$10^{-4}M$) was added to a deaerated solution of NH_4Cl ($7.5 \times 10^{-3}M$, final *p*H 8.7) and the solution was kept for 28 days. The mixture was hydrolyzed with 3*M* HCl, desalted, hydrolyzed again with 3*M* HCl, and quantitated on the amino acid analyzer. The yields were 15 percent methionine, 0.5 percent glutamic acid, 0.5 percent α,γ-diaminobutyric acid, and 13 percent α-hydroxy-γ-aminobutyric acid, based on the added acrolein. The same experiment omitting the CH_3SH gave 1.5 percent glutamic acid and 0.8 percent α,γ-diaminobutyric acid. These results show that CH_3SH adds to acrolein in preference to NH_3 or HCN under the conditions of the experiment. The relative yields of the amino acids in the primitive ocean would depend on the concentrations of CH_3SH, HCN, and NH_3 as well as the temperature and hydrolytic conditions.

It appears likely that acrolein was a key intermediate in prebiotic amino acid synthesis, being a precursor not only of methionine but also of glutamic acid, homocysteine, homoserine, α,γ-diaminobutyric acid, and α-hydroxy-γ-aminobutyric acid (Fig. 2).

There have been a number of speculations that methionine is not a "primitive" amino acid (*16*). These speculations are based primarily on the fact that methionine has only one codon, and that methionine could not be a primitive amino acid if it was very unstable. Methionine is indeed unstable in the presence of air (*17*), but seems to be quite stable under anaerobic conditions (*18*). Our results indicate that substantial amounts of methionine may have been present in the primitive ocean; therefore the possibility of methionine being a primitive amino acid cannot be excluded.

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19. Supported by NSF grant GB 25048. The gas chromatograph–mass spectrometer was obtained, in part, with NSF grant GP 18245. We thank G. Pollock for the OV-225 gas chromatography column.

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Genetic Mapping of a Murine Leukemia Virus–Inducing Locus of AKR Mice

Abstract. *The chromosomal location of one of the two murine leukemia virus–inducing loci of AKR mice has been determined. The locus, which appears to be the integrated genome of the virus, is designated* Akv-1, *and is on linkage group I, 12 map units from* Gpi-1, *with gene order* c–Gpi-1–Akv-1. *This identification of a closely linked gene whose phenotype is independent of virus expression should facilitate analysis of the biologic importance of the* Akv-1 *locus.*

The high-leukemic mouse strain AKR (*1*) is characterized by lifelong infection with murine leukemia virus (MuLV), with virus being first detectable during late embryonic or early postnatal life (*2*). We have shown that two phenomena are responsible for this: that the potentially infectious MuLV genome is present, in unexpressed form, in all cells of the AKR embryo, and that it can undergo spontaneous induction, resulting in liberation of infectious virus which can infect the uninduced cells (*3*). The induction rate is immensely increased by treatment of the cells with either 5-iododeoxyuridine (IdU) or 5-bromodeoxyuridine (*4*). In recent experiments we have found that up to 50 percent of the cells of certain tissue culture lines of AKR cells initiate synthesis of viral antigen or antigens, and as many as 2 percent produce infectious virus within a few days after IdU treatment.

Low-leukemic strains of mice also contain MuLV genetic material, which may be manifested by appearance of small amounts of infectious virus late in life, or only by appearance of virus-specific antigens (*5*, *6*). In the case of one low-leukemic strain, BALB/c, it has been shown that the MuLV genome is present in all cells (*7*), as in AKR. The IdU induction of tissue cultures of embryo cells from low-leukemic strains gives rise to two patterns of response, both of which are different from that of AKR cells. Either little or no viral antigen or virions are produced (NIH Swiss mice, for example) (*8*), or, as in the case of BALB/c, relatively large amounts of viral antigens (*8*) and virions (*7*) are induced; but the virus particles are far less infectious than those from AKR cultures.

By studies of the genetic transmission of the high-virus phenotype, we have demonstrated that AKR mice possess two independently segregating chromosomal loci, either of which leads to the appearance of MuLV early in life (*9*, *10*). These loci also appear to confer the ability to synthesize infectious MuLV after induction with IdU. All available evidence is compatible with these loci being chromosomally integrated viral genetic determinants.

One of these virus-inducing loci, referred to as V_1, has been shown to be on linkage group I, about 30 map units from the loci for the genes for albino (*c*) and the β chain of hemoglobin (*Hbb*) genes (*9*). The other locus, V_2, has not been mapped.

Analysis of these loci has been facilitated by their isolation in backcross lines (*9*). In the first backcross (BC1) generation C57BR × (C57BR × AKR)-

F_1, a low-virus × a (low-virus × high-virus) cross, three-fourths of the mice are virus-positive, because of the independent segregation of the two virus-inducing loci from AKR. Of the virus-positive BC1 mice, one-third contain both the V_1 and V_2 loci, one-third V_1 only, and one-third V_2 only. By crossing BC1 mice to low-virus C57BR or NIH Swiss mice, determining which of the backcross mice transmit virus to only 50 percent of the progeny (meaning that only one locus is being transmitted), and testing these progeny for correlation or lack of correlation between presence of virus and inheritance of the *c* or *Hbb* (or both) loci (indicating whether V_1 or V_2 is the locus being transmitted), we have been able to separate these loci from one another. These lines, referred to as V_1 and V_2 families according to whether they show linkage to *c* and *Hbb* or not, are being maintained by serial backcrossing to C57BR or NIH Swiss mice.

In one of the V_1 families, No. 2-4, the BC1 parent was a recombinant between *c* and *Hbb*, having the V_1 and *c* loci from AKR, and the *Hbb* locus from C57BR; this suggested that the gene order is *Hbb*–*c*–V_1 (*9*).

The locus for the isozymes of glucose phosphate isomerase (*Gpi-1*) (*11*) is also on linkage group I, 25 to 30 map units from *c* with gene order *Hbb*–*c*–*Gpi-1* (*12*). Thus, testing of V_1 families for segregation of *Gpi-1* alleles in relation to virus transmission can readily confirm or disprove the gene order inferred from hybrid 2-4, and can determine the map distance between V_1 and *Gpi-1*. Also, by means of a suitable three-point cross, it can provide the means to map the gene order of *c*, *Gpi-1*, and V_1 as well.

Both AKR and C57BR have the same *Gpi-1* genotype (*Gpi-1*^{a}); however, the NIH strain was found to be *Gpi-1*^{b}. NIH mice were crossed with BC1, BC2, or BC3 mice of several V_1 and V_2 families, and virus-positive male progeny were backcrossed to NIH females. The *Gpi-1* type of the progeny was determined by starch-gel electrophoresis of erythrocyte lysates (*11*), and presence of virus was determined by testing extracts of tail tissue (*2*, *9*) for plaque production in NIH mouse embryo tissue cultures (*13*). Table 1 shows that in the two families classed as V_1 on the basis of linkage to *c*, there was close linkage of the virus-inducing locus with *Gpi-1*, the two loci being 12 map units apart. As was expected, the two V_2 families showed no association of virus with *Gpi-1* type, proving that the strong correlation seen in the V_1 families is not due to a gene near *Gpi-1* being highly permissive for induction or replication of virus located elsewhere in the cell, but must reflect linkage.

The virus-positive (NIH × V_1 hybrid)-F_1 males used in these matings had been selected for being nonalbino; that is, their genotype is +/*c*, *Gpi-1*^{a}/*Gpi-1*^{b}, V_1/—. Thus, they provided a three-point cross for determining the gene order of *c*, *Gpi-1*, and V_1. The data in Table 2 show that the gene order is *c*–*Gpi-1*–V_1, since the alternative model, *c*–V_1–*Gpi-1*, would postulate that 14 of the mice were double recombinants and none were single recombinants between *Gpi-1* and V_1.

The three-point cross was also done on embryonic mice: *c* was scored by the eye color, *Gpi-1* by testing the cells either before or after growth in culture, and the virus-inducing loci by induction of the tissue culture cells with IdU. Individual embryos from two crosses of NIH × (NIH × V_1 hybrid) were cultured, and the inducibility of infectious MuLV by IdU was determined on primary cultures by a procedure that measures the number of cells producing virus by 5 days after a 42-hour exposure to 5 μg of IdU per milliliter (*4*) (Table 2). The cultures generally showed either that 100 to 400 cells were induced per culture, or that no cells were induced. Although only 19 embryos were studied, the segregation and recombination patterns were in full accord with those observed in the studies in vivo. This result provides strong confirmation that at least in the case of V_1, the spontaneous appear-

Table 1. Correlation of transmission of virus and *Gpi-1* type in progeny of (*Gpi-1*^{b},— × *Gpi-1*^{a}, V_1/*Gpi-1*^{b},—), and lack of correlation in progeny of (*Gpi-1*^{b},— × *Gpi-1*^{a}, V_2/*Gpi-1*^{b},—) matings.

Family	(No. with virus)/(No. in category), by *Gpi-1* type					
	Gpi-1^a/*Gpi-1*^{b}		*Gpi-1*^{b}/*Gpi-1*^{b}		Total	
	No.	%	No.	%	No.	%
	Families showing linkage to c (V_1 *families*)					
1-4	29/33	88	4/30	13	33/63	52
1-2	17/22	77	1/28	4	18/50	36
Total	46/55	84	5/58	9	51/113	45
	Families showing no linkage to c (V_2 *families*)					
4-1	7/16	44	7/17	41	14/33	42
1-8-7*	5/11	45	3/12	25	8/23	35
Total	12/27	44	10/29	34	22/56	39

* 1-8-7 is a V_2 family derived from a BC1 mouse that transmitted both V_1 and V_2 (*9*). The origins of the other families are given in (*9*).

Table 2. Three-point cross for determination of gene order. NIH females (genotype *c*, *Gpi-1*^{b},—) were mated with (NIH × V_1 hybrid) males (genotype +, *Gpi-1*^{a}, V_1/*c*, *Gpi-1*^{b},—), and the progeny were scored for color, *Gpi-1* type, and either presence of MuLV in tail extracts at 6 weeks of age, or inducible MuLV in tissue culture of the embryos. The numerals are the number of mice in each category.

Inferred genotype*			Tests in vivo			Tests of embryo cells in vitro		
c	*Gpi-1*	V_1	Family 1-4	Family 1-2	Total†	Family 1-4	Family 1-2	Total‡
			Parental					
+	*a*	V_1	24	13	37	2	4	6
c	*b*	—	19	20	39	4	2	6
			Recombinant c–Gpi-1					
+	*b*	—	7	7	14	1	0	1
c	*a*	V_1	5	4	9	1	3	4
			Recombinant Gpi-1–V_1					
+	*a*	—	4	5	9	1	0	1
c	*b*	V_1	4	1	5	0	1	1
			Double recombinant					
+	*b*	V_1	0	0	0	0	0	0
c	*a*	—	0	0	0	0	0	0

* Inferred genotype of linkage group I from the hybrid parent. The postulated gene order is *c*–*Gpi-1*–V_1. All mice have a parental chromosome of genotype *c*, *Gpi-1*^{b},—. † Recombinational frequencies in the in vivo studies are: *c*–V_1, 37/113 = 33 ± 4.4 percent; *c*–*Gpi-1*, 23/113 = 20 ± 3.8 percent; *Gpi-1*–V_1, 14/113 = 12 ± 3.1 percent. ‡ The estimates of recombinational frequencies from the tissue culture studies are: *c*–V_1, 7/19 = 37 percent; *c*-*Gpi-1*, 5/19 = 26 percent; *Gpi-1*–V_1, 2/19 = 11 percent.

ance of virus in vivo, and the ability of the cells to be induced by IdU are functions of the same genetic locus.

Thus, it can be concluded that one of the virus-inducing loci of AKR mice is on linkage group I, 12 map units from *Gpi-1*, with the gene order *c–Gpi-1*–V_1, and that this locus determines both the spontaneous and IdU induction rates. We propose the formal designation of this locus as *Akv-1* (AKR virus-inducing locus-1).

The finding of relatively close linkage between *Akv-1* and *Gpi-1* is important in several respects. First, it provides direct proof that the virus-inducing factor being studied in these crosses is a chromosomal locus. Second, testing for *Gpi-1* linkage provides a convenient and rapid means to test for allelism (or identity) between *Akv-1* and other, still unmapped loci involved in expression of MuLV virus and antigens (*14*). Third, since *Gpi-1* is expressed in tissue culture cells, it can be used as a marker for following the *Akv-1* locus in somatic hybridization studies with tissue culture cells; questions such as whether this type of locus is the integration site for a superinfecting MuLV genome may be answerable by this means. And fourth, *Gpi-1* testing may provide a unique means of examining the most crucial, and the most difficult to test, portion of the oncogene hypothesis (*6*), that is, that subinfectious expression of the inherited MuLV genome is a major determinant of malignancy—not only of leukemia, but of solid tumors as well. Inbred mouse strains differ markedly in the incidence of various spontaneous and carcinogen-induced tumors; the oncogene hypothesis would presumably predict that these differences are due, in large part, to genetic differences between the integrated viral genomes in the various strains. If the integrated defective and nondefective viral genomes are at allelic sites in different mouse strains, tracing their transmission in segregating crosses by means of a closely linked genetic marker provides a way to examine whether inheritance of a particular viral genome is correlated with susceptibility to a particular type of tumorigenesis. Since the expression of the marker, in this case *Gpi-1*, is independent of the viral genome, this test could be done even with mouse strains in which the viral genome is so highly defective that its expression is not detectable by any available technique.

This approach is complicated by the existence of at least one other chromosomal site containing viral genetic material (V_2) (*9*). However, if a similar linked marker can be found for this locus, the genetic approach to the oncogene hypothesis should be feasible.

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Location of the Second Gene Required for Expression of the Leukemia-Associated Mouse Antigen G_{IX}

Abstract. *Some mouse strains express G_{IX} antigen on their thymocytes; others do not. Expression depends on two genes,* Gv-1 *and* Gv-2, *in linkage groups IX and I, respectively. Cells producing leukemia virus, however, express G_{IX} antigen regardless of their inherited* Gv-1 *and* Gv-2 *genotype.*

G_{IX} is a cell surface antigen found on the thymocytes of some (G_{IX}^+) mouse strains and absent from the thymocytes of other (G_{IX}^-) strains (*1*). Thymocytes are typed for G_{IX} antigen by the cytotoxicity test, with antiserum to G_{IX}, in the same way as H-2, TL, Thy-1 (*θ*), and Ly alloantigens, which are also found on thymocytes (*2*). Expression of G_{IX} antigen on the thymocytes of normal mice is controlled by two unlinked Mendelian genes, *Gv-1* and *Gv-2* (*3*); at each locus, every mouse carries either the positive allele for expression of G_{IX} antigen or (presumably) the alternative allele for nonexpression of G_{IX} antigen. In order for a mouse to have G_{IX} antigen on its thymocytes, it must inherit the positive allele at both the *Gv-1* and *Gv-2* loci (which we shall refer to here simply as the *Gv-1*+ and *Gv-2*+ alleles, as contrasted with alleles *Gv-1*− and *Gv-2*−).

The special importance of G_{IX} antigen in relation to leukemia virus and leukemogenesis is the following: Leukemia cells, cells of the spleen, and possibly other cell types of *any* mouse strain will express G_{IX} antigen if they become productively infected with murine leukemia virus (MuLV) (*4*), regardless of whether the cells originated from a G_{IX}^+ mouse or a G_{IX}^- mouse. Moreover, when rats (which do not normally possess G_{IX} antigen) are inoculated with MuLV at birth, their thymocytes and ensuing leukemias become G_{IX}^+. Thus MuLV causes G_{IX} antigen to be expressed on cells of genotypes which normally yield the G_{IX}^- phenotype, that is, on cells of mice that lack either one or both of the *Gv-1*+ and *Gv-2*+ alleles. [There is a provocative parallel here with the anomalous expression of TL thymocyte antigens on leukemia cells of mice whose thymocytes are normally TL^- (*5*).]

To recapitulate: In the absence of overt MuLV infection, G_{IX} antigen appears as a simple Mendelian character controlled by two chromosomal genes, whereas productive MuLV infection