mouse

Chromosome	Gene	Enzyme	Reference
1	Dip-1	Dipeptidase-1	14
4	Pgd-1, Pgm-2	6-Phosphogluconate dehydrogenase phosphogluco-mutase-2	14
5	Pgm-1	Phosphoglucomutase-1	15
	Dip-3	Dipeptidase-3	This report
6	Tpi	Triose phosphate isomerase	16
7	Gpi	Glucose phosphate isomerase	17
8	Gr, Aprt	Glutatione reductase, adenine phosphoribosyltransferase	13 18
9	Mod-1, Mpi	Malate oxidoreductase decarboxylating, mannose Phosphate isomerase	19 20
10	Trip-1	Tripeptidase-1	16
11	Galk	Galactokinase	21 22
14	Np-1, Es10, Adok	Nucleoside phosphorylase-1 esterase-10, adenosine kinase	23 24
17	Glo I	Glyoxylase I	25
18	Dip-2	Dipeptidase-2	26
X	α-gal	Alpha galactosidase	27

observed that the sensitivity of different enzyme assays varies significantly, introducing a possible source of error.

Parental cells were treated for release of endogenous MuLV by XC cell assay. Hybrid clones were also assayed for release of endogenous ecotropic virus when they were tested for their ability to support exogenous N and B tropic virus replication. None of the hybrid clones spontaneously released complete infectious particles. Several were tested for viral group specific antigens (data not included) and were also negative by that test.

Correlation of Susceptibility to MuLV and VSV(MuLV). Mouse-Chinese hamster hybrids were analyzed for their susceptibility to infection by ecotropic MuLV by means of two different assays. The VSV(MuLV) pseudotype susceptibility assay measured interaction of the MuLV glycoprotein with the cell receptor and utilized pseudotypes made by superinfecting Moloney MuLV-producing cells with a VSV mutant that had a temperature sensitive glycoprotein. After heating, only VSV(MuLV) particles were left; their ability to absorb to and replicate in cells was assayed by an infective center procedure (Materials and Methods). In all cases, the pseudotypes were shown to be neutralized by an anti-MuLV serum and results were scored by the ratio of plaques without neutralization to plaques after neutralization. A positive test was scored if a ratio of 20 or more was observed. The other test used, the XC test, measured a series of steps in viral replication. It was only positive in those circumstances in which complete infectious particles were released and was susceptible to host cell regulation at all levels of viral replication. These include absorption and penetration (receptor), synthesis of proviral DNA, integration into host cell DNA, transcription of viral message, translation, release, and spread.
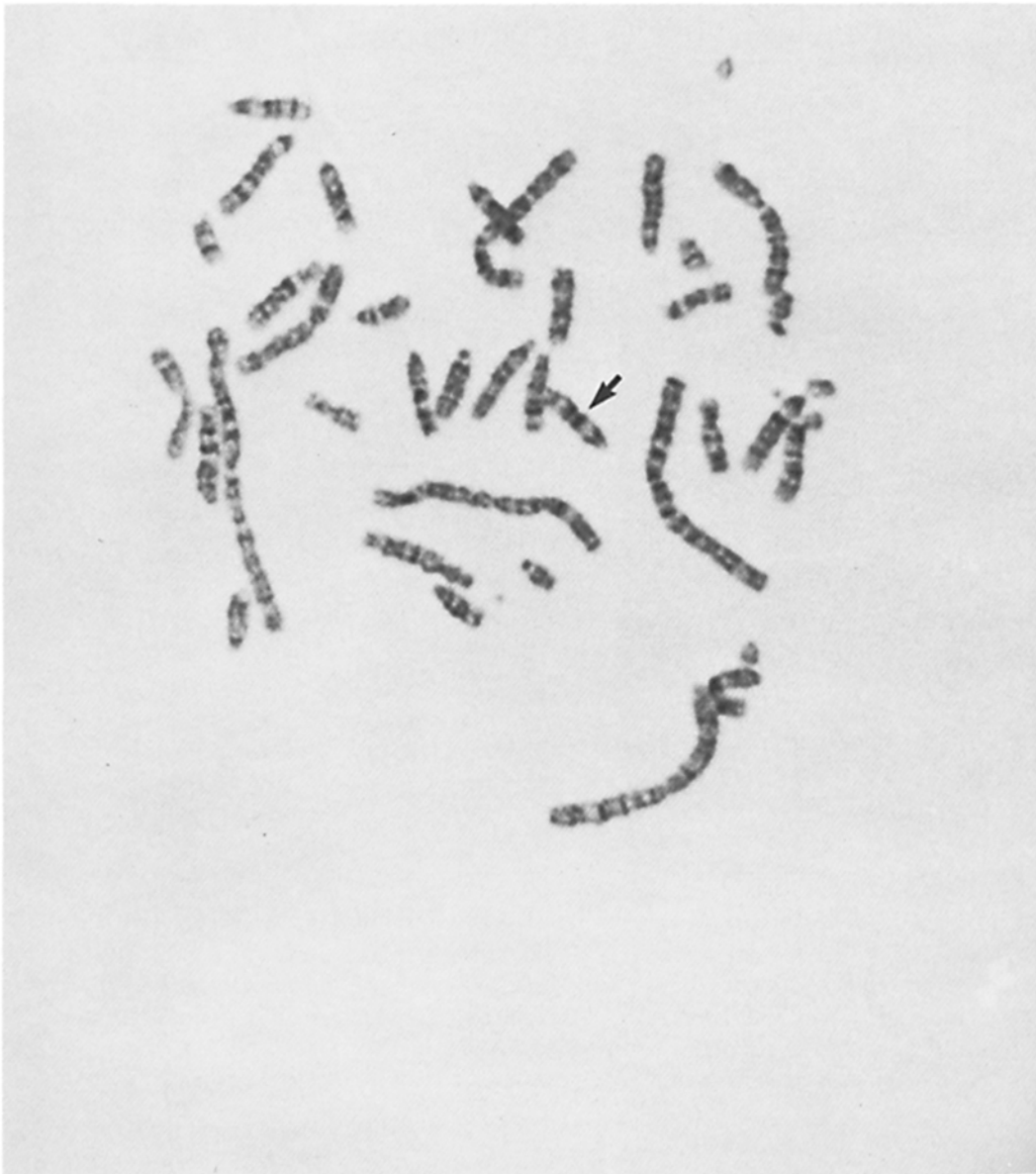
Fig. 1.   Metaphase spread of BEM-1, a somatic cell hybrid between BALB/c mouse embyro fibroblasts and E36. The chromosomes were banded with Giemsa as described in Materials and Methods. Chromosome 5 is indicated by the arrow.

The positive correlation between the two assays in our study strongly suggests that the function studied in this paper is the mouse coded receptor for ecotropic MuLV. E36 was negative for endogenous mouse ecotropic virus and for replication of exogenous ecotropic MuLV by both assays (Table II). Murine parental BALB/c and A/HeJ fibroblasts were negative for spontaneous release of endogenous MuLV, supported replication of exogenous B tropic virus, and

TABLE II

Correlation of XC Tests and VSV(MuLV) Pseudotype Assays

Species	Mouse strain	Cell line	MuLV Sus- ceptibility (XC plaques)	VSV (MuLV) Susceptibil- ity (no an- tibody/anti- MuLV)
Chinese hamster	–	E36	0	2.3
Mus musculus	A/HeJ	Embryo fibroblasts	1,850	ND
		3A	100	300
		3B	500	200
		3B5A	0	2
		3B9C	138	420
		3B9C4	330	100
		3B9C 4-4	0	1
		4A6-2	0	1
		2A-4	0	4
		4E4	100	460
		7A13-2b	66	30
		7A13-4a	6	3
		10-2	0	1.8
		14	0	0.8
		15-1	0	1
		132-Az	0	2
Mus musculus	BALB/c	Embryo fibroblasts	2,000	ND
		BEM-1	220	61
		BEM-1-2	200	110
		BEM-1-ps	0	2
		BEM-2	0	4
Mus poschiavinus	I	PIM-3A	2	4
		PIM-12B	320	600

absorbed the VSV(MuLV) pseudotype. In fact, agreement between the two tests was quantitative. Cells which yielded a high virus titer after infection as determined by XC test, could also be infected by a large number of VSV(MuLV) pseudotype virus particles. The latter is expressed as a high ratio when the number of VSV plaques obtained without antibody is compared with the number after anti-MuLV antibody treatment.

The Assignment of the Gene for the Receptor for Ecotropic MuLV (Rev) to Chromosome 5

Statistical Analysis. The susceptibility of mouse-Chinese hamster hybrid cells to infection by ecotropic MuLV was analyzed with regard to chromosome constitution. 20 core clones which represent 12 separate hybridization events were thoroughly studied with respect to viral replication as assessed by both the XC test and VSV(MuLV) pseudotype absorption assay, and for chromosome constitution by karyotype and analysis of 19 isozymes. These 20 clones were generated by hybridizing E36 with cells from two strains of *Mus musculus* (BALB/c and A/HeJ) and the I strain of *Mus poschiavinus*. 80 additional clones and subclones were analyzed by isozyme and XC test, and approximately half of these were further tested for absorption and penetration of VSV(MuLV)

TABLE III

Chromosome Frequency and Susceptibility to Ecotropic Virus Infection in Mouse-Chinese Hamster Hybrids

Species	Mouse strain	Cell line	Viral repli-cation	Chromosome frequency																			
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
														%									
Mus muscu-lus	A/HeJ	4A63	−	44	93	0	0	0	0	93	0	41	0	0	0	0	7	74	70	15	15	96	0
		4A6	−	52	70	0	35	0	35	70	0	30	9	0	0	13	30	70	65	22	13	87	17
		3B9C4	+	54	14	0	10	48	72	52	50	30	0	0	0	30	48	70	38	62	32	32	18
		2A2	−	53	90	22	43	2	69	69	33	65	51	0	73	22	82	90	80	82	55	82	41
		4B31AZ3	−	0	24	0	0	0	0	40	0	0	0	0	0	0	0	68	48	60	56	80	0
		4A62	−	0	94	0	3	0	0	55	0	48	42	0	0	0	6	82	79	45	6	70	21
		3B9C41	−	60	93	0	0	7	87	60	53	53	0	0	0	0	60	93	53	53	60	80	0
		3B9C43	−	79	96	0	4	4	92	79	83	83	0	0	0	25	67	92	92	75	54	83	4
		3B9C44	−	46	75	4	14	0	86	71	57	61	0	0	7	54	50	89	79	50	32	68	18
		2A1	−	62	85	0	38	0	50	69	27	58	35	0	81	4	62	77	73	69	19	69	46
		15	−	67	79	27	33	0	76	67	42	58	9	0	67	36	9	76	82	82	48	97	30
		3A	+	43	90	67	48	33	57	14	52	48	14	0	95	71	38	62	76	86	29	81	33
		14	−	100	100	69	62	0	69	85	69	23	38	0	92	62	54	77	69	100	46	62	54
Mus muscu-lus	BALB/c	BEM1	+	61	83	56	33	64	72	0	50	0	3	0	78	61	42	61	64	58	3	75	50
		BEM1PS	−	33	89	11	39	0	28	0	6	0	0	0	72	39	33	67	72	72	0	17	89
		BE22	−	67	33	0	0	0	93	27	0	0	20	0	87	0	60	0	0	0	67	33	47
		BEM1-4	+	46	96	88	0	77	92	4	81	0	0	0	92	73	69	73	65	85	0	38	73
		BEM1-6	+	63	81	81	63	56	69	0	38	0	25	0	94	38	50	88	69	100	31	88	81
Mus poschia-vinus	I	PIM7AB	−	5	81	19	81	0	71	81	24	71	5	0	86	0	0	10	95	71	29	62	19
		PIM9B	+	59	71	6	0	47	47	76	29	0	24	0	6	0	0	59	35	47	6	18	18

Mouse-Chinese hamster hybrids were assayed for replication of ecotropic MuLV by XC test and VSV(MuLV) pseudotype assay. Positive viral replication was defined as more than 10 plaques on an XC test and a VSV(MuLV) ratio of >20. An average of 25 cells was examined for each karyotype.

pseudotype. Data from the 20 core clones is presented in Table III, and a statistical analysis is shown in Table IV. The positive correlation between the presence of an individual chromosome in a clone and that clone's ability to be infected by ecotropic MuLV was computed with three different measures of dependence. The conclusion from an analysis of the data by all three methods is that the presence of mouse chromosome 5 is essential for viral replication. The Phi value of +1 indicates a perfect gene assignment. The Chi square of 20 is more than 10 times greater than that of most other chromosomes, and the OR score of 14.86 is also very high. Generally an OR score of 7 or above is considered evidence for a positive assignment (33). The high OR value of 12.69 for chromosome 11 is misleading and is due to the preferential loss of that chromosome in all hybrid lines (21).

Segregation Analysis. In the section above, evidence for the assignment of *Rev* to mouse chromosome 5 was obtained through the analysis of a core series of 20 clones representing 12 independent hybridization events. In this section, we present data obtained through the analysis of subclones. In such an experiment, one follows the loss of a trait, in this case the receptor for ecotropic MuLV, concurrent with the loss of a particular chromosome, detected by karyotype or isozyme analysis. We have carried out two separate subcloning experiments with the BEM (BALB/c × E36) and 3B (A/HeJ × E36) clones.

BEM-1 was a hybrid clone which was not releasing endogenous ecotropic virus and was capable of being infected by exogenous virus when assayed both by the XC test (200 B tropic plaques, 6 N-tropic plaques) and by the VSV(MuLV) absorption test (ratio of 61). The parent line continued to segregate chromosomes and subclones were prepared. Seven subclones and the parent after

TABLE IV

Statistical Analysis of the Correlation between Individual Mouse Chromosomes and Susceptibility to Ecotropic MuLV Infection in Mouse-Chinese Hamster Hybrid Clones

Mouse chromo-somes	Viral replication plus		Viral replication minus		Statistical scores		
	Chromo-some plus + +	Chromo-some mi-nus + −	Chromo-some plus − +	Chromo-some mi-nus − −	Phi	Chi square	Overall OR
			Number of clones				
1	6	0	11	3	0.28	1.51	3.56
2	5	1	14	0	−0.35	2.46	1.78
3	4	2	3	11	0.43	3.78	7.72
4	3	3	7	7	0.00	0.00	4.70
5	*6*	*0*	*0*	*14*	*1.00*	*20.00*	*14.86*
6	6	0	11	3	0.28	1.51	3.39
7	2	4	13	1	−0.63	7.94	1.44
8	6	0	8	6	0.43	3.67	5.00
9	2	4	11	3	−0.43	3.78	2.12
10	2	4	5	9	−0.02	0.01	6.53
11	0	6	0	14	0.00	0.00	12.69
12	4	2	7	7	0.15	0.47	5.11
13	5	1	6	8	0.37	2.78	6.12
14	5	1	9	5	0.19	0.73	4.69
15	6	0	12	2	0.22	0.95	3.22
16	6	0	13	1	0.15	0.45	2.66
17	6	0	12	2	0.22	0.95	3.05
18	3	3	9	5	−0.13	0.36	3.92
19	5	1	13	1	−0.15	0.42	2.22
X	4	2	7	7	0.15	0.47	5.21

The raw data regarding chromosome frequency and susceptibility to ecotropic MuLV was analyzed statistically for 20 of the primary clones presented in Table III. These scores were calculated with a base line of 20% chromosome frequency. Comparable results were obtained at other frequencies.

segregation (MEM-1-ps) were assayed for viral replication and isozyme expression (Table V). Karyotypes were also prepared for several of the key clones. Only BEM-1-ps lost the ability to absorb and replicate virus, though chromosome segregation was documented in many other subclones as well. BEM-1-ps lost PGM-1, TPI, GR and NP after segregation. Only TPI and PGM-1 were uniquely lost by BEM-1-ps in comparison with the other segregating subclones which were still receptor positive. The loss of TPI (chromosome 6) was coincidental, because that chromosome was ruled out by statistical analysis of the core clones (Table IV). Since PGM-1 is on chromosome 5, these data provide further strength for the assignment of *Rev* to that chromosome.

3B, a line derived by hybridization of A/HeJ peritoneal exudate cells with E36, was negative for spontaneous virus release, and could be infected by exogenous ecotropic MuLV (3,000 B tropic plaques, 81 N tropic plaques). The pseudotype absorption test was also positive with a ratio of 200. The line was subcloned (3B9C) and further subcloned. The only isozymes that the five negative subclones lost in common were PGM-1 and DIP-3 (Table VI). These

TABLE V

Segregation of Viral Replication with PGM-1 in BEM-1 Subclones of BALB/c × E36 Hybrids

Isozyme	Subclones								
	BEM-1*	BEM 1-2	BEM-1-4*	BEM 1-5	BEM 1-6*	BEM 1-8	BEM 1-9	BEM 1-10b	BEM-1-ps*
DIP-1	+	+	+	+	+	+	+	+	+
6PGD	+	+	−	+	+	+	−	−	+
PGM-2	+	+	−	+	+	+	−	−	+
PGM-1	+	+	+	+	+	+	+	+	−
DIP-3	+	+	+	+	+	+	−	+	+
TPI	+	ND‡	+	+	+	+	ND	+	−
GPI	−	ND	−	−	−	−	−	−	−
GR	+	+	+	+	+	+	ND	+	−
APRT	+	+	+	+	+	+	+	+	+
MPI	−	−	−	−	−	−	−	−	−
MOD-1	−	−	−	−	−	−	−	−	−
TRIP-1	−	−	−	−	−	−	−	−	−
GALK	−	−	−	−	−	−	−	−	−
NP	+	−	−	+	−	+	+	+	−
ES-10	+	+	+	+	+	+	+	+	+
ADOK	+	+	+	+	+	−	+	+	+
GLO-1	+	+	+	+	+	+	+	+	+
DIP-2	−	−	−	−	−	−	−	−	−
α-GAL	+	+	+	+	+	+	+	+	+
Viral susceptibility									
XC B tropic	220	200	370	50	87	90	15	104	0
N tropic	6	14	20	2	0	6	33	98	0
B/N	37	14	19	25	>87	15	0.45	1.06	0
VSV(MuLV)	+	+	+	+	+	+	+	+	−
Conclusion	+	+	+	+	+	+	+	+	−

* Karyotypes were analyzed for these lines. Some are included in Table III.
‡ Not done.

data again confirm the conclusion that chromosome 5 is essential for ecotropic virus replication.

Confirmation of the Importance of the Fv-1 Locus on Ecotropic MuLV Replication. A murine cell's susceptibility to B tropic or N tropic virus is influenced by its genotype at the *Fv-1* locus, a gene on chromosome 4 linked to 6PGD (1). *Fv-1^{nn}* mice preferentially support the replication of N tropic virus and are relatively restrictive for B tropic virus. The opposite holds true for *Fv-1^{bb}* mice. This restriction, which is not absolute, is reflected as a 10–100 times greater number of plaques in an XC test. Restriction is dominant and cells of *Fv-1^{nb}* mice support neither N tropic nor B tropic viruses, but can be infected by NB tropic viruses, such as Moloney MuLV.

The Fv-1 phenotype of the parent mouse lines was expressed in the hybrids made with E36 and A/HeJ or BALB/c. That is to say, the ratio of B tropic to N tropic plaques was high when replicate dishes were inoculated with the same number of PFU. This pattern was maintained in all hybrid clones from these strains except when 6PGD was lost in two BEM-1 subclones (Table V). Thus,

TABLE VI

Segregation of Viral Replication with PGM-1 in 3B9C4 Subclones of A/HeJ by E36 Hybrids

Isozyme	Subclones						
	3B9C4*	3B9C4-1*	3B9C4-2-1a	3B9C4-3*	3B9C4-4*	3B9C4-4-1	3B9C4-4-3
DIP-1	+	+	+	+	+	+	+
6PGD	+	ND‡	ND	ND	+	+	ND
PGM-2	+	−	+	−	+	+	+
PGM-1	+	−	+	−	−	−	−
DIP-3	+	−	+	−	−	−	−
TPI	+	+	+	+	+	+	+
GPI	+	+	+	+	+	+	+
GR	+	+	+	+	+	+	+
APRT	+	+	+	+	+	+	+
MPI	+	+	+	+	+	+	+
MOD-1	−	+	+	−	ND	ND	+
TRIP-1	−	−	−	−	−	−	−
GALK	−	−	−	−	−	−	−
NP	+	+	+	+	+	+	+
ES-10	+	+	+	+	+	+	+
ADOK	+	+	+	+	+	+	+
GLO-1	+	+	+	+	+	+	+
DIP-2	+	+	+	+	+	+	+
Viral susceptibility							
XC B tropic	330	0	90	3	0	0	7
N tropic	9	0	4	0	0	0	0
B/N	37	0	23	>3	0	0	>7
VSV(MuLV)	+	−	ND	ND	−	ND	−
Conclusion	+	−	+	−	−	−	−

* Karyotypes for these lines are included in Table III.

‡ Not done.

when the markers on chromosome 4 were present and viral replication occurred due to the presence of Rev on chromosome 5, the ratio of B tropic to N tropic virus ranged from 15 to >87. When 6PGD was lost in subclones BEM 1-9 and BEM 1-10b, the ratio dropped to 0.45 and 1.06. Subclone BEM 1-4 is an unexplained exception to this pattern, since it had lost chromosome 4 by isozyme and karyotype analysis and yet preferentially replicated B tropic virus (B/N = 19). Fv-1 restriction was maintained in all positive 3B9C4 subclones. Those which had lost 6PGD (3B9C4-1 and 3B9C4-3) were also negative for viral replication due to the loss Rev (Table VI).

An interesting phenomenon was observed vis-a-vis the Fv-1 question in the hybrids between *Mus poschiavirus* and E36. The Fv-1 genotype of the mouse used in this cross is unknown. We suspect it to be $Fv-1^{nb}$ from its cross with Swiss mice and an analysis of isozyme and viral susceptibility of 4 of the hybrids (Table VII). The karyotype of two of these lines is also included with the core data in Table II. PIM-3A and PIM-7AB could not be infected by B tropic or N tropic virus, did not express PGM-1 and retained 6PGD. The lack of viral susceptibility was not due to *Fv-1* restriction because the cells were not permissive for Moloney NB tropic virus or VSV(MuLV) pseudotype. PIM-9B expressed PGM-1

TABLE VII

Isozyme Analysis and Viral Susceptibility in Hybrids between Mus Poschiavinus and E36

Isozyme	PIM-3A	PIM-7AB*	PIM-9B*	PIM-12B
DIP-1	+	−	+	+
6PGD	+	+	−	+
PGM-2	+	+	−	+
PGM-1	−	−	+	+
DIP-3	ND‡	ND	+	+
TPI	+	+	+	+
GPI	+	+	+	+
GR	−	+	+	−
APRT	ND	+	ND	ND
MPI	−	+	+	+
MOD-1	−	+	+	+
TRIP-1	+	−	+	+
GALK	−	−	ND	ND
NP	+	−	−	+
ES-10	+	ND	−	+
ADOK	ND	ND	−	+
GLO-1	+	+	+	±
DIP-2	−	ND	−	+
Viral susceptibility				
XC B tropic	2	0	19	0
N tropic	0	0	1	7
Moloney	ND	0	ND	320
VSV (MuLV)	−	ND	ND	++
Conclusion	−	−	+	+

* Karyotypes for these lines are included in Table III.
‡ Not done.

and 6PGD and was not susceptible to N tropic or B tropic virus. It could be infected by the same number of PFU of Moloney NB tropic virus and had a VSV(MuLV) ratio of 600, one of the highest in this study (Table II). We conclude that the clone was not permissive for N tropic or B tropic virus due to Fv-1 restriction. Once the latter was overcome, the cells were optimally permissive due to the presence of the receptor for ecotropic MuLV.

Discussion

In this communication, we have assigned the gene for the receptor for ecotropic murine leukemia virus to chromosome 5 in the mouse. This has been accomplished by means of an analysis of a series of somatic cell hybrids between mouse primary cells and those of a Chinese hamster established line, E36, which segregates mouse chromosomes. The hybrid cells' ability to absorb and replicate both VSV(MuLV) pseudotype virus and ecotropic MuLV segregated with chromosome 5 as determined by karyotype and isozyme analysis. This function has been defined as the receptor for ecotropic MuLV, and we have designated the gene *Rev*.

Gazdar et al. (8) reported a genetic function in somatic cell hybrids which controlled an undefined step in the regulation of MuLV replication. This

function which was also assigned to chromosome 5, was monitored by reverse transcriptase activity and expression of viral antigens. On the basis of these assays, the function was designated replication of ecotropic MuLV, and the gene was termed *Rec*. The information supplied in the present communication defines a more precise function, the viral receptor. Although receptor correlated precisely with replication, implying that our analysis and that of Gazdar et al. measured the same function, we have chosen to define the gene we assigned as *Rev* both to denote the precise function involved and to avoid confusion with a class of bacterial mutants which is already denoted by the term *rec*.

The results of experiments presented in this communication were interpreted statistically using the OR model developed by Cowmeadow and Ruddle (33). This model assumes that a function studied in somatic cell hybrids is controlled by one *OR* another chromosome. Other possibilities include the *AND* model which assumes that the marker is entirely duplicated on another chromosome. The *PART* model assumes that genetic elements exist on two different chromosomes, both of which are necessary for marker expression. Our data do not support the AND model because chromosome 5 was the only one that was absent in each negative clone (with the exception of chromosome 11 which was absent in *all* clones).

The PART model remains an intriguing possibility in light of the data presented here and those of Gazdar et al. (8). In both hybrid series, preferential retention of certain chromosomes lends weak support to that model. All positive clones scored for chromosome segregation in this report retained in addition to chromosome 5, chromosomes 1, 6, 8, 15, 16, and 17. The karyotype of 3C-1 permits us to eliminate chromosome 16, since positive clones for viral replication were found which possessed 5, but lacked 16 (34). Clone series 7A13 (10 related subclones) is not included in the core data presented here because these cells lacked an intact identifiable chromosome 5. However, isozyme analysis of this clone series suggested that the *Rev* marker on chromosome 5 had translocated to mouse chromosome 18. In this series, clones were detected which were virus replication positive, but negative for murine chromosomes 1, 6, and 8 both by cytogenetic and isozyme analysis. PIM 12B was not karyotyped, but was positive for viral replication and PGM-1, but negative for GR (chromosome 8). Thus, we can also eliminate chromosomes 1, 6, and 8. Gazdar et al. (8) also found a preferential retention of certain chromosomes in addition to 5 in positive clones, namely X, 12, 15, and 17. When the data from both communications are considered together, chromosomes 15 and 17 are retained in addition to 5 in all positive clones. Negative clones exist which have 15 or 17, but none with 5. These combined data are not inconsistent with the PART model and leave open the possibility that a gene on chromosome 5 codes for an essential component of the receptor, and that genes located on chromosome 15 and/or 17 code for another section of the structure. This is particularly intriguing in light of observations concerning the role of the H-2 antigens (coded by chromosome 17) in lymphocyte cytotoxicity against virus infected cells (35). Until it is possible to obtain clones which are positive for both viral replication and chromosome 5 and negative for chromosomes 15 and 17, or clones which are negative for viral replication and contain chromosome 5 but lack 15 or 17, this problem cannot be solved. Another possibility is that the proteins coded by 15 and 17 are essential

cellular structural components and bear little direct relatinship to the receptor for ecotropic MuLV.

One interesting aspect of viral replication which deserves consideration is the contribution of the Chinese hamster parent. It would be surprising if all cellular functions required for MuLV replication, including the virus integration site, were coded by genes on mouse chromosome 5. Nevertheless, in our study, no clones were encountered which were positive for the chromosome and receptor by the VSV(MuLV) pseudotype test, and negative for viral replication by the XC test. This implies that either all viral replicative functions are contributed by the mouse parent, or that once penetration has occurred, the Chinese hamster parent can support MuLV replication. Future studies will concentrate more fully on the role of the non-segregating parent.

On the basis of results reported here, it is apparent that the gene which regulates the receptor for ecotropic MuLV has been conserved in murine speciation. In the present study, it has been mapped to the same chromosome in two inbred strains of *Mus musculus* and one strain of *Mus poschiavinus*. We believe that the isolation and analysis of this entity will provide insight into mechanisms of differentiation and oncogenesis.

Summary

The gene for the receptor for ecotropic murine leukemia virus (*Rev*) has been assigned to mouse chromosome 5. This determination was made possible by an analysis of somatic cell hybrids between mouse and Chinese hamster cells. The parents of these hybrids were A/HeJ or *Mus poschiavinus* peritoneal exudate cells or BALB/c primary embryo fibroblasts and E36, a Chinese hamster lung fibroblast deficient in hypoxanthine guanine phosphoribosyltransferase. Segregation of mouse chromosomes in these hybrids was analyzed by chromosome banding and isozyme expression. Cells were tested for their ability to absorb and replicate vesicular stomatitis virus (murine leukemia virus [MuLV]) pseudotype particles and ecotropic MuLV as measured by the XC test. The presence of chromosome 5 was essential for receptor expression as determined by three statistical procedures. Segregation of the receptor for ecotropic murine leukemia virus was also followed in two series of subclones. In both, receptor expression was syntenic with phosphoglucomutase-1, an isozyme which has been mapped to mouse chromosome 5.

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