are best adapted to an environment requiring the O_2 of aerobic conditions and the reduced forms of sulfur present only in anoxic conditions. The horizon of maximum CO_2 uptake that we have described represents this transition from oxygenated to underlying anoxic conditions (*9*).

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10. We thank Dr. R. M. Moore for critically reading the manuscript. The work was supported by the National Research Council of Canada (grants A6508, A8358, and G0078).

8 September 1978; revised 22 December 1978

Genetic Mapping of the Ecotropic Murine Leukemia Virus–Inducing Locus of BALB/c Mouse to Chromosome 5

Abstract. *By means of an approach that combined the techniques of somatic cell genetics and Mendelian breeding studies, the inducibility locus, designated* Cv, *for ecotropic murine leukemia virus in BALB/c mice, was mapped to chromosome 5, 23 units from the locus for phosphoglucomutase-1, with gene order* Cv-Pgm-1-Gus. *This low-efficiency inducibility locus is therefore not allelic with the chromosome 7 loci previously described for two other mouse strains with high virus inducibility. These studies provide further evidence that endogenous ecotropic viruses represent viral genomes inserted at different chromosomal sites in the various mouse strains.*

Many inbred mouse strains carry the genetic information for production of ecotropic (mouse-infectious) murine leukemia viruses (MuLV's); however, there are marked differences among strains in the level of virus expression. Crosses between high-lymphoma, high-virus strains such as AKR and virus-negative strains have shown that high-virus mice generally carry multiple genetic loci for virus induction (*1*), and that these loci represent chromosomally integrated viral genomes (*2*). In contrast to high-virus mice, many strains with a lower incidence of leukemia, such as BALB/c, yield virus sporadically, in low titer, and relatively late in life. Similarly, virus can be induced by 5-iododeoxyuridine (IdU) with far greater efficiency from cell cultures from high-virus mice than from low-virus strains (*3*). Genetic studies with BALB/c mice have suggested that there is single gene control of ecotropic virus inducibility (*4*), and that different genes govern the inducibility of ecotropic and xenotropic virus (*5*). More recent studies with BALB/c mice have shown that embryo fibroblasts of backcross mice inducible for ecotropic virus contain the DNA sequences specific for this virus while cells of non-inducible segregants do not (*6*). These findings indicate that, as in AKR, the BALB/c viral inducibility locus contains viral structural genes.

In this report we describe the assignment of the inducibility locus for the infectious ecotropic virus of BALB/c mice to chromosome 5 using somatic cell genetics, and we confirm this assignment and establish the intrachromosomal location of this gene by Mendelian breeding studies. Our studies complement the work of Ihle *et al.* (*7*), in which the C3H/HeJ and BALB/c endogenous virus loci identified by serological assays and DNA hybridization were also mapped to chromosome 5.

Somatic cell hybrids were made between the peritoneal cells of BALB/c mice and cells of the Chinese hamster cell line, E36, by methods previously described (*8*). Thirty-nine primary hybrid clones were isolated in HAT (*9*) selective medium in two independent fusion experiments. Parallel cultures of each hybrid clone were used to test for expression of mouse isozyme phenotypes and ecotropic virus inducibility. Cell extracts were assayed for the expression of ten isozyme markers by vertical starch gel electrophoresis (*10*); dipeptidase-1 (*Dip-1*; chromosome 1), dipeptidase-2 (*Dip-2*; chromosome 18), tripeptidase-1 (*Trip-1*; chromosome 10), phosphoglucomutase-1 (*Pgm-1*; chromosome 5), phosphoglucomutase-2 (*Pgm-2*; chromosome 4), glucose phosphate isomerase (*Gpi-1*; chromosome 7), purine nucleoside phosphorylase (*Np-1*; chromosome 14), soluble malic enzyme (*Mod-1*; chromosome 9), glutathione reductase-1 (*Gr-1*; chromosome 8), and triose phosphate isomerase (*Tpi*; chromosome 6). Activity of hypoxanthine-guanine phosphoribosyl transferase (*Hprt*; X chromosome) was detected indirectly by monitoring hybrid cell growth in selective medium (*11*).

Thirty-six hybrid clones were tested for ecotropic virus induction. Subconfluent cultures were exposed to 20 μg of IdU per milliliter for 40 to 48 hours, and SC-1 cells (*12*) were then added. Two weeks later and at weekly intervals thereafter, the mixed cultures were passaged and monitored for virus production by the XC test (*13*). In positive cultures, virus generally became detectable by 3 to 4 weeks. Virus-negative clones showed no XC foci 6 weeks after induction.

Nineteen of the 36 hybrids were inducible for mouse ecotropic virus; in all cases tested the virus was N-tropic. None of the virus-inducible hybrids tested produced ecotropic virus without induction. A comparison of isozyme expression and virus induction showed that only phosphoglucomutase-1 segregated with a high degree of concordance with virus induction (Table 1). Both phenotypes were concordantly expressed in 30 of 33 primary clones tested. Of the three remaining clones, one virus-negative clone showed weak enzyme activity, and one clone lacking mouse phosphoglucomutase-1 produced only trace amounts of virus. These clones may therefore represent false discrepancies in which the chromosome frequencies were near the threshold level necessary for detectable gene expression. The other mouse enzyme phenotypes were all highly asyntenic with virus induction (31 to 50 percent discordance).

Forty-six secondary clones were derived from seven virus-inducible primary clones. Only two of these secondary clones produced detectable levels of ecotropic virus by the XC test and only those two subclones had mouse phosphoglucomutase-1 activity (Table 1). In contrast, activity for each of the other nine mouse isozymes was detected in at least 30 percent of these 46 hybrids. Thus, virus inducibility and phosphoglucomutase-1 activity were concordantly lost or retained in 76 of the 79 primary and secondary clones examined, provid-

0036-8075/79/0406-0069$00.50/0

ing strong evidence for the assignment of this locus to chromosome 5. The absence of discrepant clones which clearly lacked virus inducibility in the presence of phosphoglucomutase-1 activity suggested that no other mouse chromosome is necessary for virus production.

Somatic cell hybrids induced with IdU were also assayed for production of xenotropic MuLV. After exposure to IdU, hybrid cells were cocultivated with mink lung cells (American Type Culture Collection CCL64) and the presence of the xenotropic virus was assayed by focus formation on mink S+L− cells (*14*). Of the 79 clones, 63 produced xenotropic virus by this assay, and perfect concordance was observed with expression of the mouse dipeptidase-1 phenotype. This is consistent with our previous asignment of the xenotropic virus-inducing locus of BALB/c to a position on chromosome 1 near *Dip-1* (*15*). There was no observed correlation between the induction of the two types of virus. This is consistent with previous studies in which it was determined that ecotropic and xenotropic virus inducibility are under separate genetic control (*5*).

Staining with the fluorochrome Hoechst 33258 (*16*) was used to identify individual mouse chromosomes in metaphase spreads prepared from four independently isolated clones which lacked both virus inducibility and mouse phosphoglucomutase-1 activity. None of the four retained chromosome 5. Eight of the nine chromosomes not monitored by enzyme expression (chromosomes 2, 3, 12, 13, 15, 16, 17, and 19) were observed to be present in frequencies above 17 percent in at least two hybrids. Thus, ecotropic virus inducibility segregated discordantly with these chromosomes. Chromosome 11, which is rarely retained in hybrids between E36 hamster cells and primary mouse cells (*17*), was not identifiable in the karyotypes of any of these virus-negative clones, and therefore it alone could not be specifically eliminated as necessary for ecotropic virus inducibility.

Sexual genetic crosses were carried out to examine the segregation of ecotropic virus inducibility with genetic markers on chromosome 5. (SWR/J × BALB/cN)F_1 animals were mated with females of the inbred NIH Swiss line NFS/N. The SWR and NFS strains are negative for ecotropic virus inducibility. Tail cultures were prepared from individual animals (*18*), induced and tested for ecotropic virus as described above for hybrid cells. F_1 animals and 27 of the 71 NFS × (SWR/J × BALB/cN)F_1 mice were inducible for virus. This is compatible with single gene control as indicated in previous genetic studies (*3*).

Kidney extracts of the segregants were typed for two isozyme markers on chromosome 5, *Gus* and *Pgm-1* (*19*), by vertical starch gel electrophoresis. The results (Table 2) show that virus inducibility is linked to *Pgm-1* ($r = 24 \pm 5.1$ percent) and not to *Gus* ($r = 39 \pm 5.8$ percent). Consistent with the designation of the AKR loci as *Akv-1* and *Akv-2*, we recommend naming the single ecotropic virus inducibility locus of BALB/c as *Cv*, C being the standard abbreviation of BALB/c. The results in Table 2 show that the gene order of the chromosome 5 loci is *Cv-Pgm-1-Gus*.

Previous genetic studies have assigned virus inducibility loci in two high ecotropic virus strains, AKR and C3H/Fg, to nonallelic sites on chromosome 7 (*20*). The mapping of *Cv* to chromosome 5 represents the first assignment of a low-efficiency virus-inducing locus. This assignment emphasizes the fact that the virus-inducing loci are located at nonallelic sites and on different chromosomes in the genome of various inbred mouse strains.

The biochemical evidence of Robbins and his colleagues (*6*) strongly suggests that the *Cv* gene, like *Akv-1*, represents the chromosomally integrated viral genome rather than a regulatory gene that controls expression of viral genomes elsewhere in the mouse genome. Indeed, the somatic cell hybrid studies provided no indication of any gene interactions in virus induction. On the one hand, no mouse chromosome other than 5 was apparently required for ecotropic virus induction, and on the other, all the virus-positive hybrids showed the low-efficiency virus inducibility pattern characteristic of BALB/c. This provides further evidence for the concept that the efficiency of virus inducibility is determined by the locus itself.

It is of interest that a locus has been described on chromosome 5 which confers on hamster × mouse somatic cell hybrids sensitivity to infection with ecotropic MuLV's (*21*); this locus presum-

Table 1. Correlation of ecotropic MuLV induction with mouse phosphoglucomutase-1 (PGM-1) activity in somatic cell hybrid clones. Primary hamster × mouse hybrids were isolated in two separate fusion experiments, designated BE and BM. Seven virus-positive primary BE clones were used to generate secondary clones.

Hybrid series	Number tested	Virus/PGM-1				Percentage discordant
		+/+	−/−	+/−	−/+	
		Primary clones				
BE	14	7	5	1	1	
BM	18	7	10	1		
Total	32	14	15	2	1	9
		Secondary clones				
1	6	1	5			
2	10		10			
3	8		8			
6	6	1	5			
8	10		10			
9	4		4			
14	2		2			
Total	46	2	44			0

Table 2. Recombination between *Cv*, *Pgm-1*, and *Gus* in NFS/N × (SWR/J × BALB/cN)F_1 mice. All mice have one chromosome 5 from the NFS/N parent, of genotype Cv^- *Pgm-1*^{a} *Gus*^{b}. The other chromosome, contributed by the F_1 parent, is of genotype Cv^+ *Pgm-1*^{a} *Gus*^{b}/Cv^- *Pgm-1*^{b} *Gus*^{a}.

Progeny classes	Inheritance of BALB/c allele			
	Cv	*Pgm-1*	*Gus*	Number of mice
Parental (nonrecombinant)	+	+	+	11
Parental (nonrecombinant)	−	−	−	28
Recombinant (*Cv-Pgm-1*)	+	−	−	6
Recombinant (*Cv-Pgm-1*)	−	+	+	7
Recombinant (*Pgm-1-Gus*)	+	+	−	8
Recombinant (*Pgm-1-Gus*)	−	−	+	7
Double recombinant	+	−	+	2
Double recombinant	−	+	−	2

Percentage recombinant: *Cv-Pgm-1*, $r = 17/71 = 24 \pm 5.1$
Cv-Gus, $r = 28/71 = 39 \pm 5.8$
Pgm-1-Gus, $r = 19/71 = 27 \pm 5.3$

ably codes for a cell surface viral receptor (*22, 23*), and is shared by all strains tested. It would be of interest to determine the relationship, if any, between this receptor locus and *Cv*.

The BALB/c mouse has been extensively used for research in immunology and oncology. These mice carry one locus for ecotropic virus and one locus for inducible xenotropic virus. Now, with known chromosomal assignments for both loci, it will be possible to explore more extensively the etiological role of these genes in carcinogenesis. In particular, we intend to develop congenic strains of BALB/c mice lacking these loci for use in studies of tumor induction by viruses and chemical carcinogens.

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8 November 1978; revised 25 January 1979

Genetic Linkage of C3H/HeJ and BALB/c Endogenous Ecotropic C-Type Viruses to Phosphoglucomutase-1 on Chromosome 5

Abstract. *The genetic linkage of the endogenous C3H/HeJ C-type ecotropic virus to phosphoglucomutase-1 (0.28, recombinant fraction) on chromosome 5 was established by means of serological assays of backcrossed mice. With a combination of serological techniques and DNA-DNA hybridization the BALB/c endogenous ecotropic virus was shown to be either closely linked or allelic with the C3H/HeJ locus.*

Murine C-type viruses, structurally similar to the virus associated with leukemia in the AKR strain of mice, are genetically transmitted in a variety of inbred strains of mice (*1*). Although the genetics of the AKR-type viruses has been extensively studied (*2–4*), the genetics of the C-type viruses in low-virus strains has received little attention. This is partly because of technical difficulties: virus expression in most low-virus strains cannot be assayed by standard methods. Therefore, the more tedious procedures of DNA-DNA hybridization or activation of virus expression in cell cultures derived from backcrossed mice must be used. From these types of experiments it has been shown that most of the low-virus strains that transmit an endogenous ecotropic virus probably have only a single locus (*5–7*) and that in BALB/c mice this locus is not linked to the inducible xenotropic virus (*5*). An important question is whether the single loci found in various strains are allelic. Presumably, if they are allelic in independently derived inbred strains of mice, a strong argument could be made for evolutionary derivation of the virus or at least for preferred sites of integration. If, however, they are not allelic, it is possible that these viruses have only recently been acquired by germ-line integration, and that the site of such integration is variable.

We have demonstrated (*7, 8*) that serological techniques can be used to study the genetics and phenotypes of expression of endogenous C-type viruses in strains such as C3H/HeJ. For these assays we utilized the observation that one consequence of the spontaneous expression of murine leukemia virus (MuLV) in vivo is the induction of both cellular (*9*) and humoral (*10, 11*) immune responses. Both responses have been characterized and shown to be serologically specific for the endogenous ecotropic virus. Using these assays we have shown that the ecotropic viruses are expressed in vivo with distinctly different phenotypes in various inbred strains and that this serologically defined phenotype for expression is dependent only on spontaneous expression and not on infectious replication of the virus (*7, 8, 12*). In the case of the C-type virus transmitted in C3H/HeJ mice, the phenotype of virus expression segregates with the structural gene for the virus (*7*). We now report the apparent allelism of the BALB/c and C3H/HeJ viral loci on chromosome 5.

The serological phenotypes of C3H/

Table 1. Serological phenotypes of C3H/HeJ, C57BL/6 F_1 and backcross mice. Symbols: + indicates the presence of antibody in titers of > 1:100 serum dilution; − indicates the lack of detectable antibody.

Strain or cross	Antibody titer at age* (in months)					Phenotype
	1	2	3	4	5	
C3H/HeJ	−	+	+	+	+	C3H/HeJ
C57BL/6	−	−	−	−	−	BL/6
C57BL/6 × C3H/HeJ	−	+	+	+	+	C3H/HeJ
C57BL/6 × (C57BL/6 × C3H/HeJ), animal:						
1	−	−	+	+	+	C3H/HeJ
2	−	−	−	−	−	BL/6
3	−	+	+	+	+	C3H/HeJ
4	−	+	+	+	+	C3H/HeJ
5	−	−	−	−	−	BL/6
6	−	−	−	−	−	BL/6
7	−	+	+	+	+	C3H/HeJ
8	−	−	−	−	−	BL/6
9	−	−	+	+	+	C3H/HeJ
10	−	+	+	+	+	C3H/HeJ

*The presence of antibody against the endogenous ecotropic virus was assayed by a radioimmune precipitation assay with [^{3}H]leucine-labeled intact AKR-MuLV as described (*19*). Individual mice were assayed at monthly intervals by tail bleeding.

0036-8075/79/0406-0071$00.50/0