Segregation of Loci for C-Type Virus Induction in Strains of Mice with High and Low Incidence of Leukemia

Abstract. *Multiple genetic loci for induction of murine leukemia viruses are demonstrated in cells of the high leukemic incidence C58 mouse strain. The biologic properties of viruses at C58 inducibility loci are clearly distinguishable from those of viruses activated from mouse cells containing a locus for virus induction of the low leukemia incidence BALB/c strain. These findings are consistent with the hypothesis that the genes for virus induction in normal mouse embryo cells represent viral structural information.*

There is a large body of evidence linking RNA C-type leukemia viruses with naturally occurring tumors in avian and a number of mammalian species (*1*). Several in vivo (*2*) and in vitro studies (*3*) have indicated that these viruses are vertically transmitted. The demonstration that clonal lines of virus-negative mouse embryo cells can be induced by chemicals in tissue culture to produce murine leukemia virus has established that the viral genome is stably associated with each cell (*4*). The mechanism of transmission of this unexpressed viral information and its location within the cell are of biologic interest.

In studies in vivo, two independently segregating loci that affect virus expression have been reported in the AKR mouse strain, which has a high incidence of leukemia (*5*). In tissue culture, cells of the BALB/c strain, which has a low incidence of leukemia, have been shown to contain at least one locus for virus inducibility (*6*). An inducibility locus in the AKR strain has been specified to a chromosome by linkage to genetic markers (*7*). None of these studies, however, has revealed whether the loci so far detected represent viral structural information or regulatory genes that affect virus expression.

We have shown that the biologic properties of virus inducible from cells of the high leukemic incidence C58 mice are different from those of an endogenous virus of BALB/c cells (*8*). Further, cells of one mouse strain (NIH Swiss) have not as yet been shown to be activated by iododeoxyuridine (IdU) in tissue culture to produce leukemia virus (*6, 8*). The fact that embryo cells derived from F_1 hybrids of either inducible strain with the noninducible NIH Swiss strain yield virus of the inducible parental type indicates that inducibility is a dominant genetic characteristic (*6*). In the present report we have approached the question of whether virus inducibility loci are structural or regulatory genes by examining the biologic properties of viruses activated from a series of second generation embryos obtained from backcrosses involving NIH Swiss with (NIH Swiss × BALB/c)F_1 and (NIH Swiss × C58)F_1 hybrids.

Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10 percent calf serum (Colorado Serum Company). The methods used for chemical activation of murine leukemia virus from mouse embryo cells have been reported (*6*). Biologic activity was measured by a plaque assay, the mixed culture (XC) test (*9*), on NIH/3T3 cells (*10*). Quantitation of virus by means of the reverse transcriptase assay (*11*), and a radioimmunoprecipitation method which detects mouse leukemia virus group-specific (gs) antigen (*12*) have been described. The following abbreviations are used: murine leukemia virus (MuLV); BALB/c (B); and NIH Swiss (N).

In previous reports, activation of C-type virus was tested in embryo cell lines derived from a series of backcross involving B and N strains, N × (N × B)F_1. The finding that 50 percent were inducible demonstrated the existence of one segregating gene for virus induction in B cells (*8*). We have examined an analogous series of backcross generation embryo lines [N × (N × C58)F_1] to determine the number of loci for virus induction in C58 cells. Because the endogenous viruses of C58 and B parental mouse strains were distinguishable with the use of quantitative biologic

Table 1. Activation of RNA C-type virus from N×(N×C58)F_1 backcross mouse embryo cultures. Secondary embryo cell cultures containing approximately 3×10^6 cells were exposed to IdU (20 μg/ml) for 24 hours. After 4 weeks of incubation at 37°C with weekly subculture, the number of virus-positive cultures was determined by assaying the supernatants for reverse transcriptase activity and for XC plaque formation on NIH/3T3 cells.

Genotype	Lines examined (No.)	Observed inducible lines		Expected percent inducible for:*		
		Total	Percent	Two loci	Three loci	Four loci
N	10	0	0	0	0	0
C58	5	5	100	100	100	100
(N×C58)F_1	5	5	100	100	100	100
N×(N×C58)F_1	55	51	92.7	75	87.5	93.7

* Percentage of virus positive embryo cultures expected following IdU activation for two, three, or four independently segregating genetic loci for virus inducibility.

Table 2. Comparison of biological properties of viruses induced from individual N × (N × C58)F_1 and N × (N × B)F_1 backcross mice. The infectivity of each virus isolate was tested on NIH/3T3 cells, which have been shown to be more susceptible than other mouse cell lines to infection by the endogenous viruses of both B and C58 parental mouse strains. The XC plaque assay was performed according to the method of Rowe *et al.* (*9*). The polymerase induction assay was performed (*11*) by infection of NIH/3T3 cells with serial tenfold virus dilutions. After 7 days at 37°C, virus reverse transcriptase was measured in 100-fold concentrated tissue culture fluids of infected cells. The end point for induction of polymerase activity was determined by extrapolation and was defined as the titer in polymerase-inducing units (PIU) per milliliter. The biologic activity of each virus in either XC plaque-forming units (PFU) per milliliter or PIU per milliliter was then standardized on the basis of the total number of nanograms of virus gs protein per milliliter. The results of tests with multiple individual virus preparations are expressed as the mean value ± 1 standard error; L, large; S, small.

Virus	Isolates examined (No.)	Infectivity per 0.1 ng of viral gs protein		XC plaque morphology
		XC PFU	PIU	
MuLV*	1	150	40	L
Activated from:				
C58	1	75	25	L
(N × C58)F_1	1	50	30	L
N × (N × C58)F_1	25	63 ± 23	31 ± 12	L
B	1	0.7	0.4	S
(N × B)F_1	2	0.4	0.2	S
N × (N × B)F_1	8	0.5 ± 0.3	0.2 ± 0.4	S

* Standard wild-type strain (Kirsten).

and biochemical methods (*8*), the viruses obtained from individual backcross lines involving either inducible strain could be compared.

A total of 55 cell lines was established from individual $N \times (N \times C58)F_1$ backcross embryos. Cultures of each were treated with IdU (20 μg/ml) for 24 hours, and supernatants were assayed for C-type virus by both the reverse transcriptase assay and the XC plaque assay at weekly intervals for up to 1 month after treatment. Induction of virus was detected with 51 out of 55 lines. Each of the four $N \times (N \times C58)F_1$ backcross lines, which had remained virus-negative, were subsequently treated again in three separate experiments. However, in each case, they remained noninducible. In Table 1, the percentage of virus-inducible backcross $N \times (N \times C58)F_1$ lines (92.7 percent) is compared with the percentages expected for two, three, or four loci. The results fit most closely with the percentage expected for three or four independently segregating dominant alleles for virus inducibility in C58 cells.

Proof that virus inducibility loci represent viral structural rather than regulatory information would first require the demonstration that all virus isolates at one locus were identical and yet distinct from those activated at some other locus. Thus, the biologic properties of viruses activated from cell lines of 8 individual $N \times (N \times B)F_1$ backcrosses, containing the BALB/c inducibility locus (*8*), were compared with viruses activated at loci for induction in 25 individual $N \times (N \times C58)F_1$ backcross lines. These viruses were also compared with isolates obtained from parental as well as $(N \times B)F_1$ and $(N \times C58)F_1$ hybrid lines. The amount of murine leukemia virus gs antigen was determined for each virus preparation. Since there is a uniform amount of gs protein per virion, the total gs protein in each virus stock provided a measure of the number of physical particles. Each virus was also assayed for its reverse transcriptase activity. These biochemical and immunologic measurements provided a means of standardizing the different viruses prior to tests of their biologic activity.

As can be seen from the results summarized in Table 2, the biologic properties of each $N \times (N \times B)F_1$–activated virus were indistinguishable from those of virus induced from cells of the parental B strain. Each formed very small XC plaques; as determined by both the XC plaque assay and by measurement of virion-associated reverse transcriptase in tissue culture fluids of infected cell cultures, each was around 100- to 200-fold less infectious than a standard wild-type strain. In contrast, each of 25 isolates from activatable $N \times (N \times C58)F_1$ embryo lines formed large XC plaques and, like the virus activated from C58 parental cells, were almost as infectious per nanogram of viral gs protein as the standard wild-type strain. Thus, the biologic properties of each of several viruses activated from backcross embryo cells containing an induction locus of the B strain, while indistinguishable from each other, were quite different from those of viruses obtained from backcross lines containing virus induction loci of the C58 strain.

The foregoing results indicate that the inducibility loci detected may represent C-type viral structural information and that regulatory factors necessary for their activation are either present in all cell strains or closely linked to the viral structural loci. Alternative possibilities are that each class of endogenous virus is present in an integrated state within cells of all mouse strains or that the endogenous viruses of a particular strain exist in multiple extrachromosomal copies. The loci observed could then be regulatory genes that allow virus activation. The possibility of extrachromosomal virus location appears to be excluded by recent biochemical evidence indicating that mouse embryo cells contain murine leukemia virus-specific DNA which is covalently linked to host cell sequences in the high molecular weight fraction of cellular DNA (*13*).

The multiple (more than two) loci for murine leukemia virus detected in cells of the C58 strain are more than have been observed in genetic studies with other mouse strains (*5*, *6*). The very infectious nature of the endogenous viruses of the C58 cell undoubtedly contributes to the ease of their detection. It is possible, therefore, that in other mouse strains, there may be additional alleles representing viruses with altered or defective properties, which cannot be detected by available methods. Whether the large number of biologically highly active endogenous viruses in the C58 strain can be causally linked to the very high leukemia incidence observed in that mouse strain remains to be determined.

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Fordilla troyensis Barrande: The Oldest Known Pelecypod

Abstract. *Specimens of the small bivalved animal* Fordilla troyensis *Barrande from New York State show that this fossil is the oldest known pelecypod mollusk and not a conchostracan arthropod. This finding extends the range of the class Pelecypoda backward in time from the Early Ordovician (about 495 million years ago) to the Early Cambrian (about 540 to 570 million years ago). The morphology of* Fordilla troyensis *suggests that it lived infaunally and that it was ancestral to the pelecypod subclasses Heteroconchia and Isofilibranchia.*

Fordilla troyensis Barrande (*1*) is a small bivalved invertebrate best known from Lower Cambrian rocks of New York State; it also occurs in rocks of the same age in Newfoundland, Greenland, and perhaps England, Denmark, and Portugal. Although in recent years there has been general agreement that this species is a crustacean and not a pelecypod, there was a long debate as to its zoological placement. Opinion was divided as to whether *Fordilla* is a pelecypod (*1–4*), possibly a pelecypod (*5*, *6*), or a bivalved conchostracan crustacean (*7*).

The Early Cambrian age of *Fordilla*