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Genetics

A mutant murine leukemia virus with a single missense codon in *pol* is defective in a function affecting integration

(transposable elements/viral replication/endonuclease/retrovirus)

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ABSTRACT **We have used site-directed mutagenesis of cloned Moloney murine leukemia virus (MuLV) DNA to define a function encoded in the 3′ region of the viral *pol* gene and required for efficient integration of viral DNA. One mutant, MuLV-SF1, contained a single base substitution (C to T at base 4950) that resulted in an arginine to cysteine change in a region highly conserved among retroviruses. Mutant DNA, introduced into rat cells by cotransfection with a herpes simplex virus thymidine kinase gene (HSV *tk*), directed production of virus particles with reverse transcriptase activity. Infection of cells with these particles led to synthesis of full-length linear and circular forms of unintegrated viral DNA; however, integrated viral DNA was decreased at least by a factor of 10 when examined by DNA hybridization, and the mutant particles were less efficient then wild-type virus at establishing an infection by a factor of at least 300. Pseudotypes formed with the proteins of MuLV-SF1 and the genome of a replication defective marker MuLV, carrying the HSV *tk* gene, were less effective by at least a factor of 100 in producing tk$^+$ colonies than pseudotypes formed with proteins encoded by wild-type virus. When the MuLV-SF1 pseudotypes did produce tk$^+$ cells, most of the proviruses were integrated aberrantly. We conclude that the MuLV-SF1 *pol* gene is defective for a function that is required for normal integrative recombination and dissociable from DNA synthesis.**

The proviruses of vertebrate retroviruses resemble mobile genetic elements in diverse eukaryotic and prokaryotic organisms; a coding domain is flanked by long terminal direct repeats (LTRs), the LTRs end with short inverted repeats, and a short host sequence at the site of insertion is duplicated to flank the element (for review, see ref. 1). Although proviruses differ from most other mobile elements by originating from an RNA template, retroviruses provide unusual biochemical and genetic access to mechanisms by which DNA of this structural type is inserted into host chromosomes.

Efforts to decipher the retroviral integrative mechanism must take into consideration several established features of the retrovirus life cycle (2). Three major species of unintegrated viral DNA appear soon after infection: a linear species with LTRs, each of which is derived from sequences unique to both ends of viral RNA, and two closed circular species, containing either one LTR or two LTRs in tandem. Many sites in host DNA can accommodate a provirus, but the proviral DNA is always joined to host DNA at a position 2 base pairs (bp) from the ends of the LTRs, and the length of the duplicated host sequence (4, 5, or 6 bp) is invariant for each retrovirus (1).

Because the recognition sites in viral DNA and the sizes of host duplications are virus specific, it seems likely that at least some of the factors mediating the precise breakage and reunion events during integrative recombination are virus-encoded. The 3′ portion of the retroviral *pol* gene has been proposed to encode an integrative function, because the products of this region of the Rous sarcoma virus (RSV) genome display endonuclease and DNA binding activities *in vitro* (3, 4). An endonuclease activity has also been detected in a 40-kDa protein apparently encoded by the 3′ end of the murine leukemia virus (MuLV) *pol* gene (5, 6).

We have made site-directed mutations in the predicted endonuclease coding domain of cloned MuLV DNA, and we have isolated and characterized a missense mutant defective in integration. When introduced into cultured cells by DNA transformation, the mutant DNA directs production of virus particles containing reverse transcriptase activity. Upon entering new cells, these particles make linear and circular DNAs of normal size, but little DNA is integrated, and the frequency of productive infection is markedly decreased. Proteins encoded by the mutant genome can form pseudotypes with the genome of a replication-defective MuLV vector carrying a selectable metabolic marker, but viral DNA found in the rare cell biochemically transformed by the phenotypically mixed particles is often improperly integrated. We conclude that the 3′ end of MuLV *pol* encodes a function required for correct integration of retroviral DNA.

MATERIALS AND METHODS

Cells and Viruses. Rat-2 cells (7) were grown in Dulbecco's modified Eagle's medium containing 5% calf serum (GIBCO) and 5% fetal calf serum (GIBCO); NIH 3T3 cells were grown in the same medium supplemented with 10% calf serum. Moloney MuLV and derived mutants were obtained from transfection of the plasmid pZAP onto rat-2 cells. pZAP, which contains an infectious integrated MuLV provirus cloned as an *Eco*RI fragment in pBR322 (8), was a gift of C. Tabin. Virus stocks were harvested 16–48 hr after the medium was changed. Mutant viruses were sometimes concentrated by pelleting at 35,000 rpm in an SW41 rotor for 1 hr, resulting in a loss of some infectivity. Infections with MuLV or MuLV mutants were carried out with 5 ml of virus stock on 100-mm plates of 25%–50% confluent cells in the presence of 8 μg of Polybrene per ml (Aldrich) for 2 hr at 37°C. Thymidine kinase-positive (tk$^+$) transformants were assayed for colony formation in selective medium (hypoxanthine/aminopterin/thymidine; HAT) (9). G418-resistant colonies were selected by incubation in 250 μg of G418 per ml (GIBCO).

Reverse Transcriptase Assays. For conventional reverse transcriptase assays, pelleted virus or virus-containing medium was assayed according to Verma *et al.* (10) using poly(rC)-oligo(dG) (Collaborative Research, Waltham, MA) as exogenous template primer. The rapid reverse transcriptase assays were according to Goff *et al.* (11), with the conventional assay ingredients (10).

Cloned DNAs. pMuLV-KT, containing an integrated Moloney MuLV provirus with an inserted herpes simplex

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Abbreviations: MuLV, murine leukemia virus; LTR, long terminal repeat; HSV *tk*, herpes simplex virus thymidine kinase gene; RSV, Rous sarcoma virus; kb, kilobase(s); bp, base pair(s).

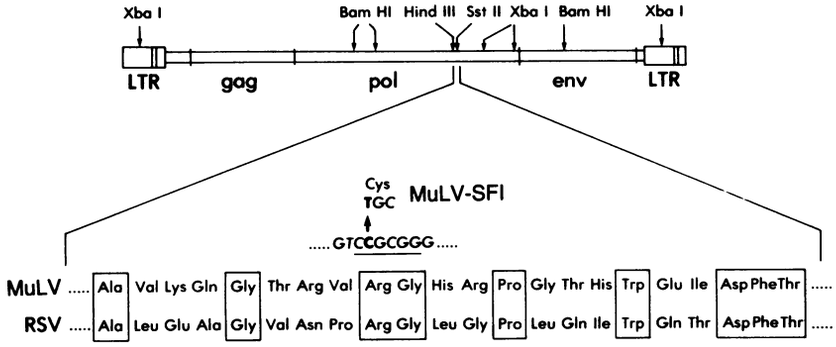

FIG. 1. The MuLV-SF1 mutation. The coding domain and relevant restriction sites in Moloney MuLV DNA are shown (*Upper*). The amino acid codons around the *Sst* II site of MuLV are shown in the enlargement (*Lower*) (21) and compared to the homologous region in the Prague C strain of RSV DNA (23, 24). Identical amino acids are boxed. The single C to T change in MuLV-SF1 is shown in boldface directly above the amino acid sequence of MuLV. The arginine to cysteine codon change is also indicated. The six nucleotides of the *Sst* II site at positions 4949–4954 of the MuLV sequence (21) are underlined.

virus *tk* gene (HSV *tk*) (12), was a gift of R. Weinberg. pMuLV(8.2), containing a permuted clone of circular MuLV DNA with a single LTR (13), was a gift of S. Goff. pAG60, containing a neomycin-resistance gene (14), and pFG5, containing the HSV *tk* *Bam*HI fragment (15), were obtained from A.-C. Garapin.

***In Vitro* Mutagenesis.** Site-directed mutagenesis of the *Sst* II site of pZAP (MuLV) was performed as described by Shortle and Nathans (16). Mutagenized pZAP plasmids were used to transform *Escherichia coli* strain HB101 by a modification of the Hanahan procedure (ref. 17; M. Scott, personal communication). Individual ampicillin-resistant colonies were screened for resistance to *Sst* II and 10 colonies were amplified in preparation for DNA transfections (18).

DNA Transfections. DNA transfections were performed as described by Graham and Van der Eb (19). pFG5 or pAG60 at 100 ng pMuLV-KT, pZAP, or cloned mutagenized derivatives of pZAP at 1 μg, and salmon sperm DNA at 20 μg were cotransfected as calcium phosphate precipitates onto 10^6 rat-2 cells for 4–6 hr.

DNA Sequencing. DNA sequencing was done as described by Maxam and Gilbert (20). The nucleotide sequences of four *Sst* II-resistant clones of pZAP, 40 bp on each side of the *Sst* II site, were determined and then compared to the wild-type MuLV sequence of Shinnick *et al.* (21).

RESULTS

Generation and Initial Characterization of MuLV Mutants. The unique *Sst* II site of the MuLV genome, located in the 3′ region of the *pol* gene (the putative coding region for the viral endonuclease), was the target for the site-directed *in vitro* mutagenesis procedure of Shortle and Nathans (16). Ten independently isolated *Sst* II-resistant plasmids were cotransfected onto rat-2 (TK^-) cells along with the HSV *tk* gene. Each cotransfected plate, after HAT selection, produced 10–50 HAT-resistant (TK^+) colonies; these were grown *en masse* and tested for production of virus particles by assay of the medium for reverse transcriptase. Cells transfected by each of the *Sst* II-resistant mutants produced 20%–70% as much reverse transcriptase as cells transfected by pZAP. A lack of secondary virus spread by noninfectious mutants (see below) may account for the lower levels of reverse transcriptase in medium from cells transfected with certain mutant genomes. When particle production was assayed by dot hybridization for viral RNA (22), the amounts of viral RNA correlated closely with levels of reverse transcriptase activity in medium from cells transfected with mutant and wild-type DNAs; thus, the specific activity of reverse transcriptase in the mutant particles is normal or near normal (data not shown).

The virus particles produced by the transfected cells were next examined for infectivity. Medium from the transfected cells was added to uninfected rat-2 and NIH 3T3 cells, and production of reverse transcriptase-positive particles was measured 3 and 6 days later. Two of the 10 *Sst* II-resistant mutants were fully infectious, whereas the other 8 mutants were unable to establish a productive infection. The defect in infectivity was not corrected by infection at lower tempera-

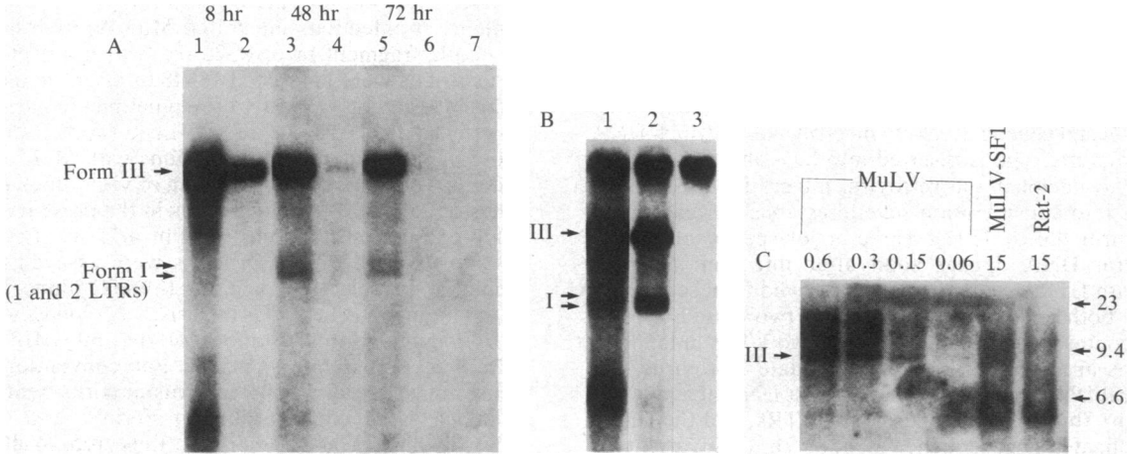

FIG. 2. Synthesis of MuLV and MuLV-SF1 DNA after infection of rat and mouse cells. NIH 3T3 or rat-2 cells were infected with MuLV or MuLV-SF1. Hirt supernatant and high molecular weight DNA were prepared from each culture at various times after infection, and undigested or endonuclease-digested DNA was electrophoresed in 0.8% agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled MuLV (p8.2) DNA (26, 27). (*A*) Hirt supernatant DNA from rat-2 cells infected for the indicated times with virus prepared from 37 ml of MuLV (lanes 1, 3, and 5) or MuLV-SF1 (lanes 2, 4, and 6); uninfected rat-2 cells (lane 7) served as controls. (*B*) Unintegrated viral DNA forms in Hirt supernatant DNA of NIH 3T3 cells 24 hr after infection with 5 ml of a diluted MuLV stock (lane 1) or with 5 ml of concentrated MuLV-SF1 stock (lane 2). Uninfected NIH 3T3 DNA (lane 3) served as a control. (*C*) Viral DNA in *Eco*RI-digested high molecular weight DNAs from Hirt pellets from the same rat-2 cells used in *A*, 72 hr after infection. The amounts of DNA (μg) used in each lane are indicated at the top; DNA from uninfected rat-2 cells served as a control. Size markers to the right are in kb.

tures or prolonged passage, implying that the mutants are not temperature sensitive or unstable (data not shown).

Four Noninfectious Mutants Have the Same Genotype. Four of the cloned DNAs that produced noninfectious virus particles after transfection were sequenced in the region of the mutagenized *Sst* II site. All four clones had the same single point mutation—a C to T transition at base 4950 of the MuLV genome (Fig. 1). The base change converts an arginine codon to a cysteine codon, and the mutant genotype has been designated MuLV-SF1. The arginine residue in the wild-type protein is conserved among several retroviral genomes sequenced in this region to date (24, 25).

Cells Infected by MuLV-SF1 Contain Normal Unintegrated DNA, but Less Integrated DNA. To further define the replication defect in MuLV-SF1, we examined its capacity to synthesize intracellular forms of viral DNA. Rat-2 and NIH 3T3 cells were infected with approximately equivalent amounts of MuLV and MuLV-SF1 (as measured by reverse transcriptase assay), and Hirt supernatant DNA was analyzed 8, 48, and 72 hr after infection for unintegrated viral DNA (26, 27) (Fig. 2*A*). By 8 hr post-infection, both wild-type and mutant virus have synthesized readily detectable levels of full-length linear viral DNA, although the wild-type virus synthesized approximately 10-fold more DNA than did the mutant virus. By 48–72 hr after infection, MuLV-SF1 DNA virtually disappeared, while wild-type MuLV DNA was still present in significant amounts, probably reflecting the ability of wild-type virus to spread by secondary infection.

MuLV-SF1 is able to generate both major species of circular DNA, in addition to full-length linear DNA. This is illustrated by the analysis of Hirt supernatant DNA isolated from NIH 3T3 cells 24 hr after a second set of infections with MuLV and MuLV-SF1 (Fig. 2*B*). Both mutant and wild-type viruses synthesize species of DNA migrating in the positions appropriate for closed circular viral DNA with one or two LTRs. Thus, MuLV-SF1 is able to synthesize the three major forms of unintegrated viral DNA.

We next examined the efficiency with which MuLV-SF1 DNA is integrated into cellular DNA. High molecular weight DNA prepared from the rat-2 cells used for the experiment in Fig. 2*A*, was digested with *Eco* RI (an enzyme that does not cleave Moloney MuLV DNA). With DNA from cells infected for 72 hr with wild-type MuLV, we observed a heavy smear of *Eco*RI fragments >8.8 kilobases (kb), the size of free linear DNA contaminating the Hirt pellet. These heterogeneous large fragments presumably result from *Eco*RI cleavage in cellular DNA adjacent to MuLV proviral DNA at many different sites in host DNA, and they were detected using as little as 0.15 μg of DNA (Fig. 2*C*). In contrast, 15 μg of DNA from cells infected with MuLV-SF1 for 72 hr (when most of the free DNA has disappeared; cf. Fig. 2*A*) yield viral DNA-specific fragments (>8.8 kb) in amounts only slightly greater than is seen with 15 μg of uninfected rat-2 DNA. Similar results were obtained after cleavage with *Bam*HI, which produces internal fragments from MuLV DNA (data not shown). It appears that integration of MuLV-SF1 DNA is at least 90% less efficient than integration of MuLV DNA after correction for differences in efficiency of DNA synthesis during the first 8 hr of infection (Fig. 2*A*).

The Infectivity of Newly Synthesized MuLV-SF1 DNA Is Impaired at Least 300-Fold. Infrequent integration of MuLV-SF1 DNA would not be detected easily in the type of experiment shown in Fig. 2*C*. We therefore devised an infectivity assay to estimate the likelihood of establishing a functional provirus from newly synthesized viral DNA. Rat-2 cells were infected with various amounts of wild-type virus, and a parallel culture was infected with MuLV-SF1. The amount of unintegrated DNA in cells infected for 8 hr by MuLV-SF1 was similar to that in cells infected with about 0.2 ml of wild-type virus stock (Fig. 3*A*). Cells from each of the infected cultures were seeded into a series of 96-well dishes at a density of 4 cells per well, grown to confluence, and tested for production of reverse transcriptase-containing particles (Fig. 3*B*). Most of the wells with cells infected with 0.1 or 0.3 ml of wild-type virus were positive in this assay, as were 5%–10% of wells with cells infected with as little as 0.01 ml of wild-type virus; however, no positive cultures were found in four 96-well dishes of cells (a total of ≈1600 cells) infected with MuLV-SF1 (Fig. 3*B*; data not shown). The few wells that gave ambiguous results were reassayed for reverse transcriptase (and tested for integrated MuLV-SF1 DNA) with negative findings (data not shown). Thus, when normalized for their ability to synthesize unintegrated viral DNA, MuLV-SF1 is less effective than wild-type virus in establishing a productive infection by a factor of at least 300.

MuLV-SF1 Can Complement a Marker Virus for DNA Synthesis, but Not for Correct Integration. We next asked whether the mutant could complement a replication-defective genome carrying a selectable marker; in this way, even rare infections could be scored and the proviral DNA could be analyzed. As a marker genome, we used MuLV-KT, constructed by Tabin *et al.* (12) in which 3.3 kb of MuLV sequences are replaced by the complete HSV *tk* gene in the opposite transcriptional orientation to the LTRs. This genome, defective for *pol* and *env*, can be complemented by wild-type MuLV after DNA transfection, producing a pseu-

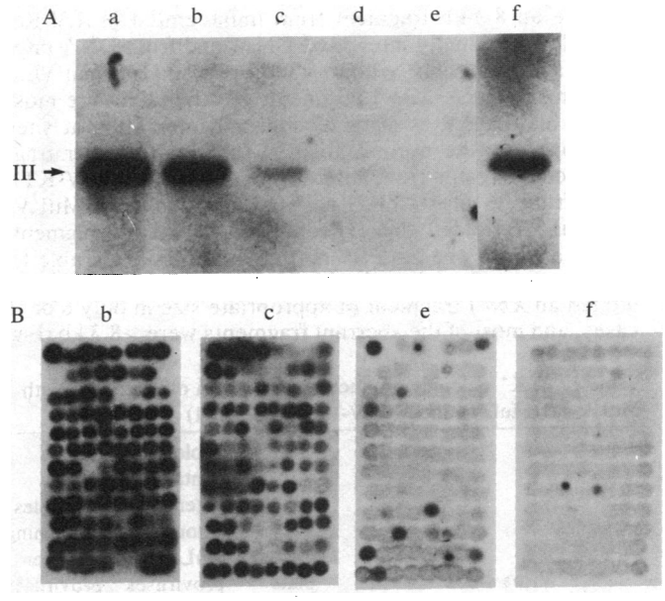

FIG. 3. Efficiency of infection by MuLV-SF1. Rat-2 cells were infected with various quantities of wild-type MuLV stock and 5 ml of 12-fold concentrated MuLV-SF1. Eight hours after infection, Hirt supernatant DNA was prepared from each culture, electrophoresed on 0.8% agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled MuLV DNA (p8.2). Portions of the infected cells were seeded into 96-well plates at a density of 4 cells per well and grown to confluence. Medium from each well (10 μl) was assayed for reverse transcriptase activity according to the procedure of Goff *et al.* (11). (*A*) Measurement of unintegrated viral DNAs in MuLV- and MuLV-SF1-infected cells. Lanes a–e, DNA from cells infected with 1.0, 0.3, 0.1, 0.03, and 0.01 ml, respectively, of MuLV-containing medium; lane f, DNA from cells infected with MuLV-SF1. (*B*) Production of reverse transcriptase by cells infected with MuLV and MuLV-SF1. Panels b, c, and e contain reverse transcriptase assays of 96-well dishes of cells infected with 0.3, 0.1, and 0.01 ml of MuLV-containing medium, respectively; panel f shows a reverse transcriptase assay of cells infected with MuLV-SF1. The cells were derived from samples assayed in lanes b, c, e, and f in *A*.

dotype virus, MuLV-KT(MuLV), that can infect TK$^-$ cells and convert them to a TK$^+$ phenotype (12).

Rat-2 cells already producing MuLV or MuLV-SF1 were cotransfected with MuLV-KT DNA and the neomycin (G418)-resistance plasmid, pAG60 (14). Five to 20 colonies resistant to the antibiotic G418 were pooled on each plate and tested for production of infectious MuLV-KT(MuLV) and MuLV-KT(MuLV-SF1) pseudotypes that could confer HAT resistance upon rat-2 cells. (As expected, medium from all plates was positive for reverse transcriptase, indicating that the cotransfected cells retained and expressed MuLV or MuLV-SF1 DNA.) Stock from the MuLV-KT(MuLV) cultures produced hundreds of TK$^+$ colonies on each infected plate of rat-2 cells, but stocks from 13 separately transfected cultures producing MuLV-KT(MuLV-SF1) yielded only 0–5 colonies per plate (Table 1). No TK$^+$ colonies were obtained after infection of rat-2 cells with MuLV or MuLV-SF1 alone. However, TK$^+$ colonies were infrequently obtained after infection of rat-2 cells with medium from cells transfected with MuLV-KT DNA alone. High molecular weight DNA from these colonies contained no detectable MuLV-KT proviruses (data not shown).

To prove that the TK$^+$ cells had acquired MuLV-KT DNA and to determine whether it was integrated like a conventional provirus under the influence of wild-type and mutant proteins, we examined high molecular weight DNA from cells biochemically transformed with MuLV-KT(MuLV) and MuLV-KT(MuLV-SF1). DNAs derived from individual TK$^+$ colonies were digested with *Xba* I, which cleaves once within each LTR of MuLV-KT DNA, to generate an 8.3-kb fragment from unintegrated MuLV-KT DNA, from normally integrated DNA, and from DNA integrated aberrantly but within ≈300 bp of the normal viral junction sequence. *Xba* I fragments of other sizes are most readily interpreted as signs of aberrant integration at sites >300 bp from the normal junction (although smaller fragments could result from internal deletions in MuLV-KT). DNA from 16 of 16 TK$^+$ colonies infected with MuLV-KT(MuLV) yielded the expected 8.3-kb *Xba* I fragment, detected with probe for HSV *tk* (Fig. 4*A*, lanes a–e; Table 1). In contrast, cells infected with MuLV-KT(MuLV-SF1) exhibited an *Xba* I fragment of appropriate size in only 6 of 16 cases, and most of the aberrant fragments were >8.3 kb (Fig. 4*A*, lanes f–j; Table 1), indicating that the majority of MuLV-KT proviruses were abnormally integrated. We also examined the status of helper virus DNA in the TK$^+$ colonies. *Xba* I cleavage of properly integrated (or unintegrated) MuLV generates two major viral internal fragments of 2.4 and 5.5 kb (cf. Fig. 1). Analysis of DNA from MuLV-KT(MuLV) and MuLV-KT(MuLV-SF1)-infected cells indicated that all 16 MuLV-KT(MuLV)-infected cells contained apparently normal MuLV DNA (Fig. 4*B*, lanes a–e; Table 1), while none of the 16 MuLV-KT(MuLV-SF1)-infected colonies contain apparently normal MuLV-SF1 DNA (Fig. 4*B*, lanes f–j; Table 1). In sum, these results demonstrate that MuLV-SF1 is deficient in a function required for correct integration of its own DNA or the DNA of the pseudotyped genome MuLV-KT.

Table 1. TK$^+$ colonies produced by infection of rat-2 cells with MuLV-KT(MuLV) and MuLV-KT(MuLV-SF1)

Virus stock	TK$^+$ colonies/plate	Colonies containing potentially normal MuLV-KT proviruses	Colonies containing helper proviruses
MuLV-KT(MuLV) [4]	300–600	16/16	16/16
MuLV-KT(MuLV-SF1) [13]	0–5	6/16	0/16
MuLV	0	—	—
MuLV-SF1	0	—	—
MuLV-KT(−)	0–1	—	—

Virus-containing medium (5 ml), with similar amounts of reverse transcriptase activity for each infection, was used to infect 10^6 rat-2 cells. HAT selection was begun 24 hr later, and TK$^+$ colonies were scored after 10 days. High molecular weight DNA from individual TK$^+$ colonies was digested with *Xba* I and examined for 8.3-kb fragments hybridizing to HSV *tk* probe, indicating potentially normal MuLV-KT proviruses. These DNAs were also examined for 2.4- and 5.5-kb fragments hybridizing to MuLV probe, indicative of potentially normal MuLV (or MuLV-SF1) proviruses. See Fig. 4 and text for details. Numbers in brackets represent numbers of tested virus stocks of each type. Each stock was derived from MuLV- or MuLV-SF1-producing rat-2 cells independently transfected with pAG60 and pMuLV-KT.

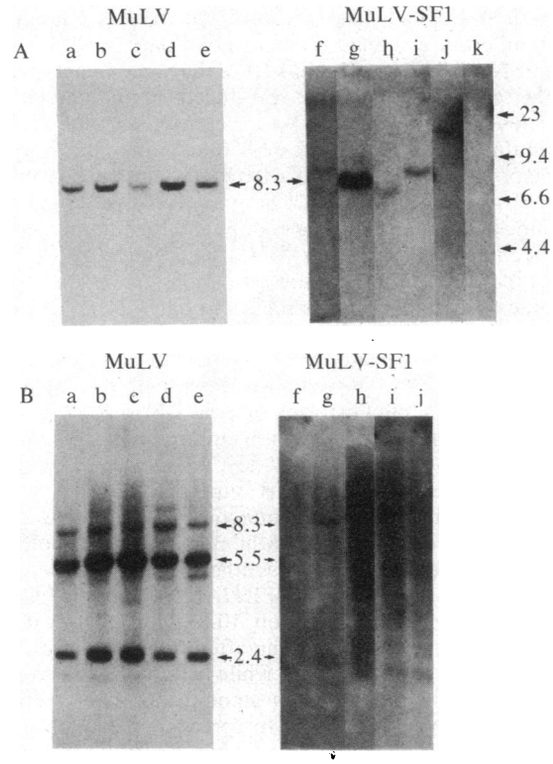

FIG. 4. Analysis of MuLV-KT and helper proviruses in TK$^+$ cells infected with MuLV-KT(MuLV) and MuLV-KT(MuLV-SF1). TK$^+$ colonies were generated by infection with MuLV-KT(MuLV) (lanes a–e) or MuLV-KT(MuLV-SF1) (lanes f–j). DNA from each colony (≈10 μg) was cleaved with *Xba* I, electrophoresed in 0.7% agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled HSV *tk* probe (pFG5) in *A* or ^{32}P-labeled MuLV (p8.2) DNA in *B*. DNA from uninfected rat-2 cells served as a control in lane k of *A*; rat-2 DNA analyzed with MuLV probe in a separate experiment produced a pattern indistinguishable from that seen in lanes h–j of *B* (not shown). Differences in intensity of endogenous background bands of lanes f–j are due primarily to different exposure times.

DISCUSSION

We have generated a missense mutation in the region of the MuLV *pol* likely to encode p40^{pol}, a protein associated with DNA endonuclease activity (5, 6). The lesion in MuLV-SF1 makes a single nonconservative amino acid alteration (arginine to cysteine) in the center of a region of *pol* that shows considerable homology among retroviruses and may, therefore, be functionally important (refs. 24 and 25; Fig. 1). The mutation renders the genome nonconditionally defective for replication, and the available evidence strongly indicates a specific impairment in the integrative mechanism. (*i*) Late events in the virus life cycle seem to be unaffected, because

cells transfected with mutant DNA produce virus particles in near normal amounts, as measured by reverse transcriptase and RNA hybridization assays (unpublished data). (*ii*) The mutant can conduct the early events of the life cycle—absorption, penetration, and synthesis of viral DNA—because unintegrated DNAs of normal size and structure are made (Fig. 2*B*). In cells infected by mutant particles, we observed 1/5th to 1/10th as much free DNA as expected on the basis of reverse transcriptase assays *in vitro*, implying that the DNA synthetic mechanism may be modestly unstable or inefficient (Fig. 2*A*). (*iii*) DNA synthesized during infection by mutant virus is less likely to establish a productive infection than is DNA made during wild-type infection by at least a factor of 300 (Fig. 3). Our inference that the failure to replicate is due to impaired integration is supported by the significant decrease in viral sequences in high molecular weight DNA from mutant infected cells (Fig. 2*C*). More compelling, in cells metabolically selected for the presence of viral DNA synthesized by a MuLV-SF1 pseudotype, we found aberrantly integrated species in most instances (Fig. 4*A*). Finally, *pol* mutants generated in other laboratories appear to have a similar phenotype to MuLV-SF1 (34; T. Shinnick, A. Panganiban, and H. Temin, and D. Grandgenett, personal communications). Thus, we conclude that MuLV-SF1 is unable to perform correct integration of viral DNA.

The MuLV *pol* gene encodes at least two major proteins, an RNA-dependent DNA polymerase (80 kDa) and a DNA endonuclease (40 kDa). We believe the MuLV-SF1 mutation affects $p40^{pol}$ rather than reverse transcriptase, for several reasons. (*i*) The position of the lesion in MuLV-SF1, 930 nucleotides upstream of the 3′ end of *pol* and 2532 nucleotides downstream from the sequence encoding the amino terminus of the reverse transcriptase (28), is likely to reside 3′ of the coding domain for $p80^{pol}$. (*ii*) The MuLV-SF1 DNA polymerase can synthesize the major forms of unintegrated viral DNA, implying that the polymerase function is genetically dissociable from other *pol* functions, as previously illustrated by RSV *pol* mutants with unaffected endonuclease activity (29). (*iii*) Antibodies directed against a synthetic peptide derived from the RSV sequence homologous to the region spanning the MuLV-SF1 mutation precipitate RSV $pp32^{pol}$, a protein with associated endonuclease activity (30).

It is probable that the MuLV-SF1 lesion defines a function required at one or more steps beyond DNA synthesis. For correct integration, several distinct events are likely to occur: (*i*) strand scissions in viral DNA, two nucleotides from the extremities of the LTRs; (*ii*) staggered breaks in chromosomal DNA; (*iii*) alignment and ligation of the ends of viral and cellular DNA; and (*iv*) repair and ligation of the incomplete strands of host DNA generated by staggered cleavage. Panganiban and Temin have recently documented that inverted repeats are necessary for integration (31), and they have shown that the small region surrounding the junction of two LTRs in circular molecules can determine the viral site of integrative recombination (32). Hence, proper termination of DNA strands and circularization without loss of essential sequences, producing molecules with two intact LTRs in tandem, appear to be prerequisites to correct integration that could be affected by the mutation in MuLV-SF1. We believe it is more likely that scission of host or viral DNA or the juxtaposition and joining of ends are impaired by mutations in the region altered in MuLV-SF1. Recent evidence that a RSV endonuclease activity can preferentially nick single-stranded LTR DNA within the inverted repeat sequence *in vitro* (33) is consistent with the possibility that a mutation in the MuLV endonuclease domain might prevent efficient site-specific opening of the integrative precursor.

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