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.

WALLACE P. ROWE
JANET W. HARTLEY
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014
THEODORE BREMNER
Department of Botany, Howard University, Washington, D.C. 20001

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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

Table 1. Segregation of *Gv-1*, *Gv-2*, *Hbb*, and *Gpi-1* in the backcross (C57BL/6 × 129)F_1 × C57BL/6, showing linkage of *Hbb* with *Gv-2*. The results of Gpi-1 typing are not included because there was no linkage with *Gv-2*, showing that the order must be *Gpi-1 Hbb Gv-2*. Because no strain of mouse known so far has the genotype *Gv-1*$^+$ *Gv-2*$^-$, it was not possible to avoid the use of a cross in which both genes would be segregating and therefore yielding only 25 percent G_{IX}^+ segregants. The total number of mice typed for G_{IX} in this series was 345, yielding 87 G_{IX}^+ : 258 G_{IX}^- segregants (expected 86.25 : 258.75). In the early part of the study, only G_{IX}^+ segregants were typed for Gpi-1 and Hbb, hence the preponderance of G_{IX}^+ phenotypes among the 158 mice in this table (85 G_{IX}^+ and 73 G_{IX}^- were typed for Gpi-1 and Hbb). For these two reasons the calculations of significance and map distance are lengthy and complicated (copies can be had from the authors if required). The estimated distance of *Gv-2* from *Hbb* is 33.6 ± 5 units. The references for typing methods are as follows: for G_{IX} (*1*), Gpi-1 (*9*), and Hbb (*10*) (LG, linkage group).

Parental types
C57BL/6: Gv-1$^-$ Gv-2$^-$ Hbb^s Gpi-1^b
129: Gv-1$^+$ Gv-2$^+$ Hbb^d Gpi-1^a

Phenotype		Genotypes		Observed (No.)
		LG IX	LG I	
G_{IX}^+	Hbb^{ds}	*Gv-1*$^+$/*Gv-1*$^-$	*Gv-2*$^+$ *Hbb*^{d}/*Gv-2*$^-$ *Hbb*^{s}	55
	Hbb^{ss}	*Gv-1*$^+$/	*Gv-2*$^+$ *Hbb*^{s}/	30
G_{IX}^-	Hbb^{ds}	*Gv-1*$^-$/	*Gv-2*$^+$ *Hbb*^{d}/	28
		Gv-1$^+$/	*Gv-2*$^-$ *Hbb*^{d}/	
		Gv-1$^-$/	*Gv-2*$^-$ *Hbb*^{d}/	
	Hbb^{ss}	*Gv-1*$^-$/	*Gv-2*$^+$ *Hbb*^{s}/	45
		Gv-1$^+$/	*Gv-2*$^-$ *Hbb*^{s}/	
		Gv-1$^-$/	*Gv-2*$^-$ *Hbb*^{s}/	
			Total	158*

* Comprising 15 progeny of hybrid males and 143 of hybrid females. Approximately equal numbers of males and females. Segregation ratios: For *Gpi-1*, 75 (*bb*), 83 (*ab*). For *Hbb*, 75 (*ss*), 83 (*ds*). Our data indicate 31.6 ± 3.7 units between *Gpi-1* and *Hbb* [compare 32 ± 5 reported in (*11*)].

[which occurs spontaneously in association with aging, and with leukemia and other forms of malignancy; or is produced experimentally by inoculation of MuLV—for review see (*6*)] causes G_{IX} antigen to appear regardless of the inherited *Gv-1* and *Gv-2* genotypes of the virus-producing cells.

G_{IX} is probably absent from the virus itself, and it is not known whether the coding gene belongs to the viral genome or to the cellular genome. Thus the expression of G_{IX} antigen on thymocytes of normal mice, which is independent of MuLV production, could, for example, be viewed as partial expression of a viral genome integrated at either the *Gv-1* locus or the *Gv-2* locus. Alternatively, *Gv-1* and *Gv-2* may be cellular genes, with *Gv-1*$^-$ and *Gv-2*$^-$ alleles that repress G_{IX} antigen in G_{IX}^- mice; and MuLV may alter this negative control in such a way as to permit expression of G_{IX} antigen. Such possibilities have already been discussed (*1*).

One useful step that can be taken to approach this difficult problem is to find out where *Gv-1* and *Gv-2* are situated in the cellular genome; knowledge of their linkage relations enables us to establish whether they are identical with or closely linked to other genes associated with leukemogenesis or the inheritance of leukemia virus (*5*, *7*) and serves other valuable purposes outlined by Rowe (*8*).

The *Gv-1* gene has already been located in linkage group IX (chromosome 17), 36 units from *H-2* (*1*); this is why the antigen is called G_{IX}. We report here that *Gv-2* has now been located in linkage group I (chromosome 7). This was ascertained in a three-point cross with *Gpi-1* (glucose phosphate isomerase) and *Hbb* (β chain of hemoglobin). The data are summarized in Table 1, and give the order *Gpi-1 Hbb Gv-2*, with a distance of 33.6 ± 5 units between *Hbb* and *Gv-2* (*13*) (footnote to Table 1).

Another locus recently identified in linkage group I is *Akv-1* (*8*), which Rowe and his colleagues consider to be one of two unlinked sites of integration of MuLV in AKR mice (*12*). Mice which inherit the *Akv-1* allele carried by AKR exhibit high levels of MuLV production starting in early life, and their cells are characteristically susceptible to the induction of MuLV by 5-iododeoxyuridine. However, the two loci *Akv-1* and *Gv-2* are not identical, nor even closely associated, for *Akv-1* has been located on the opposite side of *Gpi-1* and *Hbb*, giving the order—centromere *Akv-1 Gpi-1 Hbb Gv-2*.

One special reservation must be recognized in connection with loci like *Gv-1* and *Gv-2* which may represent viral genes: The establishment of linkage for such a locus in a particular strain of mice carries no assurance that the same gene or its alleles will occupy the same site in the genomes of other mice. In fact the linkage of *Gv-1* with *H-2* (linkage group IX) established in 129 mice (*1*) and in A mice (unpublished) has not so far been demonstrable in AKR mice; one explanation, among others, is that *Gv-1* occupies a different site in AKR mice.

ELISABETH STOCKERT
HIDETOSHI SATO
KATSUAKI ITAKURA
EDWARD A. BOYSE
LLOYD J. OLD
Division of Immunology, Sloan-Kettering Institute for Cancer Research, New York 10021
JOHN J. HUTTON
Division of Hematology, University of Kentucky Medical Center, Lexington 40506

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3. The locus notation *Gv* (Gross virus) was adopted at the suggestion of Dr. Margaret Green, Jackson Laboratory, Bar Harbor, Maine.
4. The designation MuLV is used in this report in reference to wild-type and passage A, Gross leukemia virus (that is, excluding Friend, Rauscher, and other subtypes which are serologically distinguishable from wild-type and passage A virus and occur infrequently or not at all as natural infections of mice).
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