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Differential Expression of Murine Leukemia Virus Loci in Chemically Induced Hybrid Cells

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The time course of murine leukemia virus production after chemical induction was determined in hamster-mouse somatic cell hybrids containing the xenotropic murine leukemia virus induction locus *Bxv-1* or the ecotropic locus *Akv-2*. By using these hybrids, induction could be studied in the absence of secondary virus spread because xenotropic viruses cannot infect hybrid cells and ecotropic viruses cannot infect hybrids which have lost mouse chromosome 5. After induction, hybrids with *Bxv-1* produced only a transient burst of virus, whereas those with *Akv-2* continued to produce virus for periods in excess of 3 months. The presence or absence of other mouse chromosomes in the hybrid lines did not alter these induction patterns. Thus, endogenous murine leukemia virus loci differ in their response to induction, and both inducibility and the kinetics of virus expression are controlled at or near these proviral loci.

Genetic information for the expression of infectious ecotropic and xenotropic murine leukemia viruses (MuLVs) is present as discrete chromosomal loci (V loci) in many mice. Infectious virus can be induced from the cultured cells of these mice by treatment with chemical inducers, irradiation, or B-cell mitogens (1, 2, 11, 12, 13, 17). Studies describing the kinetics of induced virus production have shown that there are different patterns of expression for xenotropic and ecotropic V loci (3). Both types of V loci produce virus shortly after induction, but xenotropic virus production is transient, whereas a persistent ecotropic virus infection is established. This persistence has been attributed to the susceptibility of mouse cells to infection by the induced ecotropic virus (3, 15).

In this study, hamster-mouse somatic cell hybrids carrying different V loci were used to study virus induction in the absence of any secondary virus spread. The parental E36 Chinese hamster cells are resistant to infectious ecotropic and xenotropic virus. Hamster-mouse hybrids cannot be infected by xenotropic virus (6), and all but one of the hybrids used here lacked mouse chromosome 5, which carries the locus for the ecotropic virus receptor (*Rec-1*) (4).

Hybrid lines from two fusion experiments were selected for induction (8, 9). The BM and BE hybrids were derived from fusions with peritoneal cells of BALB/c mice. VE hybrids were produced with cells of an NFS.*Akv-2* congenic line which carries the AKR ecotropic locus *Akv-2* on the virus-negative NFS/N genetic background. The mouse chromosome content of each hybrid line is given in Table 1. Xenotropic virus inducibility was monitored in BALB hybrids carrying the chromosome 1 *Bxv-1* locus (10). Ecotropic inducibility was monitored in the VE hybrids carrying the chromosome 16 *Akv-2* locus (9). Although BALB also contains an ecotropic virus locus, *Cv*, induction of ecotropic MuLV could not be described independently of secondary virus spread in the BALB hybrids because both *Cv* and the ecotropic receptor locus *Rec-1* map to chromosome 5 (4, 5, 8).

For induction, hybrid cells were treated with 20 μg of 5-iododeoxyuridine (IdU) or 2 μg of 5-azacytidine (5-ACR) per ml for 48 h. After the inducer was removed, cells were maintained with daily fluid changes. Individual plates were tested for infectious ecotropic or xenotropic virus at various times thereafter. Most commonly, plates were exposed to lethal UV irradiation and overlaid with mouse SC-1 cells (for ecotropic virus) or mink lung cells (CCL64) (for xenotropic virus). Four days later, ecotropic virus was scored in the SC-1 cultures by the XC test (16), and xenotropic virus in the harvested mink cultures was titrated by focus formation on the mink S+L− line of Peebles (14). This procedure produced reproducible activation curves. The addition of SC-1 or mink cells provided a sensitive means of amplifying low levels of virus. Uninduced hybrids showed no evidence of spontaneous virus production, even after cocultivation with SC-1 or mink cells for 10 days.

The time course of xenotropic virus activation from cells with *Bxv-1* is shown in Fig. 1. With IdU as the inducer, BALB embryo fibroblasts, the cell line K-BALB (1), and all of the hybrids showed the same general pattern of induced expression. Little or no virus was detected at the time IdU was removed. Virus production reached a peak 3 to 4 days later and then declined to trace levels by 8 to 10 days. The inducer 5-ACR also induced transient virus expression, although it produced a broader activation curve as shown for hybrid BE 8-2 in Fig. 1. Results with both inducers were the same whether xenotropic virus production was scored by the amount of infectious virus in harvested cultures or by plating induced cells as infectious centers (20 μg of mitomycin C per ml, 30 min, 37°C) on mink S+L− cells (data not shown).

These results show that the transient induction of xenotropic virus typical of cultured mouse cells is also characteristic of hybrids carrying little mouse genetic material in addition to *Bxv-1*. Thus, it is unlikely that genes on other mouse chromosomes are either required for induction or alter its time course.

The kinetics of ecotropic virus inducibility from cells with the *Akv-2* locus was quite different (Fig. 2). XC virus appeared shortly after removal of the inducer. The hybrid cells then continued to produce high titers of virus for the remainder of the test period (generally 22 days). Similar results were observed with the inducer 5-ACR, although 5-ACR induced higher titers of virus than did IdU (up to 2 logs) at all time points. When IdU inducibility was monitored as the number of virus-positive infectious centers, the propor-

TABLE 1. Mouse chromosome contents of nine hybrid lines[a]

Hybrid	Presence (P, +) or absence (A, −) of mouse chromosome no.:																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
VE 9-6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
VE 6	+	−	+	−	−	+	+	−	−	−	−	−	−	−	+	+	+	+	+	+
VE 3A	P	A		P	P	P	P							A		P	P	P		P
VE 12		P		P	A	A	P							A		P	P	P		P
BE 7-2	+[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	+[b]	−	−	−	−	−
BM 20	+	−	−	−	−	+	+	−	−	+	−	+	+	−	+	+	+	+	−	+
BM 12H	+	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−	−
BE 8-2	+	−	−	+	−	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−
BM 14	+	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−

[a] The mouse chromosome contents of seven hybrids were determined by the expression of 14 marker loci on different mouse chromosomes (9) and by direct karyology, using Giemsa-trypsin banding and staining with Hoechst 33258 (7). For hybrids VE 3A and VE 12, the presence (P) or absence (A) of specific mouse chromosomes was determined solely by typing for various mouse isozyme markers.

[b] Present as a translocation.

tion of infectious centers in the induced cultures remained constant over a 2-week period (1 to 50 per 10^3 cells).

Surprisingly, this continued production of virus was characteristic of all hybrids whether or not they contained chromosome 5, which carries the ecotropic MuLV receptor locus *Rec-1*. Although virus titers from hybrids without chromosome 5 began to decrease several days after induction, virus production then stabilized at high levels. Additional experiments were done to monitor the extent of virus production over a longer time period. One hybrid which contained only mouse chromosome 16 (VE 9-6) was induced, passaged weekly, and scored for XC virus at each passage. These cells continued to produce high titers of virus over the 90-day test period (10^2 to 10^3 XC plaques per 60-mm plate).

This extended production of ecotropic virus could not be attributed to secondary virus infection. First, all but one of the hybrids (VE 3A) either lacked a karyologically identifiable chromosome 5 or expression of chromosome 5 markers *Pep-7* and *Pgm-1*. Second, no virus could be recovered from cultures of uninduced, chromosome 5-negative hybrids after exogenous infection with high titers (multiplicity of infection, 0.1 to 1.0) of Moloney ecotropic virus and cocultivation with SC-1 cells. Third, no virus was detected 4 days

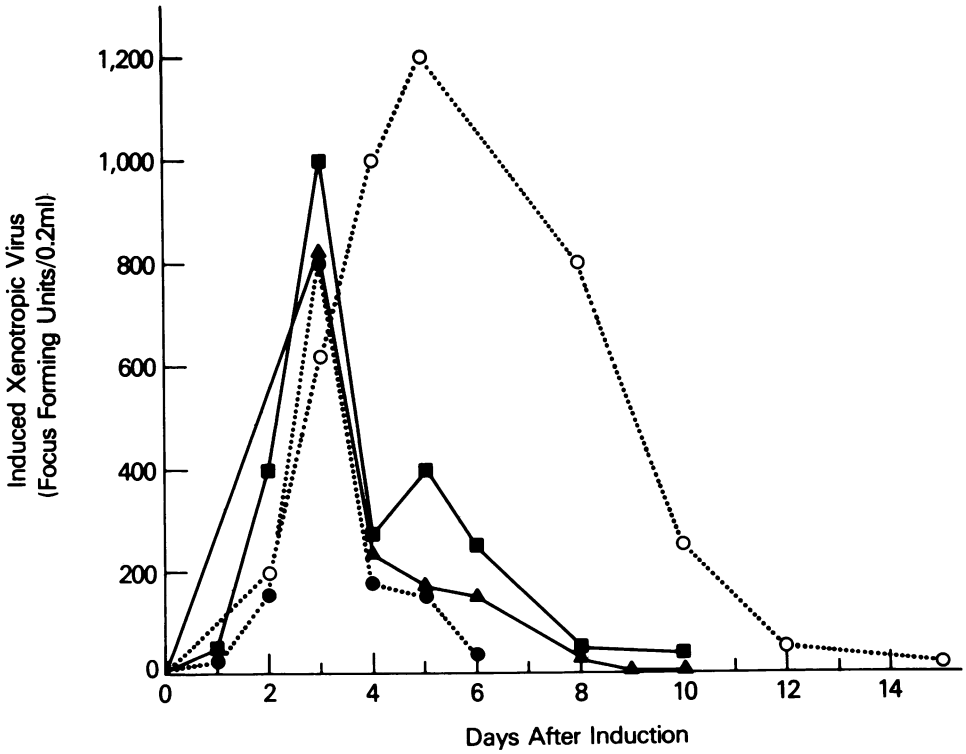

FIG. 1. Time course of xenotropic MuLV induction in mouse and hamster-mouse hybrid cells. At the specified time points after induction with IdU or 5-ACR, cells were lethally irradiated, overlaid with mink lung cells, and tested for xenotropic virus 4 days later. Data shown here represent a composite of three experiments. Hybrids BM 12H, BM 20, and BE 14 (not shown) produced similar curves with IdU, and the curve representing 5-ACR induction of BE 8-2 was typical of all five hybrids. Symbols: ▲, K-BALB; ●, hybrid BE 8-2; ■, hybrid BE 7-2. Solid symbols indicate IdU-treated cells; open circles indicate 5-ACR–treated cells.

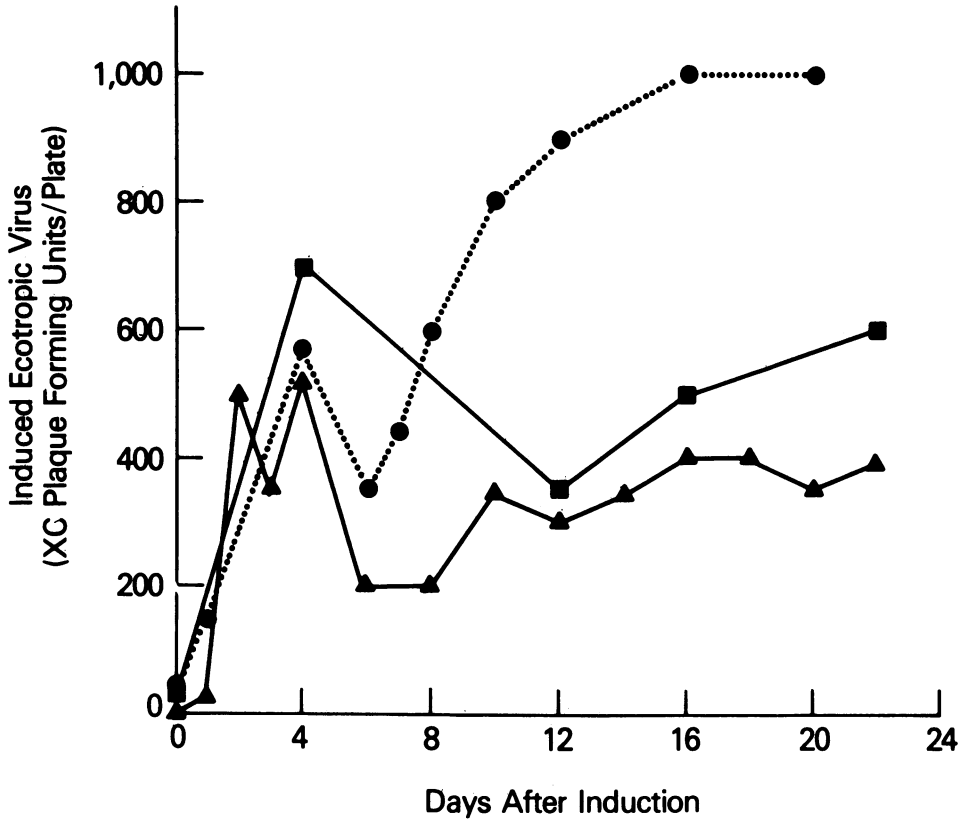

FIG. 2. Time course of ecotropic MuLV activation in somatic cell hybrids. At each time point after induction, cells were lethally irradiated, overlaid with SC-1 cells, and tested for XC virus 4 days later. Data represent a composite of two experiments. Hybrid VE 6 (not shown) produced a curve similar to those of VR 12 and VE 9-6. Symbols: ▲, hybrid VE 9-6; ●, hybrid VE 3A; ■, hybrid VE 12.

after VE 9-6 cells were added to lethally irradiated SC-1 cells chronically infected with AKV ecotropic MuLV. By these criteria, the resistance of chromosome 5-negative hybrids to exogenous virus was complete.

These results indicate that the persistence of virus after the induction of *Akv-2* is not dependent on secondary virus spread, nor is it altered by the presence or absence of any additional mouse chromosomes, including those carrying genes known to affect the in vivo or in vitro spread of virus, e.g., *H-2* (chromosome 17) or *Fv-1* (chromosome 4). These conclusions differ from those of previous studies which attributed the persistence of ecotropic virus in induced mouse cells to secondary infection (3, 15).

This analysis of ecotropic and xenotropic virus induction in somatic cell hybrids shows that the inducibility of both *Bxv-1* and *Akv-2* is controlled strictly by genes at or near the proviral loci. However, the activated *Akv-2* locus differs from *Bxv-1* in that it is not subject to stringent negative control. It remains to be determined whether this difference between *Akv-2* and *Bxv-1* is due to differences in the viral genomes integrated at these loci, to chromosomal position effects, or to linked controlling elements.

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