Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6627–6631, November 1984
Biochemistry

Integration-specific retrovirus expression in embryonal carcinoma cells

(teratocarcinoma/gene expression/vector/provirus expression)

JOE SORGE*, ANN E. CUTTING*, VALERIE D. ERDMAN*, AND JAMES W. GAUTSCH†

*Department of Basic and Clinical Research and †Department of Molecular Biology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Frank J. Dixon, July 2, 1984

ABSTRACT **The undifferentiated embryonal carcinoma (EC) cell line PCC4 aza 1 was infected with a selectable amphotropic retrovirus. Although EC cells are generally refractory to retroviral gene expression, we found that approximately 1 of 5,000–10,000 recombinant proviruses was transcribed in these cells. Cells containing an active recombinant provirus were cloned and expanded. Nucleic acid analysis revealed approximately one intact provirus per cell. Viral RNA levels were different for each cell clone examined [between 0.05% and 0.5% of the poly(A)$^+$ RNA], and transcription was initiated and terminated in the long terminal repeats as in permissive differentiated cells. The cells retained an undifferentiated phenotype and remained positive for stage-specific embryonic antigen 1 and negative for H-2 surface antigens. The cells retained the block to the expression of other proviruses integrated at other chromosomal locations. The data suggest that a *cis*-acting genetic element, at or near the chromosomal site of integration, or mutations in the viral control elements themselves may be responsible for provirus expression in these cells.**

The ability to transfer cloned genes into preimplantation embryos has opened the way for the study of gene expression in the maturing organism. Unfortunately, much of the early work has suggested that many cloned genes do not carry the information necessary to guarantee their proper expression during differentiation (1–4). Similarly, the expression of cloned retroviral genes introduced into preimplantation embryos seems to depend on the chromosomal site of integration (5). Most retroviral genomes introduced in this way are not expressed at all and become highly methylated (5).

Undifferentiated embryonal carcinoma (EC) cells provide a useful model system for studying early gene expression. As in early embryos, retroviral genomes are not expressed in undifferentiated EC cells (6–8). Yet EC cells present no block to viral penetration, reverse transcription, or viral DNA integration (9, 10). Although newly integrated proviruses become methylated in EC cells (10), this modification occurs several days after integration, suggesting that methylation may be a result, not a cause, of provirus inactivity (11, 12). Thus, a provirus that has integrated into a transcriptionally active region of a chromosome might escape inactivation—at least while that region remained transcriptionally active.

With this in mind, we infected undifferentiated EC cells with a selectable recombinant retrovirus (13) and asked if any cells would express the selectable provirus. Rare cells were found that contained an active recombinant provirus.

METHODS

Cells and Viruses. NIH 3T3 and PCC4 aza 1 (PCC4) cells were grown in Dulbecco-modified Eagle medium containing 10% fetal bovine serum. In some cases the medium was supplemented with 0.1 μM molar retinoic acid as indicated in the text. The antibiotic G418 was added at a concentration of 300 μg/ml (14). NIH 3T3 cells were infected with murine amphotropic virus 4070 (15) by transfecting with a plasmid, prA8, containing the nonpermuted amphotropic 4070 genome (Fig. 1). A recombinant amphotropic retrovirus genome, cistorneo (*cis*-acting vector, cistor, and *neo* gene), was constructed by replacing the *gag*, *pol*, and *env* of prA8 with the Tn*5* gene for neomycin resistance (ref. 13; Fig. 1). This recombinant genome replicates in the presence of amphotropic helper virus. Reverse transcriptase levels were measured as described (16).

Infections. Culture supernatant containing defective cistorneo particles was adjusted to 8 μg of Polybrene per ml and passed through a 0.45-μm filter. The filtrate was added to plates of NIH 3T3 or PCC4 target cells for 2 hr. The plates were then supplemented with regular culture media. Certain PCC4 lines containing cistorneo proviruses were superinfected with amphotropic virus 4070 at a multiplicity of infection (moi) of 2.5.

Nucleic Acid Analysis. Routine DNA blotting procedures were used to detect integrated proviruses (17). Briefly, 10 μg of cellular DNA was digested with appropriate restriction enzymes and electrophoresed on 0.8% agarose gels. DNA was denatured and transferred to nitrocellulose paper. The paper was then incubated for 3 days at 42°C in a 50% formamide solution containing a radioactive reverse-transcribed probe. The paper was then washed extensively and exposed to x-ray film (17).

S1 nuclease analysis was performed by using end-labeled probes as described (18–20). Briefly, 30 μg of total cellular RNA was incubated with 0.1 pmol of end-labeled denatured DNA in 80% formamide at 48°C for 3 hr. Hybridization complexes were digested with 300 units of S1 nuclease at 28°C for 1 hr. Digestion products were denatured and electrophoresed on acrylamide/urea gels.

Phage λ Cloning. The protocols described by Maniatis *et al.* were followed (17).

Immunofluorescence. Cells were removed from plates with 0.05% trypsin in 0.4 mM EDTA, washed, and suspended in a dilution of monoclonal antibody at 4°C. Monoclonal antibodies were anti-stage-specific embryonic antigen 1 (SSEA-1) (21), which is specific for undifferentiated EC cells, and SF-1 diluted 1:50, which is histocompatibility complex antigen H-2K^b. After 20 min, cells were washed and exposed to a 1:10 dilution of fluorescein isothiocyanate-conjugated rabbit anti-mouse F(ab')$_2$ fragment (Zymed Laboratories, Burlingame, CA) at 4°C for 20 min. The cells again were washed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: moi, multiplicity of infection; SSEA-1, stage-specific embryonic antigen 1; EC cells, embryonal carcinoma cells; LTR, long terminal repeat; PCC4, cell line PCC4 aza 1; cistorneo, vector containing the gene for neomycin resistance.

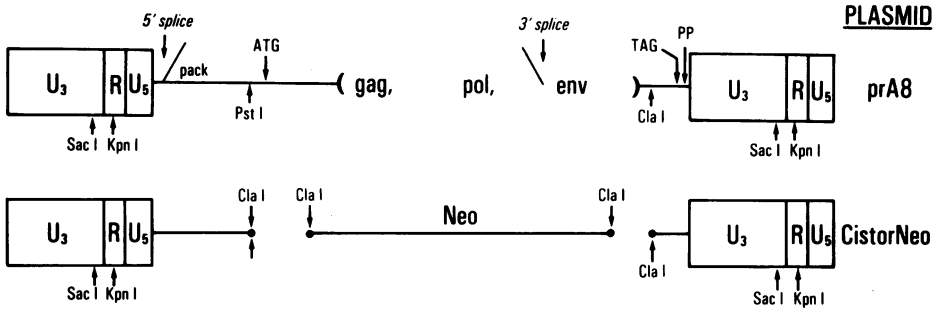

FIG. 1. Maps of viral DNA. Plasmid prA8 contains a nonpermuted clone of amphotropic virus 4070 DNA in pUC9 (only viral DNA drawn). ATG represents the initiation codon for the *gag* gene; *pack*, a *cis*-acting locus necessary for viral RNA packaging; and PP, the polypurine tract. The plasmid cistorneo was derived from prA8 by deleting the region between the *Pst* I and *Cla* I sites shown on the prA8 map (13). The neomycin-resistance gene from Tn*5* was inserted as shown (by converting natural *Hin*dIII and *Sma* I sites to *Cla* I sites). The upstream *Cla* I site of cistorneo corresponds to nucleotide 550 of the RNA transcript.

three times in phosphate-buffered saline and examined by fluorescent epiillumination.

RESULTS

Growth of Amphotropic Retrovirus neo Vector. Replication-defective amphotropic retroviruses containing the selectable neomycin-resistance gene (Fig. 1) were grown by using a helper cell line as described (13). Once removed from the helper cell line, the recombinant viruses can infect target cells and express their integrated genomes but lack the ability to synthesize new virus particles (13).

PCC4 Cells Are Also Resistant to Productive Infection of Amphotropic Retrovirus. Although it has been shown with other murine retroviruses that there is no block to viral penetration or DNA integration in PCC4 cells, we exposed PCC4 cells to either our recombinant virus or to wild-type amphotropic virus at a moi of 2.5 and then performed a DNA blot hybridization to detect integrated proviruses. With use of a neomycin-specific probe for the recombinant retrovirus and an envelope-specific probe for the wild-type amphotropic virus, our data concurred with previous studies (10–12) showing that there was no block to either penetration or integration of the amphotropic viruses in PCC4 cells (data not shown).

cistorneo Is Expressed in G418-Resistant PCC4 Cells. Cells infected with the recombinant viruses were placed in media containing antibiotic G418 at 300 μg/ml 2 days after infection. Cell colonies were counted 10–14 days later. Approximately one EC cell colony was seen for every 5,000–10,000 NIH 3T3 cells colonies, which correlated linearly with virus dilution. G418-resistant PCC4 colonies were cloned to single cells by limiting dilution, and cell clones were expanded. To determine the provirus copy number, cellular DNA was digested with restriction enzymes that released an internal proviral fragment, and blot-hybridization band intensities were compared to lanes containing known amounts of plasmid DNA. Cellular DNA from a G418-resistant clone digested with *Cla* I released the neomycin-resistance gene (1350 bp) from the provirus (Fig. 1). Lanes B and C of Fig. 2 contained recombinant proviral DNA digested with *Cla* I in amounts equivalent to one and five copies per cell, respectively. Lane E contained cellular DNA digested with *Eco*RV, which cleaves once in each long terminal repeat (LTR) and releases a 2350-bp internal fragment. Lanes F and G contained *Eco*RV-cleaved recombinant proviral DNA in amounts approximating one and five copies per cell, respectively. Fig. 2 demonstrates that the G418-resistant PCC4 cells contain roughly one recombinant provirus per cell and that there were no large deletions or insertions in the provirus. Cellular DNAs also were digested with *Eco*RI, which does not cleave the recombinant provirus. A different-size band was seen in each G418-resistant cell line examined, and bands varied in size from 5 to 12 kbp (data not shown). The large size of these bands demonstrated that the recombinant proviruses were integrated in cellular DNA.

Correct Proviral Transcripts Are Made in G418-Resistant PCC4 Cells. Cellular RNA was analyzed by S1 nuclease mapping to determine if the viral transcripts were initiating and terminating in LTRs using the retroviral 5′ initiation and 3′ termination signals. The map in Fig. 3 shows that the DNA probe was 5′-end-labeled at a *Bgl* II site in the neomycin gene, and, after hybridization to cellular RNA and S1 nuclease digestion, transcripts originating in the R region of the LTR should yield a 720-nucleotide band. Lane A demonstrates the presence of this RNA species in the G418-resistant PCC4 cells. The same RNA species is seen with infected G418-resistant NIH 3T3 cells (lane B). A second, larger band can be seen in both lanes as well. Although the larger band could be interpreted as representing transcripts initiating upstream of the LTR, it also could represent upstream hybridization of the probe to the 3′ terminus of viral transcripts.

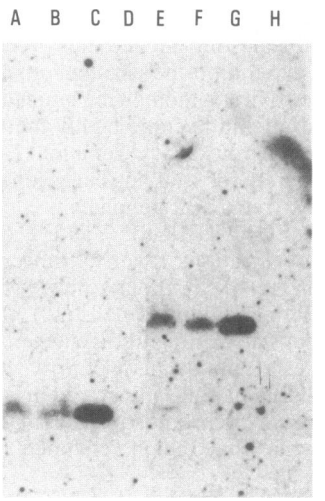

FIG. 2. DNA blot hybridization of chromosomal DNA isolated from PCC4 cells infected with cistorneo using a probe specific for the neomycin-resistance gene. The G418-resistant PCC4 cells were cloned as single cells before expanding the culture to make DNA. Ten micrograms of DNA was digested to completion with *Cla* I, which releases the 1350-bp neomycin gene from the provirus (lanes A–D) or with *Eco*RV, which cleaves once in each LTR and releases a 2350-bp fragment (lanes E–H). Lanes: A and E, DNA from G418-resistant PCC4 cells infected with recombinant virus; B and F, uninfected PCC4 DNA with 25 pg of viral plasmid DNA; C and G, uninfected PCC4 DNA with 125 pg of viral plasmid DNA; D and H, uninfected PCC4 DNA.

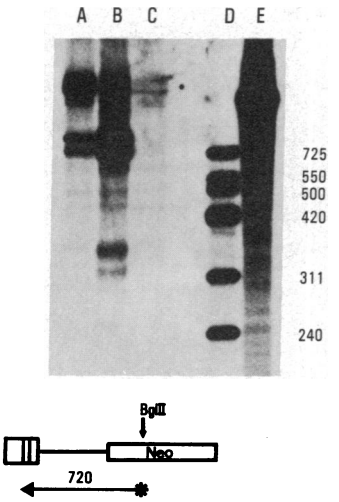

FIG. 3. S1 nuclease analysis. Plasmid DNA containing the neomycin-resistant recombinant provirus, cistorneo, was cleaved with Bgl II and 5'-end-labeled. Cellular RNA (30 μg) was hybridized to 0.1 pmol of labeled DNA in each case. S1 digestion products were electrophoresed on an 8% acrylamide/urea gel. Lanes: A, RNA from G418-resistant PCC4 cells infected with recombinant retrovirus; B, RNA from NIH 3T3 cells infected with recombinant retrovirus; C, RNA from uninfected PCC4 cells; D, *Hinf* I-digested ϕX174 phage size markers; E, DNA probe undigested. Sizes are shown in nt.

Since both bands were seen in G418-resistant PCC4 and NIH 3T3 cells, it most likely represents hybridization to the 3' terminus of viral transcripts rather than aberrant transcription initiation upstream of the LTR. In either case, lane A demonstrates initiation of a major transcript in the cistorneo LTR in PCC4 cells. The bands of highest molecular weight represent S1 nuclease protection due to hybridization of the DNA probe, which includes prokaryotic sequences. These bands are unrelated to viral transcripts. An analogous study of the 3' LTR showed a major transcript terminating in the R region (data not shown).

Superinfection with Retrovirus Does Not Result in Expression of Input Provirus. To test if the expression of the recombinant provirus was due to a *cis*- or *trans*-acting mechanism, a G418-resistant PCC4 line was subsequently infected with wild-type amphotropic virus, 4070A, at an moi of 2.5. Fig. 4, lane A shows an S1 nuclease analysis demonstrating the presence of recombinant viral transcripts but the absence of wild-type amphotropic transcripts in G418-resistant PCC4 cells; lane B demonstrates that only the recombinant provirus and not the wild-type provirus was transcribed in G418-resistant PCC4 cells superinfected with wild-type amphotropic virus. Presence of wild-type provirus in superinfected cells was confirmed, as in Fig. 2, by DNA blotting of several cell generations after infection (roughly 1 provirus per cell). The superinfected cells remained reverse transcriptase-negative, and replication-competent virus could not be recovered from the culture medium of these cells, as assayed by infectivity in NIH 3T3 cells.

Neomycin-Resistant PCC4 Cell Clones Remained Undifferentiated. G418-resistant PCC4 cells containing a single recombinant provirus were removed from G418 selection for 3 weeks. The cells were then placed back into G418 selection medium. There was no evidence of cell death or altered growth properties, suggesting that the mechanism responsible for provirus expression is stably associated with the provirus and is not influenced by the presence or absence of selective pressure.

Morphologically the G418-resistant PCC4 cells appeared to be identical to the parental PCC4 cells and grew at the same rate. Fig. 5 shows that the G418-resistant PCC4 cells retained the SSEA-1 (21). In addition, the cells did not express detectable H-2 surface antigen, another characteristic of undifferentiated cells. In 0.1 μM retinoic acid (22), the G418-resistant cells differentiated, at which point H-2 antigen could be detected on their surface (data not shown). Together, these data suggest that infection of the PCC4 cells with the recombinant retrovirus and subsequent selection in G418 did not alter the undifferentiated "state" of the cells.

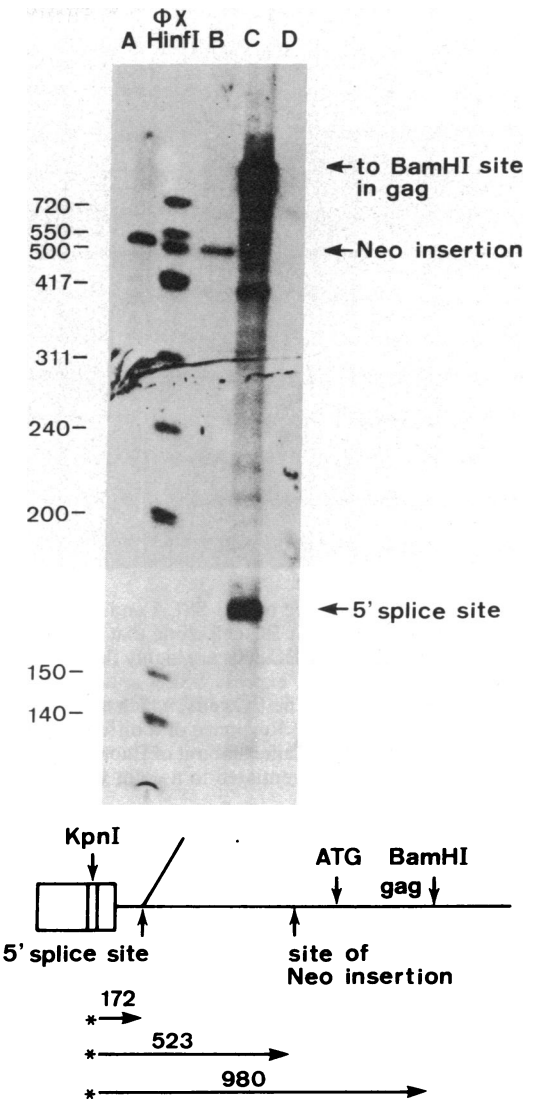

FIG. 4. S1 nuclease analysis. Plasmid DNA containing the upstream LTR and a portion of the *gag* gene (to the *Bam*HI site) was digested with *Kpn* I and 3'-end-labeled. Cellular RNA (30 μg) was hybridized to 0.1 pmol of labeled DNA in each case. S1 nuclease digestion products were electrophoresed on an 8% acrylamide/urea gel. Lanes: A, RNA from a G418-resistant PCC4 line infected with recombinant retrovirus; B, RNA from the same cells, which had been infected subsequently with wild-type amphotropic virus at a moi of 2.5; C, RNA from NIH 3T3 cells infected with amphotropic virus; D, RNA from uninfected PCC4 cells; ϕX Hinf I, *Hinf*I-digested ϕX174 phage size markers.

DNA Cloning of cistorneo Proviruses and Flanking Genes. Cellular DNA was isolated from a G418-resistant cell clone and digested partially with *Mbo* I. DNA $\approx$15,000–20,000 bp long was isolated and ligated to Charon 30/*Bam*HI arms (23). A genomic library of $\approx 1.5 \times 10^6$ plaques was established and screened with a *neo*-specific probe. Six reactive plaques were picked and purified, and the phage DNA was

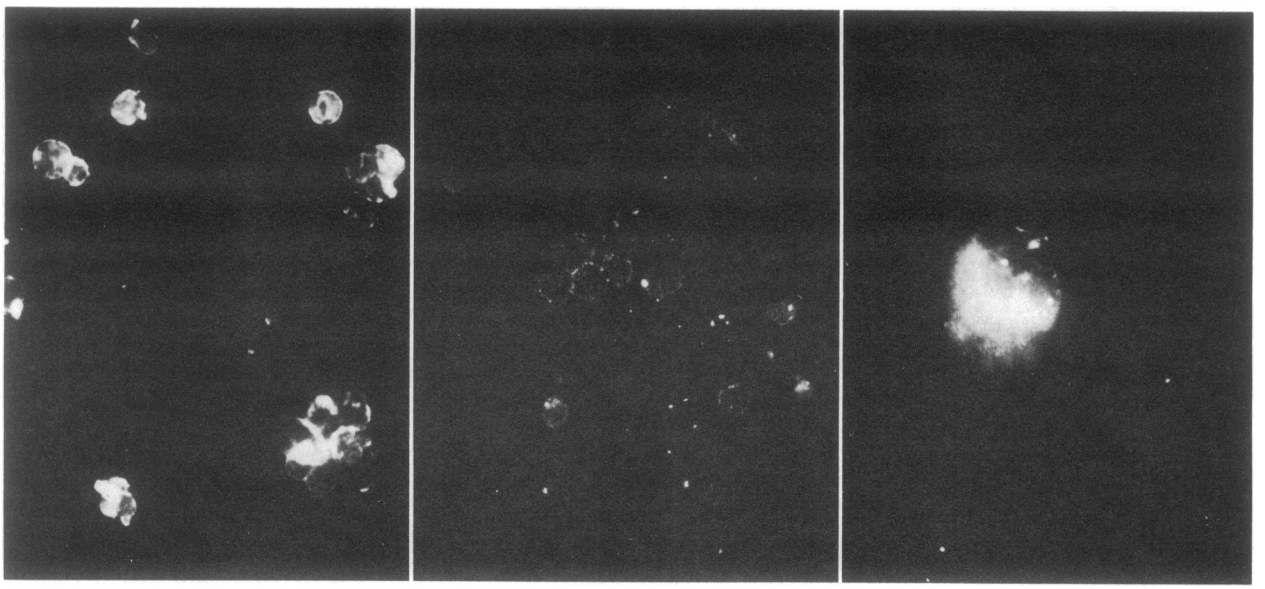

FIG. 5. Immunofluorescence of anti-SSEA and anti-H-2 antisera to surface antigens of EC and differentiated teratocarcinoma cells. (*Left*) Cells from a neomycin-resistant EC cell clone that have been exposed to anti-SSEA antisera and subsequently to fluorescein isothiocyanate-conjugated anti-mouse Ig. The EC cells are highly fluorescent and are typical of undifferentiated EC cells in expressing this surface antigen (21). Differentiated EC cells do not express SSEA antigen and were uniformly not fluorescent (not shown). (*Center*) Undifferentiated EC cells exposed to anti-H-2 antisera. The EC cells, which do not express H-2 antigens, are negative. The EC cells are faintly visible because of a long photographic exposure. (*Right*) Response of a differentiated teratocarcinoma somatic stem cell line, SSA-7, to the anti-H-2 antisera. Several cells in this field displayed the same amount of fluorescence as did the EC cells in *Center* and are not visible in this exposure, whereas the single large fluorescing cell has differentiated to a point where H-2 antigen is expressed on the cell surface.

restriction-mapped to confirm the presence of the integrated cistorneo provirus. The restriction map of each phage λ DNA was unique, although many fragments identical in size were seen.

Transfection of Phage λ Clones. In an effort to determine if the cistorneo proviruses had become favorably mutated themselves, we transfected PCC4 and NIH 3T3 cells with the λ-neo DNA clones. None of the λ-neo DNAs produced G418-resistant PCC4 cells (Table 1). Moreover, the λ-neo-1 DNA did not inhibit the generation of G418-resistant cells when cotransfected along with cistorneo.

Some of the G418-resistant NIH 3T3 cells that had been transfected with λ-neo-1 were superinfected with the amphotropic helper virus. Rescued neomycin-resistant viruses were tested for infectivity in PCC4 and NIH 3T3 cells. No difference was seen between neomycin-resistant virus generated from λ-neo-1 as compared with virus generated by the cistorneo plasmid.

DISCUSSION

Previous attempts to infect EC cells with retroviruses have produced cells containing integrated-viral genomes but no evidence of viral gene expression (6–12). If, in fact, 1 of 5000 integration events does lead to an actively transcribed provirus, why had investigators not detected low levels of virus production from infected EC cells previously? Possible explanations fall into three categories: (*i*) there is something unusual about the virus we are using, (*ii*) the viral titer from the rare producer cells is too low to detect by conventional means, and (*iii*) there is an additional block to retrovirus replication in EC cells subsequent to viral RNA expression. We find it unlikely that there is something unusual about the amphotropic virus and its derivatives. After infection of PCC4 cells with the amphotropic virus 4070, the cells remained reverse transcriptase-negative, and the culture medium remained void of infectious virus particles (assayed on permissive NIH 3T3 cells). This is analogous to what others have found with ecotropic viruses (6–12). The recombinant virus that we have used is simply a deletion mutant of the amphotropic virus 4070, and the transcriptional control elements are identical to those of the parental virus (Fig. 1). Consequently, the absence of detectable wild-type virus production in infected EC cells might then be due to either a low viral titer or a second block to replication. Since the amphotropic viral titer in NIH 3T3 cells is about 1×10^5 particles per ml, infected PCC4 cells might be expected to produce less by a factor of 5,000–10,000, or 10–20 particles per ml. It seems unlikely that these viruses would go undetected, especially when transferred to permissive cells. We are left with the possibility that retrovirus replication might be blocked also at a step subsequent to RNA expression in EC cells.

It is clear, however, that provirus RNA expression in EC cells is quite different from expression in fibroblasts. Although provirus expression in differentiated cells is influenced somewhat by the site of integration (24–27), for the most part the likelihood of individual provirus expression in permissive cells is quite high. Therefore, the retroviral genome must either contain the information necessary for gene expression from most chromosomal locations in permissive cells, or the information necessary to guarantee integration into a transcriptionally active region. In contrast, retroviruses obviously lack the information needed to guarantee expression in EC cells. This also has been demonstrated by using a transient expression assay (28).

Since the G418-resistant PCC4 cells had not become generally permissive to proviral expression, it is likely that a *cis*-acting mechanism activated the expression of the neomycin-resistance gene-containing proviruses. Although no gross mutations were detected, it is possible that a small mutation within the provirus could have affected its ability to be expressed in EC cells. Alternatively, the chromosomal region flanking the provirus could have influenced its expression. In an effort to distinguish between the two possibilities, we cloned the *neo*-containing provirus and flanking regions from a G418-resistant PCC4 cell line. As seen in Table 1, it requires ≈5 μg of cistorneo plasmid DNA to obtain one G418-resistant PCC4 cell. Since the molecular weight of the

Table 1. Transfections with *neo*-containing proviruses

DNA	Amount, μg	Recipient cell type	G418-resistant colonies
cistorneo	1	NIH 3T3	10–20
cistorneo	15	NIH 3T3	10–20
cistorneo	1	PCC4	0
cistorneo	15	PCC4	3
λ-neo-1	15	PCC4	0
λ-neo-2	15	PCC4	0
λ-neo-3	15	PCC4	0
λ-neo-4	15	PCC4	0
λ-neo-1/cistorneo	15/1	PCC4	0
λ-neo-1/cistorneo	15/15	PCC4	1
λ-neo-1	15	NIH 3T3	15–20

Approximately 2×10^6 NIH 3T3 or PCC4 cells were transfected with the DNAs listed. After 48 hr the cells were placed into G418 selection media, and resistant colonies were counted after 10 to 12 days. Each transfection was performed twice.

phage λ DNAs is 7 times that of cistorneo, one might not expect to see colonies produced by 15 μg of λ-neo DNA, unless the λ-neo DNAs have significantly more "activity" on a molar basis. Since the λ-neo DNAs were equally active on a molar basis in NIH 3T3 cells but did not show increased activity in PCC4 cells, we conclude that the neo-containing provirus had not acquired a significant mutation that could be detected easily by transfection.

We also rescued the neo-containing viral genome from NIH 3T3 cells transfected with λ-neo-1 using the amphotropic helper virus. This rescued neo-containing virus was no more active than cistorneo virus in PCC4 or NIH 3T3 cells. Although favorable mutations in the upstream U_3 region of the λ-neo-1 provirus would not have been passed on to the rescued genomes, this test does rule out favorable mutations in the upstream U_5 and downstream U_3 regions.

Although these data do not firmly disprove the hypothesis that the neo-containing proviruses have themselves undergone alterations to favor expression in PCC4 cells, they do make the hypothesis less likely. The hypothesis that flanking cellular control elements influence cistorneo expression is also made more likely by the observation that a portion of nonviral flanking PCC4 DNA isolated from one of the λ-neo clones hybridizes to a highly expressed poly(A)$^+$ RNA species in uninfected PCC4 cells (unpublished data).

Recent evidence suggests that endogenous enhancer elements, or related genetic elements, may be cell-type specific (29, 30). Consistent with our data is the possibility that retroviruses do not carry the information necessary for gene expression in EC cells, but if they happen to integrate within the functional domain of such a tissue-specific embryonic control element they become activated. Further examination of the proviruses with the phage λ clones and/or their flanking DNA sequences may enable isolation of the region of DNA responsible for cistorneo activation in these embryonic cells.

We thank Dr. Ernest Beutler for his support, Dr. Linda Sherman for monoclonal antibody SF-1, and Drs. Davor Soltor and Barbara Knowles for the monoclonal antibody to SSEA-1, and the Basic and Clinical Research Word Processing Center for their efforts. This work was supported by Grants AM25696 and R01 CA36448 to J.S. and by Grant CA35326 and March of Dimes grants to J.G. This is publication no. 3461BCR from the Research Institute of Scripps Clinic (La Jolla, CA).

1. Constantini, F. & Lacy, E. (1981) *Nature (London)* **294,** 92–94.
2. Gordon, J. W. & Ruddle, F. H. (1981) *Science* **214,** 1244–1246.
3. Harbers, K., Jahner, D. & Jaenisch, R. (1981) *Nature (London)* **293,** 540–542.
4. Wagner, E. F., Stewart, T. & Mintz, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5016–5020.
5. Jaenisch, R., Jahner, D., Nobis, P., Simon, I., Lohler, J., Harbers, K. & Grotkopp, D. (1981) *Cell* **24,** 519–529.
6. Teich, N. M., Weiss, R. A., Martin, G. R. & Lowy, D. R. (1977) *Cell* **12,** 973–982.
7. Speers, W. C., Gautsch, J. W. & Dixon, F. J. (1980) *Virology* **105,** 241–244.
8. Peries, J., Alves-Cardoso, E., Canivet, M., Debons-Guillemin, M. C. & Lasneret, J. (1977) *J. Natl. Cancer Inst.* **59,** 463.
9. D'Auriol, L. D., Yang, W. K., Tovaly, J., Cavalieri, F., Peries, J. & Emanoil-Ravicovitch, R. (1981) *J. Gen. Virol.* **55,** 117–127.
10. Stewart, C. L., Stuhlmann, H., Jahner, D. & Jaenisch, R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4098–4102.
11. Gautsch, J. W. & Wilson, M. C. (1983) *Nature (London)* **301,** 32–37.
12. Niwa, O., Yokota, Y., Ishida, H. & Sughara, T. (1983) *Cell* **32,** 1105–1113.
13. Sorge, J., Wright, D., Cutting, A. & Erdman, V. (1984) *Mol. Cell. Biol.* **4,** 1730–1737.
14. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1,** 327–341.
15. Chattopadhyay, S. K., Oliff, A. I., Linemeyer, D. L., Lander, M. R. & Lowy, D. R. (1981) *J. Virol.* **39,** 777–791.
16. Goff, S., Traktman, P. & Baltimore, D. (1981) *J. Virol.* **38,** 239–248.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
18. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
19. Weaver, R. F. & Weismann, C. (1979) *Nucleic Acids Res.* **5,** 1175–1193.
20. Sorge, J., Ricci, W. & Hughes, S. H. (1983) *J. Virol.* **48,** 667–675.
21. Solter, D. & Knowles, B. B. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5565–5569.
22. Strickland, S. & Mahdavi, V. (1978) *Cell* **15,** 393–402.
23. Rimm, D. L., Horness, D., Kucera, J. & Blattner, F. R. (1980) *Gene* **12,** 301–309.
24. Fan, H., Jaenisch, R. & MacIsaac, P. (1978) *J. Virol.* **28,** 802–809.
25. Ringold, G. M., Shank, P. R., Varmus, H. E., Ring, J. & Yamamoto, K. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 665–669.
26. Quintrell, N., Hughes, S. H., Varmus, H. E. & Bishop, J. M. (1980) *J. Mol. Biol.* **143,** 363–393.
27. Berkower, A. S., Lilly, F. & Soeiro, R. (1980) *Cell* **19,** 637–642.
28. Linney, E., Davis, B., Overhauser, J., Chao, E. & Fan, H. (1984) *Nature (London)* **308,** 470–472.
29. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33,** 717–728.
30. Banerji, J., Olson, L. & Schaffner, W. (1983) *Cell* **33,** 729–740.