Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4098–4102, July 1982
Developmental Biology

De novo methylation, expression, and infectivity of retroviral genomes introduced into embryonal carcinoma cells

(transfection/unintegrated viral DNA/5-azacytidine)

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Communicated by J. D. Watson, March 29, 1982

ABSTRACT **We have investigated the block to expression of Moloney murine leukemia virus in murine embryonal carcinoma (EC) cells. Infected EC cells were found to contain up to 100 integrated proviral genomes. However, expression of virus as measured by XC plaque and virus-specific RNA synthesis did not occur at significant levels, in contrast to productively infected differentiated cells. Analysis of the DNA in the infected EC cells revealed that the proviral genomes were highly methylated, as shown by their resistance to cleavage by *Sma* I. Integrated proviral genomes in infected differentiated cells were readily cut by *Sma* I and thus were not methylated at these sites. Transfection of DNA from infected EC cells to cells permissive for virus expression failed to induce virus expression. The proviral genomes, however, were potentially infectious because they induced XC plaques when the recipient cells for transfection were treated with 5-azacytidine. This drug is believed to interfere with DNA methylation. We conclude that expression of proviral genomes introduced into EC cells is suppressed and that this inactivation can be correlated with the *de novo* methylation of the viral DNA. *De novo* methylation activity thus may be a characteristic of early embryonic cells.**

In eukaryotes, methylation of cytosine residues at the 5′ position has been extensively studied as a possible mechanism involved in the control of gene expression and these studies have shown that an inverse correlation exists between gene expression and the level of methylation (1–3).

As a means to study gene expression in mammals, we have introduced the murine retrovirus Moloney murine leukemia virus (M-MuLV) into the germ line of mice. A number of substrains, each carrying one M-MuLV genome at a different chromosomal position, have been derived and the expression of the M-MuLV genome has been shown to differ between some of them (4). A previous report (5) showed that the germ line-transmitted copy of the viral genome was methylated and noninfectious, whereas the somatically acquired copies, derived from the germ-line copy, were hypomethylated and infectious. Furthermore, cloning of the germ-line gene, which removes the methyl group from the cytosine bases, rendered the germ-line copy infectious (6). Because the proviral DNA introduced into the mouse embryos to derive the Mov-substrains was nonmethylated, *de novo* methylation of the integrated proviral genome must have occurred at some point during derivation of the substrains. This may have occurred either during the development of the infected embryo or as a consequence of transmitting the proviral genome through the germ line to the next generation.

To investigate the early steps of virus infection in embryonal cells, we have studied the integration and expression of M-MuLV in murine embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas. EC cells are a useful system for study of embryonal development because they have many features in common with the embryonic ectoderm cells of the early mouse embryo (7–9), and their injection into mouse blastocysts has demonstrated that some EC cells are developmentally totipotent, giving rise to functional germ cells (10, 11). Furthermore, EC as well as preimplantation mouse embryos are nonpermissive for the replication of a number of tumor viruses (12–14), including M-MuLV (15–17).

Here, we show that EC cells can be efficiently infected with M-MuLV to result in some cells containing up to 100 integrated proviral copies. The viral DNA becomes methylated on integration into the EC cells but not on integration into differentiated cells. This methylation correlates with the loss of infectivity of the viral genome and with the lack of expression of the virus in the EC cells.

MATERIALS AND METHODS

Cell Cultures. The following cloned cell lines were used: EC cell line F9-41 (18), differentiated cell line EB 22/20 derived from a culture of differentiated PSA-4 EC cells (ref. 19; M. L. Hooper and R. Morgan, personal communication), and the fibroblast line NIH 3T3. Cl-1A cells (20) were used for virus production. All cells were grown as described (21) in Dulbecco's modified Eagle's medium with 10% fetal calf serum (heat-inactivated at 56°C for 30 min before use).

Infection of the Cells with M-MuLV. Two protocols were used to infect cells with M-MuLV. (*i*) F9 EC cells were infected by cocultivation with mitomycin C-treated (10 μg/ml for 3 hr) Cl-1A cells for 7 days; mitomycin C-treated Cl-1A cells died within 7–10 days after treatment. (*ii*) The cells were exposed to Cl-1A virus at a multiplicity of infection of 3 for 10 hr in the presence of Polybrene (2 μg/ml; Aldrich). Infected F9 EC cells were cloned as described (22).

Differentiation of F9 EC Cells. Differentiation of the EC cells was induced by culturing them in 0.1 μM retinoic acid (*all-trans*, Eastman) dissolved in dimethyl sulfoxide (23). After 5 days, the medium was replaced with normal medium. Differentiation was assessed by gross changes in the morphology of the cells, the loss of EC-specific antigen ECMA-7 (24), and the appearance of H-2 antigens on the differentiated derivatives (25).

Induction and Measurement of Viral Synthesis. Virus production was induced by a 24-hr exposure of the infected cells to various concentrations of 5-azacytidine (Sigma) dissolved in phosphate-buffered saline. Virus gene expression was measured by the infectious center assay and by quantitating the levels of stable M-MuLV RNA present. In the infectious center

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Abbreviations: M-MuLV, Moloney murine leukemia virus; EC, embryonal carcinoma; kb, kilobase(s).

assay, 10^6 infected cells were plated onto NIH 3T3 cells at a ratio of 1:10. Five days later the cells were tested by the XC plaque assay (5). RNA was isolated and hybridized to representative M-MuLV cDNA under conditions as described (26).

Restriction Enzyme Analysis and Blotting. DNA from cultured cells was obtained as described (27) and was digested with enzymes (3 units/μg) for 12–16 hr under the conditions recommended by the suppliers (Boehringer Mannheim; Bethesda Research Laboratories). The completeness of the digestion was controlled by the addition of cloned M-MuLV DNA to part of the digestion mixtures. Transfer of DNA onto nitrocellulose, synthesis of labeled DNA, and hybridization were as described (5, 27).

Transfection Assay. Infectivity of DNA was tested as described with 30 μg of cellular DNA per assay. Infectious centers were scored by the XC plaque assay 1 week after transfection (5).

RESULTS

Expression of M-MuLV in the Infected Cell Lines. Infected F9 EC cells did not produce virus (Table 1). In contrast, both the differentiated cell line EB 22/20 and NIH 3T3 cells were productively infected. When differentiation of the infected F9 EC cells was induced by retinoic acid treatment, only 1 infectious center per 10^6 differentiated cells was produced, even after the cells had been maintained in the differentiated state for 3 weeks. 5-Azacytidine, an inhibitor of methylation (28), induced approximately 50 plaque-forming units/10^6 cells in both the infected F9 EC cells and their retinoic acid-induced differentiated derivatives. Uninfected F9 EC cells as well as their differentiated derivatives did not produce any virus after treatment with 5-azacytidine.

To characterize further virus expression in the different cell lines, the levels of stable viral RNA were measured by hybridizing labeled M-MuLV cDNA to RNA extracted from the cells. The $C_rt_{1/2}$ of the reaction was used to quantitate the level of M-MuLV expression as described (26). The data in Table 1 indicate that RNA from infected F9 EC cells as well as from differentiated and from azacytidine-treated F9 cells contained significant although low levels of M-MuLV-specific RNA corresponding to approximately 0.001% of the total cellular RNA. In contrast, the infected EB 22/20 and NIH 3T3 contained 50- and 100-fold higher levels, respectively.

Integration and Methylation of M-MuLV DNA Copies. The total number of M-MuLV DNA copies present in the infected EC and 3T3 cells was determined by digesting the DNA with *Sst* I and then performing Southern blotting. The 5.6- and 2.6-kilobase (kb) *Sst* I fragments (see map in Fig. 1) are specific for M-MuLV and can be separated from *Sst* I fragments derived from the endogenous proviral copies present in the mouse genome (4, 29). Fig. 1 *Lower Left* shows the presence of the two *Sst* I-specific fragments in the F9 (lane a) and the 3T3 (lane b) cells when DNA was extracted 7 days after infection with M-MuLV (protocol *ii*). Comparison with DNA from Mov-1 liver (lane c), which contains 2 M-MuLV copies per cell, reveals the presence of approximately 10 M-MuLV DNA copies in the infected 3T3 cells and 30 copies in the infected F9 EC cells. When the EC cells were infected by cocultivation (protocol *i*), approximately 50 M-MuLV-specific copies were present. These estimates were obtained by diluting the EC DNA and comparing the intensity of the bands with those derived from the *Sst* I bands seen in the standard Mov-1 DNA (not shown).

To demonstrate stable integration of the proviral genomes, infected EC cells (protocol *i*) were cloned and the DNA of 11 randomly picked clones was analyzed by digestion with *Eco*RI (an enzyme that does not cleave in the viral genome) and subsequent hybridization to a M-MuLV-specific probe (27). All of the clones contained proviral DNA, indicating that each EC cell can be infected and maintain multiple proviral copies. Fig. 1 *Lower Right* shows multiple nonseparable bands of hybridization in the range of 9–30 kb in four representative clones, in contrast to Mov-1 DNA (lane e) which shows a single band at 27 kb corresponding to the Mov-1 locus (27). This revealed that the infected F9 EC cells carried 20–100 integrated M-MuLV proviral copies per cell.

To analyze for the presence of unintegrated viral genomes,

Table 1. Frequency of infectious centers and viral specific RNA synthesis in M-MuLV-infected cells

Cell line	pfu/10^6 cells*	RNA $C_rt_{1/2}$, mol·sec/liter
F9	0	6,000
F9+5-Aza (25 μM)	54	5,000
F9 (differentiated)	1	5,000
F9 (differentiated)+5-Aza (10 μM)	45	5,000
EB 22/20	10^5–10^6	80
NIH 3T3	10^5–10^6	40
Uninfected controls [F9, EB 22/20, NIH 3T3, F9 (differentiated)+10 μM 5-Aza]	0	>40,000

All assays for virus synthesis were performed at least twice for every cell line. F9 differentiated cells were prepared as described (23). The concentrations of 5-azacytidine (5-Aza) represent those that were found to induce a maximal number of XC plaques in the range 1–50 μM. Viral specific RNA was quantitated as described (26).
* pfu, Plaque-forming unit.

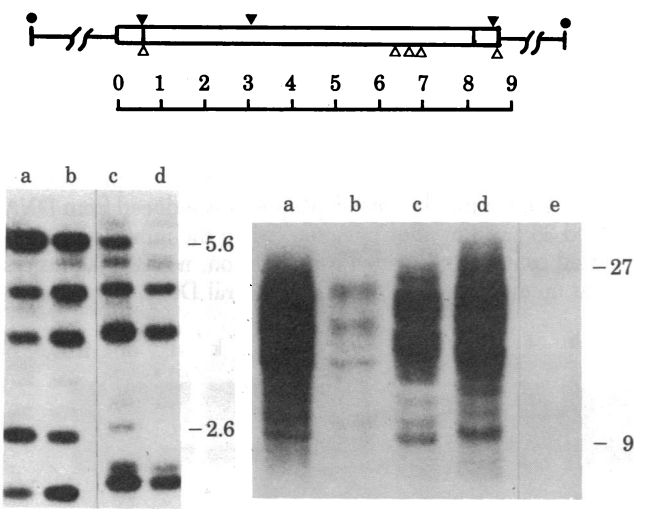

FIG. 1. (*Upper*) Restriction map of M-MuLV provirus. The locations of *Sst* I (▼) and *Sma* I (△) sites within the M-MuLV genome are indicated. The *Eco*RI sites (●) surrounding the provirus are randomly distributed in the flanking mouse DNA (27). (*Lower Left*) M-MuLV copies in infected F9 EC cells. DNA was digested with *Sst* I and run on a 0.7% agarose gel. Hybridization was with a nick-translated cloned M-MuLV DNA. F9 (lane a) and NIH 3T3 cells (lane b) were infected according to protocol *ii* and DNA was prepared 1 week after infection. Lane c, liver DNA from Mov-1 animals, containing 2 copies of M-MuLV per cell (27); lane d, liver DNA from an uninfected BALB/c mouse. Lane a contained 2.5 μg of DNA; all other lanes contained 5 μg of DNA. Size markers are in kb. (*Lower Right*) Stable integration of multiple M-MuLV copies in F9 cells. DNA (15 μg) was digested with *Eco*RI and run on a 0.5% agarose gel. Hybridization was with a M-MuLV-specific cDNA (27). Lanes a–d, DNA from F9 cells, cloned after infection (protocol *i*) with M-MuLV; lane e, DNA from liver of a Mov-1 mouse carrying 2 copies of M-MuLV per cell (27). Size marker fragments are in kb.

DNA extracted from the EC and 3T3 cells at different times after infection was subjected to Southern blot analysis without enzyme digestion. Prominent bands at 8.8 kb indicated the presence of linear viral genomes early after infection (Fig. 2). In addition, double bands at approximately 5 and 14 kb were detected at a lower level of intensity, corresponding to superhelical and circular forms with one and two long terminal repeats, respectively, as described by others (30). By comparison with the standard DNA (lane n), we estimate that the infected F9 and 3T3 cells at 24 hr after infection carry 30 and 10 unintegrated proviral copies, respectively. The amount of unintegrated viral DNA decreased with time, and no free viral DNA was detectable at 7 days after infection.

Methylation of the viral genomes was studied by digesting the DNA with the methylation-sensitive enzyme *Sma* I, which generated two large fragments of 2.1 and 5.7 kb (see map in Fig. 1). The enzyme does not cleave when the cytosine residues of the cleavage sites are methylated. Two *Sma* I fragments were generated in the DNAs from infected 3T3 cells at early and late times after infection (Fig. 3). DNA from the infected F9 cells, however, yielded the M-MuLV-specific *Sma* I fragments only early after infection when unintegrated genomes were present (lanes d–g, compare to Fig. 2).

These results suggest that all M-MuLV DNA copies become *de novo* methylated at the *Sma* I sites within 1 week after integration into the F9 EC cells. In contrast, >80% of the copies integrated into 3T3 and EB 22/20 cells remained unmethylated.

Infectivity of High Molecular Weight DNA from M-MuLV-Infected EC Cells and Differentiated Cells. The biological activity of DNA from F9 EC cells and the differentiated cell lines EB 22/20 and NIH 3T3 after infection with M-MuLV was tested by using the DNA transfection assay (Table 2). The DNA from uninfected cells was not infectious. When DNA from F9 cells at different times after infection with M-MuLV was tested, a correlation between the presence of unintegrated M-MuLV copies and infectivity was found. A high number of XC plaques was induced by DNA isolated 12 and 24 hr after infection, when 20–30 copies of unintegrated M-MuLV DNA were present (see Fig. 2). A lower number of XC plaques was induced from DNA isolated 36 or 48 hr after infection, when the number of unintegrated copies decreased. From 72 hr on, no XC plaques, as well as no unintegrated M-MuLV proviral DNA, were found.

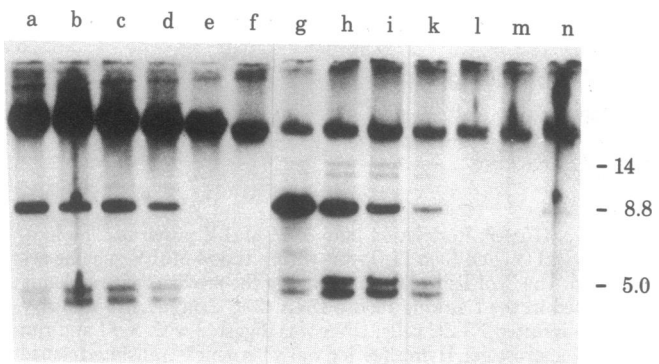

FIG. 2. Unintegrated M-MuLV DNA in infected NIH 3T3 and F9 cells. NIH 3T3 and F9 cells were infected (protocol *ii*) and 10 μg of undigested DNA was run on a 0.7% agarose gel. Hybridization was with nick-translated M-MuLV DNA. Lanes: a–e, DNA from NIH 3T3 cells was prepared 12, 24, 48, and 72 hr and 1 week after infection, respectively; g–l, DNA from F9 cells prepared 12, 24, 36, 48, and 72 hr after infection; f and m, DNA from uninfected NIH 3T3 and F9 cells, respectively; n, thymus DNA from a 60-day-old Mov-1 mouse containing approximately 1 copy of unintegrated M-MuLV DNA per cell (27) (this DNA served as a standard for quantitating unintegrated M-MuLV copies in the other DNAs). Sizes of markers are in kb.

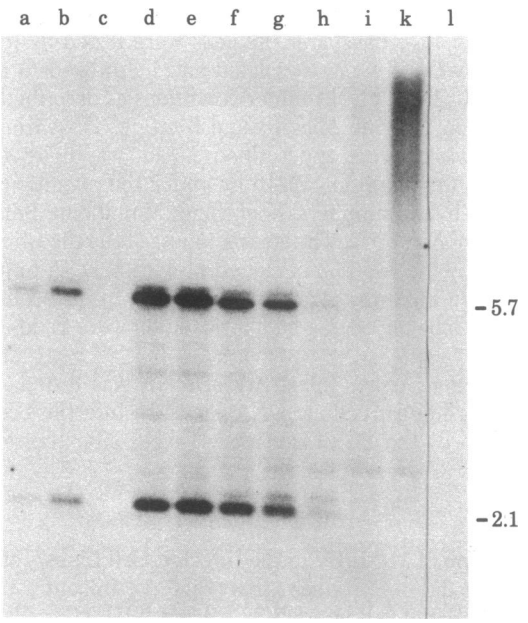

FIG. 3. *De novo* methylation of M-MuLV genomes in infected F9 cells. DNA (5 μg) from M-MuLV-infected cells (protocol *ii*) was digested with *Sma* I (6 units/μg of DNA) for 16 hr and separated on a 0.6% agarose gel. Hybridization was with a M-MuLV-specific cDNA (27). Lanes: a and b, DNA from NIH 3T3 cells 12 hr and 3 weeks after infection; c, DNA from uninfected NIH 3T3 cells; d–i, DNA from F9 cells 12, 24, 36, 48, and 72 hr and 1 week after infection, respectively; k, same DNA as in Fig. 1 *Lower Right*, lane d, containing approximately 100 M-MuLV copies per cell; l, DNA from uninfected F9 cells. Lane a contained less DNA. Weak bands in lanes d–k are due to partial methylation of *Sma* I sites. Size markers are in kb.

DNA isolated from F9 EC cells 4 weeks after infection, which contained an average of 50 copies of integrated M-MuLV per cell and no unintegrated forms, was not infectious. Thus, the infectivity of integrated M-MuLV copies in the infected F9 cells was less than 10^{-8} plaque-forming unit per viral genome (5).

In contrast to these results, infectivity was found in DNA from M-MuLV-infected NIH 3T3 and EB 22/20 cells 72 hr and 3–4 weeks after infection, when only integrated proviral copies were found (compare Fig. 2). DNA from both azacytidine-treated and retinoic acid-differentiated F9 EC cells was not infectious (data not shown). Azacytidine, a potent inducer of endogenous viral genomes (31, 32), was used in the transfection

Table 2. Infectivity of DNA from M-MuLV-infected cells

Time after infection, hr	XC plaques, no./30 μg of DNA		
	F9	NIH 3T3	EB 22/20
12	71 (+)	8 (+)	ND (+)
24	31 (+)	40 (+)	11 (+)
36	7 (+)	79 (+)	ND (+)
48	3 (+)	21 (+)	ND (+)
72	0 (−)		
3–4 wk	0* (−)	52 (−)	12 (−)
Uninfected	0	0	0

DNA was extracted from cells infected with M-MuLV (protocol *ii*) and tested twice in two independent DNA transfection assays (the mean values are shown). The number of proviral copies per cell, determined as in Fig. 1 *Lower Left* was: F9, 30 copies; NIH 3T3 and EB 22/20, 10 copies. (+) or (−), Presence or absence of unintegrated viral copies as determined in Fig. 2; ND, not done.

* DNA was from F9 cells infected according to protocol *i* and carrying 50 proviral copies per cell.

assay to determine if the M-MuLV DNA integrated in the F9 EC cells was potentially infectious. For this, NIH 3T3 receptor cells were treated either before or after transfection with DNA. Treatment of the 3T3 receptor cells with 5-azacytidine resulted in XC plaques being reproducibly found in five independent transfection assays (Table 3). When DNA from uninfected F9 cells was used for transfection, no XC plaques were induced, and treatment of the receptor cells did not increase the efficiency of transfection. From these results we conclude that the integrated M-MuLV DNA in infected F9 cells is potentially infectious and that the lack of infectivity can be correlated with methylation of the proviral genomes.

DISCUSSION

Previous reports have shown that EC cells are nonpermissive for M-MuLV production (15, 16). However, the evidence for the integration and maintenance of M-MuLV in the EC cells was unclear (15, 16, 33). Our results show that up to 100 proviral copies can integrate stably into the DNA of EC cells. Production of infectious virus was not observed and inducing the infected EC cells to differentiate did not result in virus production. However, a small fraction of infected F9 EC cells and their differentiated derivatives were induced to produce virus by treating them with 5-azacytidine (Table 1). In contrast to these results, a differentiated cell line (EB 22/20) and NIH 3T3 fibroblasts were productively infected. These cell lines were found to contain high levels of virus-specific RNA. The level of virus-specific RNA in the infected F9 EC cells was decreased to 1% and its concentration did not change significantly upon retinoic acid-induced differentiation or treatment with azacytidine. Virus-specific protein was not detected in the F9 EC cells or their differentiated derivatives by immunofluorescence or by radioimmunoassay (unpublished data), and thus we do not know if the observed M-MuLV-specific RNA is functional mRNA.

Previous results from this laboratory have shown that a correlation exists among methylation, infectivity, and expression of M-MuLV proviral genomes (5, 6). Here we have shown that the proviral genomes of M-MuLV in F9 EC cells become methylated upon integration, whereas those in EB 22/20 and NIH 3T3 cells remained unmethylated. Thus, F9 EC cells differ from the differentiated cells in being able to methylate proviral genomes *de novo* after integration.

Table 3. Effect of 5-azacytidine treatment of NIH 3T3 receptor cells on infectivity of proviral DNA in F9 cells

	XC plaque-forming units		
DNA	No treatment	Treatment before transfection	After transfection
F9 cells infected with M-MuLV	0/0/0	15/3	5/59/3
F9 cells uninfected	0/0/0	ND	0/0
pMov-3	305	ND	257

DNA (30 μg) from F9 EC cells prepared 4 weeks after infection with M-MuLV (protocol *i*) and containing ≈50 M-MuLV copies per cell was used for the transfection assay. NIH 3T3 receptor cells were treated for 24 hr with 30 μM azacytidine either 48 hr before or 24 hr after addition of the transfected DNA. The cells were tested for production of virus by the XC plaque assay after reaching confluency (6–9 days after transfection). The XC plaques were large and surrounded by secondary plaques characteristic of production of infectious M-MuLV. DNA from uninfected F9 EC cells (30 μg) and from *Eco*RI-cleaved pMov-3 DNA (5 ng), which has previously been shown to be highly infectious (6), were included as a control. ND, not done.

Methylated M-MuLV copies in the F9 EC cells failed to induce XC plaques upon transfection of 3T3 cells (specific infectivity, $<10^{-8}$ plaque-forming unit per proviral copy), whereas the unmethylated proviral genomes in EB 22/20 or NIH 3T3 were at least 100 times more infectious (specific infectivity, 1×10^{-6} plaque-forming unit per proviral copy). Thus these results argue against a *trans*-acting effect in the F9 EC cells that suppresses expression, as has been suggested by others (34).

The noninfectious methylated proviral genomes in the F9 EC cells, however, were potentially infectious and were able to induce XC plaques when the NIH 3T3 receptor cells were treated with azacytidine before or after transfection (Table 3). The observation that pretreatment of the receptor cells rendered the M-MuLV provirus in the transfected F9 DNA infectious cannot be readily explained by assuming that the biological effect of azacytidine is only due to the incorporation of the analog into transfected DNA (35). It appears possible that other, as yet undefined, mechanisms may play a role in the effect of azacytidine.

The results presented here suggest that cells of the early mouse embryo, or at least those from which EC cells are derived, are able to methylate proviral genomes *de novo*. Recent evidence (unpublished data) supports this suggestion: proviral genomes introduced into preimplantation embryos are methylated *de novo* and are not expressed. When M-MuLV was introduced into cells of postimplantation embryos, however, the proviral genomes remained unmethylated and were expressed. Thus, our results suggest that efficient *de novo* methylation may be a characteristic activity for developmentally pluripotent cells and may be less efficient in differentiated cells. So far, *de novo* methylation of DNA has only been observed in differentiated cell lines after long-term cultivation to select cell clones (2, 3, 36).

Recently, a number of different procedures, such as the use of EC cells as vectors (11, 37) and the direct injection of cloned genes into zygotes (29, 38–41), have been used to introduce new genetic information into the germ line of mice. Thus, if retroviruses are a valid model for studying gene expression, our observations suggest that genes introduced into early embryos or EC cells may be suppressed due to DNA methylation. The low transformation frequency of EC cells after DNA transfection, compared to differentiated cells, is in agreement with this hypothesis (37, 42). High transformation frequency, however, has been obtained with another cell line (43).

Finally, if there is a causal relationship between methylation and gene expression in mammals, then the *de novo* methylation activity in EC cells poses some intriguing questions concerning the control of gene expression in these cells.

We thank Dr. Starzinski-Powitz for providing us with the ECMA-7 antibody and E. Hoffmann and K. Haase for excellent technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk. The Heinrich-Pette-Institut is financially supported by Freie und Hansestadt Hamburg and Bundesministerium für Jugend, Familie und Gesundheit.

1. Razin, A. & Riggs, A. D. (1980) *Science* **210**, 604–610.
2. Wigler, M. H. (1981) *Cell* **24**, 285–286.
3. Sutter, D. & Doerfler, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 253–256.
4. Jaenisch, R., Jähner, D., Nobis, P., Simon, I., Löhler, J., Harbers, K. & Grotkopp, D. (1981) *Cell* **24**, 519–529.
5. Stuhlmann, H., Jähner, D. & Jaenisch, R. (1981) *Cell* **26**, 221–232.
6. Harbers, K., Schnieke, A., Stuhlmann, H., Jähner, D. & Jaenisch, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7609–7613.
7. Graham, C. F. (1977) in *Concepts in Mammalian Embryogenesis*, ed. Sherman, M. I. (MIT Press, Cambridge, MA), pp. 315–394.
8. Martin, G. R. (1980) *Science* **209**, 768–776.

9. Evans, M. J. (1981) *J. Reprod. Fertil.* **62,** 625–631.
10. Illmensee, K. & Mintz, B. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 549–553.
11. Stewart, T. A. & Mintz, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6314–6318.
12. Lehman, J. M., Klein, I. B. & Hackenberg, R. M. (1975) in *Teratomas and Differentiation,* eds. Sherman, M. I. & Solter, D. (Academic, New York), pp. 289–301.
13. Sekikawa, K. & Levine, A. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1100–1104.
14. Vasseur, M., Kress, C., Montreau, N. & Blangy, D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1068–1072.
15. Teich, N. M., Weiss, R. A., Martin, G. R. & Lowy, D. R. (1977) *Cell* **12,** 973–982.
16. Speers, W. C., Gautsch, J. W. & Dixon, F. J. (1980) *Virology* **105,** 241–244.
17. Jaenisch, R. & Berns, A. (1977) in *Concepts in Mammalian Embryogenesis,* ed. Sherman, M. I. (MIT Press, Cambridge, MA), pp. 267–314.
18. Bernstine, E. G., Hooper, M. L., Grandchamp, S. & Ephrussi, B. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3899–3903.
19. Martin, G. R. & Evans, M. J. (1975a) in *Teratomas and Differentiation,* eds. Sherman, M. I. & Solter, D. (Academic, New York), pp. 237–250.
20. Fan, H., Jaenisch, R. & MacIsaac, P. (1978) *J. Virol.* **28,** 802–809.
21. Stewart, C. L. (1980) *J. Embryol. Exp. Morphol.* **58,** 289–302.
22. MacPherson, I. (1973) in *Tissue Culture,* eds. Kruse, P. F. & Patterson, M. K. (Academic, New York), pp. 241–244.
23. Strickland, S. & Mahdavi, V. (1978) *Cell* **15,** 393–402.
24. Kemler, R. (1981) *Prog. Dev. Biol.* **26,** 175–181.
25. Knowles, B. B., Pan, S., Solter, D., Linnenbach, A., Croce, C. & Huebner, K. (1980) *Nature (London)* **288,** 615–618.
26. Jaenisch, R. (1979) *Virology* **93,** 80–90.
27. Jähner, D., Stuhlmann, H. & Jaenisch, R. (1980) *Virology* **101,** 111–123.
28. Jones, R. A. & Taylor, S. M. (1980) *Cell* **20,** 85–93.
29. Harbers, K., Jähner, D. & Jaenisch, R. (1981) *Nature (London)* **293,** 540–542.
30. Yoshimura, F. K. & Weinberg, R. A. (1979) *Cell* **16,** 323–332.
31. Niwa, O. & Sugahara, T. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6290–6294.
32. Groudine, M., Eisenman, R. & Weintraub, H. (1981) *Nature (London)* **292,** 311–317.
33. D'Auriol, L., Yang, W. K., Tobaly, J., Cavalieri, F., Peries, J. & Emanoil-Ravicovitch, R. (1981) *J. Gen. Virol.* **55,** 117–122.
34. Gautsch, J. W. (1980) *Nature (London)* **285,** 110–112.
35. Compere, S. J. & Palmiter, R. D. (1981) *Cell* **25,** 233–240.
36. Desrosiers, R. C., Mulder, C. & Fleckenstein, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3839–3843.
37. Pellicer, A., Wagner, E. F., El Kareh, A., Dewey, M. J., Reuser, A. J., Silverstein, S., Axel, R. & Mintz, B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2098–2102.
38. Constantini, F. & Lacy, E. (1981) *Nature (London)* **294,** 92–94.
39. Wagner, E. F., Stewart, T. & Mintz, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5016–5020.
40. Gordon, J. W. & Ruddle, F. H. (1981) *Science* **214,** 1244–1246.
41. Brinster, R. L., Chen, H. Y., Trumbauer, M., Senear, A. W., Warren, R. & Palmiter, R. D. (1981) *Cell* **27,** 223–231.
42. Linnenbach, A., Huebner, K. & Croce, C. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4875–4879.
43. Wagner, E. & Mintz, B. (1982) *Mol. Cell. Biol.* **2,** 190–198.