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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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.

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Table 2. Linkage analysis of the C3H/HeJ AKR-type MuLV locus in C57BL/6 × (C57BL/6 × C3H/HeJ) mice.

Locus	Chromosome	Phenotype	Virus phenotype		Heterozygous fraction (number/total)	Recombinant fraction (number/total)
			C57BL/6	C3H/HeJ		
*Mod-1**	9	b	18	17	0.48 (32/67)	0.49† (33/67)
Mod-1	9	ab	16	16		
Pgm-1	5	a	48	20	0.52 (73/141)	0.28 (40/141)
Pgm-1	5	ab	20	53		

*The soluble form of malate dehydrogenase (*Mod-1*) was assayed by a modification of the procedure previously described (*20*). Phosphoglucomutase-1 (*Pgm-1*) was assayed as described (*21*). †The recombinant fraction with *Mod-1* was not significantly different ($P < .05$) from the expected fraction for an unlinked locus when examined by the χ^2 test. The linkage with *Pgm-1* was highly significant ($\chi^2 = 26.4$, $P > .01$).

HeJ, C57BL/6, (C57BL/6 × C3H/HeJ) F_1, and C57BL/6 × (C57BL/6 × C3H/HeJ) mice are shown in Table 1. The C3H/HeJ mice characteristically have antibody that is specific for the AKR-type MuLV early in life such that by 2 to 3 months most mice are antibody-positive. In contrast, C57BL/6 mice develop an immune response against the virus only after 4 to 5 months of age. The C3H phenotype was completely dominant in the F_1 mice, and all the F_1 mice resemble the C3H/HeJ parental strain. In the backcross C57BL/6 × (C57BL/6 × C3H/HeJ) there is a clear segregation of the C3H/HeJ phenotype in that individual mice are either antibody-positive early in life and remain antibody-positive or they remain antibody-negative. In a group of 208 such backcrossed mice, 107 (51.4 percent) were of the C3H/HeJ phenotype, suggesting that a single gene locus controls the serological phenotype ($\chi^2 = .173$, $P = .6$ to $.7$). In previous experiments (*7*) with NIH Swiss × (NIH × C3H/HeJ), the C3H/HeJ serological phenotype segregated with AKR-MuLV proviral DNA, demonstrating that these techniques can be used to follow segregation of the viral structural gene.

Using the C57BL/6 × (C57BL/6 × C3H/HeJ) backcross we next examined the linkage of the C3H/HeJ phenotype with a variety of isoenzyme markers. As previously shown (*7*) there was no apparent linkage with serum esterase-1 (*Es-1*), serum esterase-3 (*Es-3*), glucose-6-phosphate dehydrogenase-1 (*Gpd-1*), the agouti locus (*A*), the locus for the β chain of hemoglobin (*Hbb*), or the histocompatibility locus (*H-2*). The lack of apparent linkage with *H-2* or *Gpd-1* is important, since *H-2* linked genes are known to influence immune functions that might have contributed to the phenotypes, and *Gpd-1* is linked to the gene for resistance to Friend virus (*Fv-1*) which can influence in vivo the replication of the infectious virus (*13–15*). In addition, there was no apparent linkage with malate dehydrogenase (*Mod-1*) (Table 2). In contrast, however, linkage was apparent with phosphoglucomutase-1 (*Pgm-1*) in that of 141 mice examined only 40 (28 ± 3.8 percent) were of the recombinant phenotype. These results demonstrate that the C3H/HeJ viral locus (*C3v*) is on chromosome 5 and linked to *Pgm-1*.

To determine whether the viral locus of other inbred strains was allelic with the C3H/HeJ locus, we used a combination of serological and hybridization techniques as previously described (*7*). The F_1 was obtained between two test strains, and the F_1 was mated to virus-negative NIH Swiss mice. As shown in Table 3, the cross NIH Swiss × (BALB/c × C3H/HeJ) showed a clear segregation of the C3H/HeJ phenotype (31 out of 62, 50 percent). To determine allelism we next examined the non-C3H/HeJ phenotypic mice for the presence of the late-expressing BALB/c locus by DNA-DNA hybridization using AKR-MuLV complementary DNA (cDNA) which had been absorbed with NIH Swiss mouse DNA to remove cross-reactive sequences (*7, 8*). If the loci were allelic, the F_1 would be homozygous for the presence of a viral locus, with one allele from each parent, and the cross with the NIH Swiss strain would yield offspring all of which would have one or the other allele. If, however, the viral loci were not allelic and not linked, the F_1 would be heterozygous for the presence of a viral locus at two loci, and in the cross with NIH Swiss the loci would segregate independently yielding heterozygous mice, 25 percent with both loci, 25 percent with one locus, 25 percent with the other locus, and 25 percent with none. Table 3 shows that in the BALB/c, C3H/HeJ test all of non-C3H/HeJ-type mice examined had the proviral DNA of the BALB/c virus. These results contrast with previous results (*7*) in which the test cross NIH Swiss × (C57BL/6 × C3H/HeJ) was used and only half of the non-C3H/HeJ-type mice had the proviral DNA of the C57BL/6 virus. From these results the probability is <.01 that the BALB/c and C3H/HeJ loci are not linked. With regard to the possible nonallelism but linkage of the C3H/HeJ and BALB/c loci, the probability is <.05 that the true recombinant fraction is greater than 0.20. These data therefore demonstrate that the BALB/c and C3H/HeJ loci are either allelic or closely linked on chromosome 5 in contrast to the C57BL/6 viral locus which is not allelic and probably is not linked to the C3H/HeJ locus.

Table 3. Genetic analysis for allelism of C3H/HeJ, BALB/c and C57BL/6 viral loci.

Cross	C3H/HeJ virus phenotype* (number/total)	Proviral DNA in non-C3H/HeJ-type†
NIH Swiss × (BALB/c × C3H/HeJ)	31/62	16/16‡

*The C3H/HeJ virus phenotype was assessed by the development of an immune response against the endogenous C3H/HeJ ecotropic virus as described (*7*). †The presence of the BALB/c endogenous ecotropic virus in the non-C3H/HeJ phenotypic mice was assayed by DNA-DNA hybridization with total liver DNA and cDNA prepared from AKR-MuLV which had been extensively absorbed on normal NIH Swiss mouse DNA as described (*7, 8*). For details of this cDNA probe and the hybridization kinetic characteristics see (*8*). ‡Number with proviral DNA/total.

The data presented here extend the number of C-type viral loci which have been mapped to two inbred strains of mice with low levels of virus expression. The linkage of the C3H/HeJ viral locus to *Pgm-1* on chromosome 5 is interesting in that Rowe (*3*) has shown that a strain derived from the C3H/HeJ and C3H/Fg strains has multiple ecotropic viral loci, one of which is on chromosome 7 although it is not allelic with the AKR viral locus on chromosome 7. The data also suggest that the C3H/HeJ locus is linked to *Pgm-1* and is either allelic with or closely linked to the BALB/c locus. This observation is consistent with the results of Kozak and Rowe (*16*) who demonstrated a comparable linkage of the BALB/c locus to *Pgm-1* by somatic cell genetics and the activation of fibroblasts from backcrossed mice by 5-iododeoxyuridine (IdU). By means of a three-point genetic analysis of the backcrosses, these authors mapped the viral locus on the centromeric side of *Pgm-1*.

The apparent allelism of the loci for the BALB/c and C3H/HeJ viruses and the lack of apparent allelism of the locus for the C57BL/6 virus (*7*) may be related

to the origin of these inbred strains. In particular, the C3H/HeJ strain was originally derived by Strong from a cross of a Bagg albino ♀ × DBA ♂, and the BALB/c strain was originally the Bagg albino stock (*17*). Therefore, the C3H/HeJ strain may have acquired the locus for the BALB/c virus from the initial albino cross. This can be further assessed by examining the current DBA mice, which also appear to have a single AKR-type locus for MuLV (*1*). In contrast to the BALB/c and C3H/HeJ strains, the C57BL/6 strain has a completely independent origin. These observations suggest that endogenous ecotropic viruses probably became integrated in the inbred strains of mice prior to the derivation of these strains (≃1900) and certainly within the relatively recent evolutionary history of mice. As more viral loci are mapped it will be interesting to see the extent of diversity. If, in fact, there is a great diversity, the site of viral integration may actually be an important marker for the probable origin of strains of mice of unknown or questionable derivation.

The presence of the BALB/c, C3H/HeJ viral locus on chromosome 5 is of interest because the cell receptor for the virus is also on chromosome 5 (*18*). The presence of both loci on chromosome 5 appears to be fortuitous because, unlike the C3H/HeJ C-type virus, the cell receptor is common to all the mouse strains examined including the NIH Swiss strain which genetically lacks the virus.

Although the BALB/c and C3H/HeJ loci are probably allelic and code for very similar if not identical viruses, the phenotypes of expression are quite distinct; the C3H/HeJ virus is expressed early in life, whereas the BALB/c virus is only expressed late in life. This observation argues against the possibility that the site of integration plays a major role in determining the phenotype of expression. However, since the phenotype for expression of the C3H/HeJ virus segregates with the structural gene for the virus, it appears that there probably are closely linked functions which regulate expression in vivo. Nevertheless, as new techniques are developed for examining the integrated structure of the virus, these observations will be useful in studying the cellular controls of viral expression.

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Vitamin D Deficiency and Reproduction in Rats

Abstract. *Female weanling rats from a colony maintained on a diet low in vitamin D were raised on a diet that was deficient in vitamin D but was otherwise adequate. Vitamin D deficiency was confirmed in the rats by hypocalcemia and the absence of vitamin D metabolites in blood. These females gave birth to litters that were slightly smaller than control litters from females maintained on a vitamin D–containing diet. The pups from the vitamin D–deficient mothers appeared normal throughout lactation, and at weaning had normal concentrations of calcium and phosphate in the plasma. These results indicate that vitamin D and its metabolites are not necessary for reproduction and fetal development in the rat.*

The importance of vitamin D in calcium and phosphate metabolism is well known (*1, 2*). Vitamin D is converted to a hormone, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$], before it can function in the regulation of calcium and phosphorus metabolism (*3–6*). Recent studies have shown that vitamin D is required for normal embryonic development in the chicken (*7*). Toverud (*8*), however, has found evidence that vitamin D is not essential for lactation nor for maintaining a normal level of calcium in the milk of lactating rats (*8*). We have therefore investigated whether or not mammals kept on a strict vitamin D–deficient diet from weaning can reproduce and maintain their offspring.

Female Holtzman rats were obtained as weanlings from a colony kept on a diet low in vitamin D. The weanlings were divided into two groups: both groups received a vitamin D–deficient diet that contained 0.47 percent calcium and 0.3 percent phosphorus (*9*), and one of these groups received 25 units of vitamin D_3 (orally) per day. At no time were any of the animals exposed to ultraviolet radiation, thus the possibility of vitamin D being produced endogenously in the skin was eliminated. Each group was maintained on its respective diet throughout the experiment, and at age 110 days the animals were mated with normal, vitamin D–replete males.

The number of females becoming pregnant, reaching term, and giving birth to normal-appearing offspring from each group was similar. When the newborn pups reached an age of 23 days postpartum they were weaned. At this time, four to five pups were taken randomly from each litter and killed. The remaining pups were placed in individual cages and fed the same diet that their respective mothers had received. Mothers and nonmated females from the vitamin D–deficient and vitamin D–replete groups were also killed. Plasma samples were analyzed for the vitamin D metabolites 25-hydroxyvitamin D_3 (25-OH-D_3) and 1,25-$(OH)_2D_3$ by previously established methods (*10, 11*). Plasma calcium concentration was determined by diluting 0.1-ml samples of plasma with 1.9 ml of 0.1 percent aqueous $LaCl_3$ and measur-

Table 1. Plasma concentrations of 25-OH-D_3 and 1,25-$(OH)_2D_3$ in vitamin D–deficient (−D) and vitamin D–replete (+D) rats. Data are expressed as means ± standard error (S.E.). The numbers of rats in each group is given in parentheses.

Group	25-OH-D_3 (ng/ml)	1,25-$(OH)_2D_3$ (pg/ml)
+D	21 ± 6 (N = 6)	25 ± 5 (N = 3)
−D	0* (N = 6)	0† (N = 3)

*Not detectable, < 0.5 ng/ml. †Not detectable, < 5 pg/ml.

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