Activation of Nonexpressed Endogenous Ecotropic Murine Leukemia Virus by Transfection of Genomic DNA into Embryo Cells

JAMES McCUBREY† AND REX RISSE*

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

Received 29 October 1982/Accepted 3 December 1982

We studied the infectivity of endogenous ecotropic murine leukemia virus genomes contained in high-molecular-weight DNA prepared from virus-free cells of the AKR-2B line, and from RF, BALB/c, B6, and (BALB/c × B6)F₁ mouse embryo cells. When DNA prepared from virus-free AKR-2B cells was transfected into NIH-3T3 cells, no virus-positive cultures were observed, a result consistent with previous reports. However, when DNAs from virus-free AKR-2B cells or virus-free cells containing the RF/J or BALB/c ecotropic proviruses were transfected into chicken embryo cells that were then cocultivated with SC-1 (mouse) cells, virus-positive cultures were recovered. The specific infectivities of the AKR provirus(es) contained in virus-free cells and the molecularly cloned Akv-1 provirus were similar when chicken embryo cells were used as primary recipients. Virus-positive cultures were also observed when secondary mouse embryo cells were used as recipients for DNA from virus-free AKR-2B and RF/J cells. The transfected chicken embryo-SC-1 cultures produced XC-positive murine leukemia virus that is N-tropic. Virus-positive recipient cultures were observed 10- to 100-fold more frequently when AKR-2B DNA was used than when BALB/c DNA was used as the donor DNA. Our studies indicate that some nonexpressed ecotropic murine leukemia virus proviruses are activated upon transfection into chicken embryo cells. Such studies suggest that there are different factors governing the expression of murine leukemia virus after transfection into established cell lines (NIH-3T3) and into nonestablished secondary cultures (chicken and mouse).

It has long been known that different inbred strains of mice show different patterns of expression of endogenous retroviruses. Recent work from this laboratory has documented the patterns of ecotropic murine leukemia virus (MuLV) induction *in vitro* and of spontaneous virus expression *in vivo* in low leukemic mouse strains (13a, 14, 16). We have observed increased ecotropic virus expression in induced cells from hybrid mice made between unrelated inbred strains. The genes that control this enhanced virus induction phenotype map to the positions of ecotropic proviruses in most cases (10a, 15). In the present study, we examined the infectivity of genomic DNA containing these proviruses in DNA transfection experiments.

DNA transfection experiments have been used to determine the biological function and genetic composition of proviruses contained in a

variety of genomic DNAs (2, 9). Cooper and Temin (3) investigated the infectivity of high-molecular-weight DNA prepared from chicken cells harboring complete RAV-0 proviruses and observed that the infectivities of DNAs prepared from virus-producing and virus-free cells differ in DNA transfection assays. The specific infectivity of DNA prepared from virus-positive cultures was 400-fold greater than the specific infectivity of DNA prepared from cultures producing very little virus (3). Parallel results were observed by others using DNAs from AKR-2B mouse cells and transfection of NIH-3T3 cells (4, 11). It was also observed that addition of the halogenated pyrimidine 5-iododeoxyuridine, which induces virus expression, to recipient cultures led to virus production after transfection of DNA from virus-free AKR-2B cells (11).

In the present study, we confirmed the observation that DNA from virus-free AKR-2B cells is not infectious for NIH-3T3 cells. However, we found that such DNA is readily infectious

† Present address: Basel Institute for Immunology, Basel, Switzerland.

when assayed on chicken or mouse embryo cells that are subsequently cocultivated with mouse SC-1 cells.

MATERIALS AND METHODS

Cell culture. Cell lines and primary cultures were grown in a humidified incubator containing 5% CO₂. In some instances, XC cells were also grown in roller flasks at 37°C. The AKR-2B cell line was obtained from J. Hartley and W. P. Rowe (17). Cell cultures were monitored for the production of ecotropic MuLV by the XC plaque assay (19).

Induction assays. In situ virus induction assays were performed as previously described (14). 5-iododeoxyuridine (60 μ M) (13) or 5-azacytidine (30 μ M) in 10% fetal bovine serum and McCoys medium was added to cultures for 48 h. Low concentrations of 5-azacytidine (3 μ M) were not effective in inducing ecotropic virus production, and concentrations greater than 30 μ M were toxic to mouse cell cultures (see reference 6).

Isolation of cellular DNA. Cellular DNAs were prepared as described previously (10a). DNAs, spooled out of the final ethanol solution, were suspended in 10 mM Tris-base (pH 7.4)–0.1 mM EDTA and stored at 4°C. The Akv-1 virus DNA clone (p623) was obtained from D. Lowy (12).

DNA transfection assay. One million mouse embryo (ME), rat embryo, NIH-3T3, NZB-Q, SC-1, CCL64 (mink), SIRC (rabbit), human foreskin, BSC-1 and CV-1 (monkey), MDCK (dog), or Rat-1 cells were seeded onto 60-mm petri dishes in Dulbecco minimal essential medium plus 10% fetal bovine serum. v_gs⁺ SPAFAS chicken embryo cells (6×10^5), kindly provided by S. Hellenbrand and H. M. Temin, were seeded onto 60-mm petri dishes in Temin's minimal essential medium plus 20% tryptose phosphate, and 2% fetal bovine serum was added 4 h later. The next day, the test DNAs were diluted in HEPES-buffered saline (0.14 M NaCl, 0.005 M KCl, 0.004 M Na₂HPO₄, hydrated PO₄, 0.01 M dextrose, 0.02 M N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, final pH 7.05). Test DNA with a concentration of less than 20 μ g/ml had carrier (sheared, RNase-digested calf thymus) DNA added to bring the final DNA concentration to 20 μ g/ml. These test DNAs were sheared by passage (10 times) through a syringe with a 20-gauge needle to reduce DNA aggregate formation, and CaCl₂ was added to a final concentration of 0.125 M CaCl₂ (5). After 0.5 h of precipitation at room temperature, 0.5 ml of the calcium phosphate-precipitated DNA was added to each 60-mm plate. After 4 to 6 h of incubation of the recipient cells with the DNA precipitate, the medium was removed and fresh growth medium was added. SC-1 cells (10^5) (7) in growth medium with 2% fetal bovine serum were added to xenogenic and ME transfected cultures 6 to 24 h after the addition of the donor DNA. The medium was removed after 4 days of incubation, and fresh medium was added. Cells were passaged at weekly intervals for 2 to 4 weeks. Cultures were scored for the presence of ecotropic MuLV by the XC plaque assay (19).

In some experiments, 24 h after the addition of the donor DNA, 60 μ M 5-iododeoxyuridine in growth medium was added to some of the transfected plates.

The cells were rinsed with medium 24 h later, and fresh medium containing 10^5 SC-1 cells was added.

Fv-1 typing. The tropism of virus isolates was determined by titration of the isolates on SC-1 (*Fv-1*^{+/+}), NFS ME (*Fv-1*^{+/m}), and BALB/c ME (*Fv-1*^{b/b}) cells. N-tropic virus (Akv-1) had a 100-fold higher titer on *Fv-1*^{+/m} cells than on *Fv-1*^{b/b} cells, whereas B-tropic virus (WN1802B) had a 30- to 60-fold higher titer on *Fv-1*^{b/b} cells than on *Fv-1*^{+/m} cells (8).

RESULTS

Activation of endogenous ecotropic MuLV in cells treated with base analogs. Before comparing the infectivities of DNAs from different mouse strains, we determined the frequency with which various mouse cells produce infectious ecotropic MuLV. We did this in an infectious center assay (14) by plating serial cell dilutions, treating the cells with control medium, with 60 μ M 5-iododeoxyuridine, or with 30 μ M 5-azacytidine, and scoring the frequency of virus-producing cells (Table 1). The results of that experiment indicate that spontaneous virus production in uninduced AKR-2B cells or uninduced BALB/c, C57BL/6 (B6), or (BALB/c $\times$ B6)F₁ secondary ME cells is very low. Among 3×10^7 AKR-2B cells examined for production of ecotropic MuLV, no infectious centers were observed. The same result was observed for each of the four groups of virus-free AKR-2B cells from which DNA was prepared. Similar results were also observed with uninduced BALB/c, B6, or (BALB/c $\times$ B6)F₁ mouse embryo cells.

The data in Table 1 also document the different inducibilities of AKR-2B, (BALB/c $\times$ B6)F₁, and BALB/c or B6 cells. AKR-2B cells were approximately 1,000-fold more inducible than (BALB/c $\times$ B6) F₁ cells, which were at least 10- to 20-fold more inducible than the BALB/c or B6 parental cells (14). It is also apparent from these data that ecotropic MuLV was induced with approximately equal efficiency

TABLE 1. Activation of endogenous ecotropic MuLV by treatment of cells with base analogs^a

Cell	Frequency of virus-producing cells in situ		
	Uninduced	5-Iododeoxyuridine	5-Azacytidine
AKR-2B	$\leq 3.3 \times 10^{-8}$	8.4×10^{-2}	2.1×10^{-1}
BALB/c ME	$\leq 2 \times 10^{-7}$	$\leq 4 \times 10^{-7}$	$\leq 4 \times 10^{-7}$
B6 ME	$\leq 2 \times 10^{-7}$	9×10^{-6}	8×10^{-6}
(BALB/c $\times$ B6)F ₁ ME	$\leq 4 \times 10^{-7}$	1.4×10^{-4}	2×10^{-4}

^a Various numbers (10^3 to 3×10^6) of cells were seeded onto multiple petri dishes, induced with growth medium (uninduced) or with growth medium containing 60 μ M 5-iododeoxyuridine or 30 μ M 5-azacytidine, cocultivated with SC-1 cells, and developed in an situ XC plaque assay (14).

from these cells with either 5-iododeoxyuridine or 5-azacytidine.

Transfection with DNA prepared from AKR-2B cells. To determine the sensitivity of various cells to DNA transfection, we transfected DNA isolated from virus-producing AKR-2B cells into different recipient cells. We observed that mouse NIH-3T3 cells, NFS, BALB/c, and B6 ME cells that were cocultivated with SC-1 cells, and chicken embryo cells that were cocultivated with SC-1 cells, produced ecotropic MuLV after transfection with DNA isolated from AKR cells producing MuLV. SC-1 or NZB-Q cells transfected with the same DNAs did not yield virus (Table 2). In our assays, human fibroblasts, BHK cells (hamster), SIRC cells (rabbit), CV-1 and BSC-1 cells (monkey), and MDCK cells (dog) did not yield virus-positive cultures after DNA transfection and cocultivation with SC-1 cells. Secondary rat embryo or CCL64 mink cells yielded very few ecotropic virus-positive cultures (data not shown).

Virus-positive cultures were also recovered when chicken embryo cells were transfected with DNA from SC-1 cells producing Akv-1 virus and subsequently cocultivated with SC-1 cells (four positive cultures of four tested cultures). Taken together, these results indicate that chicken embryo and ME cells, in addition to NIH-3T3 cells, are sensitive to transfection by DNA from cells producing ecotropic MuLV.

To determine if the endogenous proviruses contained in genomic DNA isolated from virus-free AKR-2B cells (V^- AKR-2B DNA) were infectious, we transfected chicken embryo cells, NFS ME cells, and NIH-3T3 cells with V^- AKR-2B DNA, and cocultivated the chicken embryo and ME cells with SC-1 cells. The data in Table 3 indicate that virus-positive cultures were recovered after transfection of chicken embryo cells and NFS ME cells with DNA

TABLE 3. Transfection of different cells with DNA isolated from virus-free AKR-2B cells^a

Recipient cell type	No. of virus-positive cultures/total no. of cultures	
	With 5-iododeoxyuridine	Without 5-iododeoxyuridine
NIH-3T3	1/30	0/31
NFS ME ^b	3/5	10/31
Chicken embryo	12/24	21/42

^a DNA (10 to 25 μ g) was added to each plate, and the cells were treated with medium alone or medium containing 60 μ M 5-iododeoxyuridine 24 h later, passaged, and scored for virus production in the XC plaque test. Chicken embryo and ME cells were cocultivated with SC-1 cells after transfection.

^b These results were pooled from six experiments in which ME cells yielded virus-positive cultures with V^+ or p623 control DNAs; in an additional seven experiments, no virus-positive cultures were obtained with V^- , V^+ , or p623 DNAs.

isolated from virus-free AKR-2B cells. In several experiments with ME recipient cells, no positive cultures were obtained with control DNAs, and so we have not included these. The source of this variation with ME cells is currently under investigation. Virus-positive cultures were not recovered when NIH-3T3 cells were transfected with V^- AKR-2B DNA (Table 3), a result consistent with earlier observations (4, 11). However, the data in Table 2 indicate that our NIH-3T3 cells yielded fewer positive cultures with virus-positive AKR DNA (V^+ AKR DNA) than observed in earlier studies. Nonetheless, the difference between transfection of NIH-3T3 cells with V^- AKR-2B DNA (0/31) and transfection of NIH-3T3 cells with V^+ AKR DNA (17/76) is significant.

To determine if treatment of the recipient cells with 5-iododeoxyuridine would increase the frequency of recovery of virus-positive cultures after transfection (11), we transfected the same cells with V^- AKR-2B DNA and then treated the cells with 5-iododeoxyuridine (Table 3). The data in Table 3 indicate that addition of 5-iododeoxyuridine to the recipient cells 24 h after transfection did not significantly increase the recovery of virus-positive cultures. Lowy (11) has observed that addition of 5-iododeoxyuridine 24 h before or at the time of DNA addition to NIH-3T3 cells results in recovery of ecotropic virus from NIH-3T3 cells transfected with V^- AKR-2B DNA. The different results of these two experiments may reflect the different protocols used or different sensitivities of substrains of NIH-3T3 cells to transfection.

Because it has been reported that shearing of DNA from cells that carried but produced very little RAV-0 virus increased the infectivity of

TABLE 2. Transfection with DNA from AKR virus-producing cells^a

Recipient cell	No. of virus-producing cultures/total no. of cultures
NIH-3T3	17/76
SC-1	0/15
NZB-Q	0/8
NFS, BALB/c, and B6 ME ^b	28/42
Chicken embryo ^b	18/29

^a DNA (5 to 25 μ g) from AKR cells producing AKR ecotropic MuLV was added to each plate, and cells were passaged and scored for virus production in the XC plaque test.

^b Chicken embryo and ME cells were cocultivated with SC-1 cells 6 to 24 h after addition of the DNA.

that DNA for chicken embryo cells, we tested the effect of shearing genomic DNA (1) on infectivity of ecotropic MuLV genomes contained in V^- AKR-2B DNA. DNA was sheared through 22- or 27-gauge needles and then shown to be significantly reduced in average molecular mass in agarose gels (kindly analyzed by I. Chen and L. Green). No significant difference was found in the infectivity of sheared DNA transfected into chicken embryo cells that were subsequently cocultivated with SC-1 cells (data not shown).

Titration of AKR-2B DNAs on chicken embryo cells and analysis of recovered virus. Table 4 and Figure 1 present the titration pattern of V^- AKR-2B genomic DNA, V^+ AKR-2B genomic DNA, and p623 DNA, a proviral clone isolated from Akv-1 virus-infected cells, on chicken embryo cells that were cocultivated with SC-1 cells. Clearly, V^+ and V^- AKR-2B DNAs show similar titration patterns on chicken embryo cells. The specific infectivity of these genomic DNAs (assuming two intact ecotropic proviruses per cell) is similar to that found with the cloned Akv-1 virus (2×10^{-7} PFU per viral genome).

The virus recovered from chicken embryo-SC-1 cultures was titered on SC-1, NFS ($Fv-I^{n/n}$), BALB/c ($Fv-I^{b/b}$), and chicken embryo cells. The data in Table 5 indicate that the virus recovered from transfection of chicken embryo cells with V^- AKR-2B DNA is N-tropic, induces XC syncytia, and does not replicate on

FIG. 1. Titration of DNA isolated from AKR-2B cells on chicken embryo fibroblasts. Chicken embryo cells were transfected with DNA as described in the text, cocultivated with SC-1 mouse cells, and scored for the production of ecotropic MuLV. Symbols: ●, V^- AKR-2B DNA; ■, V^+ AKR-2B DNA; and △, p623 DNA.

chicken embryo cells. Therefore, the virus recovered from chicken embryo cells transfected with V^- AKR-2B DNA resembles the ecotropic virus normally produced in AKR mice. Taken together, these results indicate that the endogenous ecotropic MuLV provirus(es) present in V^- AKR-2B cells is not defective and has an infectivity similar to the molecularly cloned provirus when chicken embryo cells are used as recipients.

Transfection of chicken embryo cells with DNAs prepared from cells of low leukemic mice. To determine if the activation of ecotropic MuLV in chicken embryo cells is a unique property of AKR-2B DNA, we tested the ability of DNAs prepared from low leukemic mouse

TABLE 4. Titration of DNA isolated from AKR-2B cells on chicken embryo cells

Source of DNA	µg of test DNA/plate ^a	No. of virus-producing cultures/total no. of cultures (%)
Virus-free AKR-2B cells	15	16/22 (73)
	10	21/39 (54)
	5	2/20 (10)
	2.5	2/25 (8)
	1	0/18 (0)
Virus-producing AKR-2B cells	15	18/29 (62)
	5	2/12 (17)
	2.5	1/5 (20)
	1	2/16 (12)
p623 (cloned Akv-1)	3×10^{-4}	10/14 (71)
	10^{-4}	9/24 (38)
	10^{-5}	1/6 (16)

^a Carrier DNA was added to bring the final concentration to 15 µg per culture plate. DNA transfections were carried out as described in the text.

TABLE 5. *Fv-I* tropism of viruses recovered from V^- AKR-2B-transfected chicken embryo cells^a

Isolate no.	Titer on:			
	NFS ($Fv-I^{n/n}$)	BALB/c ($Fv-I^{b/b}$)	SC-1 ($Fv-I^{-/-}$)	Chicken
4783	5×10^2	0	2.5×10^4	0
4784	25×10^2	0	5×10^3	0

^a Supernatant fluids harvested from passaged chicken embryo-SC-1 cultures which had received V^- AKR-2B DNA were titered directly on the indicated cells and scored for XC plaque formation. Units are XC syncytia per milliliter.

tissues, which do not normally produce ecotropic MuLV, to transfect chicken embryo cells. Several DNA preparations, made from virus-free cells, were tested for infectivity on chicken embryo cells cocultivated with mouse SC-1 cells. The summary of that data indicates that all V⁻ AKR-2B DNA preparations tested were readily infectious in chicken embryo cells (Table 6). Moreover, DNA prepared from cells of RF/J mice was also infectious for chicken embryo and ME cells. This strain of mouse expresses little or no ecotropic virus early in life and carries three genome-length ecotropic proviruses, located in cellular DNA sequences different from the sequences adjacent to the ecotropic proviral genomes of AKR mice (13a). However, at least one of the ecotropic proviral genomes of RF/J mice is highly inducible for ecotropic virus expression (13a). Recovery of virus after transfection of chicken and mouse cells with RF/J DNA indicates that ecotropic proviruses other than those found in AKR-2B cells are activated by this procedure.

In addition, a low percentage of cultures that received DNA containing the BALB/c ecotropic provirus were also positive. In these cultures, XC plaque-forming virus was only detected after several passages of the initial chicken-SC-1 re-

cipient culture. Cells that received the B6 ecotropic provirus did not yield positive cultures. We conclude that infectious XC plaque-forming MuLV can be recovered by transfection of chicken embryo cells with V⁻ BALB/c DNA, but with much lower efficiency than with V⁻ AKR-2B DNA or V⁻ RF/J DNA.

DISCUSSION

The data in this study present the results of a novel combination of recipient cells and genomic DNAs in transfection studies. We have observed that nonexpressed endogenous mouse retroviruses are activated with high frequency when genomic DNA is transfected into chicken embryo or ME cells that are then cocultivated with mouse SC-1 cells. The specific infectivity of ecotropic viral genomes found in virus-free AKR-2B cells is comparable to that of a molecularly cloned AKR virus, and so it seems reasonable to conclude that at least one of the nonexpressed ecotropic genomes carried in AKR-2B cells is not defective and is readily activated upon transfection. These results differ from those of Cooper and Temin (3), who found that genomic DNA of cells producing very little RAV-0 virus was much less infectious in transfection assays with chicken cells than genomic DNA from producer cultures. Therefore, it seems apparent that either the proviral genomes used in those two studies differ in their requirements for activation or chicken embryo and ME cells process input mouse proviruses differently than they do input chicken proviruses.

Previous studies by Lowy (11) and by Copeland and Cooper (4) indicated that DNA of virus-free AKR-2B cells was not infectious for NIH-3T3 cells, and this observation was interpreted, much as the Cooper and Temin (3) observation was, to mean that a change in proviral DNA was necessary for virus expression. Although we have reproduced this observation, the present results with embryo recipient cells offer an alternative or additional explanation. Because virus-positive cultures were recovered upon transfection of recipient chicken embryo or ME cells with genomic DNA from virus-free cells, it may be that virus expression after transfection depends upon both the state of input viral DNA and the nature of the recipient cells. Secondary embryo cells may process transfected DNA differently than an established line such as NIH-3T3. The topological or physical processing of that DNA in the recipient cell may be an important factor in determining the subsequent pattern of expression of introduced viral genomes. It is also possible that the differentiative state of fibroblasts or other cells present in embryo cultures determines the response of the cells to

TABLE 6. Transfection of chicken embryo cells with DNA preparations from virus-free cells^a

Source of DNA	No. of virus-producing cultures/total no. of cultures (%)
AKR-2B prep 1	33/45 (73)
AKR-2B prep 2	3/5 (60)
AKR-2B prep 3	6/17 (35)
AKR-2B prep 4	17/60 (34)
RF/J ^b	2/6 (33)
BALB/c-related cells ^c	4/71 (6)
B6-related cells ^d	0/96 (0)
(BALB/c × B6)F ₁ cells ^e	6/54 (11)
Calf thymus	0/34 (0)

^a Cells were transfected with 10 to 15 µg of test DNA, cocultivated with SC-1 cells, passaged, and scored for production of ecotropic virus.

^b This DNA preparation was also infectious for three of four cultures of NFS ME cells.

^c DNA preparations from cells of BALB/c and strains that carry only the BALB/c ecotropic provirus, e.g., CXBH.

^d DNA preparations from cells of B6 and strains that carry only the B6 ecotropic provirus, e.g. CXB D, CXB G.

^e DNA preparations from cells of mice that carry both BALB/c and B6 ecotropic proviruses, e.g. (BALB/c × B6)F₁, CXB E, CXB K.

signals which govern the expression of silent proviruses. In this regard, it is interesting to note that expression of ecotropic virus in AKR mice is first detected during late embryogenesis and does not appear uniformly throughout the organism (18). In any case, it seems apparent that the signals which prevent expression of the ecotropic proviral genome(s) in the donor AKR-2B or RF/J cell DNA are either removed or ignored in the recipient embryo cells.

It also seems reasonable to conclude that at least one of the proviral genomes carried by AKR cells is nondefective. Because infectious XC-positive MuLV was also recovered in about 5% of the cultures transfected with donor BALB/c DNA, it could be argued that this ecotropic proviral genome is also nondefective. However, XC-positive virus was not seen in the initial passage of cultures that received BALB/c DNA, in contrast to cultures that received AKR-2B DNA, and so it is entirely possible that the ecotropic genome carried in BALB/c mice is either inefficient in replication or does not induce XC syncytia (10), or both. The lower infectivity of V⁻ BALB/c or B6 DNA compared to V⁻ AKR DNA parallels the lower inducibility of ecotropic proviruses from cells of BALB/c or B6 mice compared with AKR mice (Table 1). Future transfection studies of molecular clones of these viral genomes may provide information about their *in vivo* patterns of interaction and expression.

ACKNOWLEDGMENTS

We thank S. Hellenbrand and H. M. Temin for providing chicken cells, J. Horowitz and P. Green for several DNA preparations, and J. Horowitz, H. M. Temin, and D. Lowy for a careful reading of the manuscript.

This work was supported by grants CA-22443 and CA-07175 from the National Cancer Institute. R.R. is a Scholar of the Leukemia Society of America, Inc.

LITERATURE CITED

- Cooper, G. M., and L. Silverman. 1978. Linkage of the endogenous avian leukosis virus genome of virus-producing chicken cells to inhibitory cellular DNA sequences. *Cell* 15:573-577.
- Cooper, G. M., and H. M. Temin. 1974. Infectious Rous sarcoma virus and reticuloendotheliosis virus DNAs. *J. Virol.* 14:1132-1141.
- Cooper, G. M., and H. M. Temin. 1976. Lack of infectivity of the endogenous avian leukosis virus-related genes in the DNA of uninfected chicken cells. *J. Virol.* 17:422-430.
- Copeland, N. G., and G. M. Cooper. Transfection by exogenous and endogenous murine retrovirus DNAs. 1979. *Cell* 16:347-356.
- Graham, F. L., and A. J. Van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus DNA. *Virology* 52:456-467.
- Groudine, M., R. Eisenmen, and H. Weintraub. 1981. Chromatin structure of endogenous retroviral genes and activation by an inhibitor of DNA methylation. *Nature (London)* 292:311-317.
- Hartley, J. W., and W. P. Rowe. 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. *Virology* 65:128-134.
- Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1970. Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. *J. Virol.* 5:221-225.
- Hill, M., and J. Hillova. 1974. RNA and DNA forms of the genetic material of C-type viruses and the integrated state of the DNA form in the cellular chromosome. *Biochim. Biophys. Acta* 355:7-48.
- Hopkins, N., and P. Jolicœur. 1975. Variants of N-tropic leukemia viruses derived from BALB/c mice. *J. Virol.* 16:991-999.
- Horowitz, J. M., and R. Risser. 1982. A locus that enhances the induction of endogenous ecotropic murine leukemia viruses is distinct from genome-length ecotropic proviruses. *J. Virol.* 44:950-957.
- Lowy, D. 1978. Infectious murine leukemia virus from DNA of virus-negative AKR mouse embryo cells. *Proc. Natl. Acad. Sci. U.S.A.* 75:5539-5543.
- Lowy, D., E. Rands, S. Chattopadhyay, C. Garon, and G. Hager. 1980. Molecular cloning of infectious integrated murine leukemia virus DNA from infected mouse cells. *Proc. Natl. Acad. Sci. U.S.A.* 77:614-618.
- Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus: high-frequency activation *in vitro* by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Science* 174:155-156.
- McCubrey, J., J. M. Horowitz, and R. Risser. 1982. Structure and expression of endogenous ecotropic murine leukemia viruses in RF/J mice. *J. Exp. Biol.* 156:1461-1474.
- McCubrey, J., and R. Risser. 1982. Genetic interactions in the induction of endogenous murine leukemia virus from low leukemic mice. *Cell* 28:881-888.
- McCubrey, J., and R. Risser. 1982. Allelism and linkage studies of murine leukemia virus activation genes in low leukemic strains of mice. *J. Exp. Med.* 155:1233-1238.
- McCubrey, J., and R. Risser. 1982. Genetic interactions in the spontaneous production of endogenous ecotropic murine leukemia virus in low leukemic mice. *J. Exp. Med.* 156:337-349.
- Rowe, W. P., J. W. Hartley, M. R. Lander, W. E. Pugh, and N. Teich. 1971. Noninfectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. *Virology* 46:866-876.
- Rowe, W. P., and T. Pincus. 1972. Quantitative studies of naturally occurring murine leukemia virus infection. *J. Exp. Med.* 135:429-436.
- Rowe, W. P., W. E. Pugh, and J. W. Hartley. 1970. Plaque assay technique for murine leukemia viruses. *Virology* 42:1136-1139.