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Infectivity and structure of molecular clones obtained from two genetically transmitted Moloney leukemia proviral genomes

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ABSTRACT

The Mov-2 and Mov-10 substrains of mice, each carrying Moloney leukemia virus (= M-MuLV) in their germ line at the Mov-2 and Mov-10 locus, respectively, do occasionally at a later age (Mov-2) or not at all (Mov-10) activate infectious virus. The M-MuLV proviruses with flanking mouse sequences corresponding to the Mov-2 and Mov-10 locus, respectively, were molecularly cloned. Restriction enzyme analysis revealed no major deletions or insertions in the proviral genomes of the Mov-2 and Mov-10 locus. Both cloned DNAs induced XC plaques in a transfection assay. The specific infectivity, however, was very low and 3T3 cells transfected with the Mov-2 or Mov-10 clone did not produce infectious virus. Removing part of the 5' cellular sequences from the Mov-10 clone did not increase the infectivity. The results suggest that the M-MuLV integrated at the Mov-2 and Mov-10 locus carry a mutation which prevents synthesis of infectious virus but permits XC plaque induction by partial genome expression or synthesis of non-infectious particles.

INTRODUCTION

Many mammalian species have been shown to carry the genetic information for retroviruses within their chromosomal DNA. Expression of proviral genomes may contribute to the induction of neoplastic disease (1,2). To study the mechanisms that regulate the activation of endogenous viruses in mice, we have inserted the Moloney leukemia virus (= M-MuLV) into the germ line of mice. Different substrains of mice have been derived which carry the M-MuLV genome at different chromosomal positions (= Mov-loci) and display a distinct pattern of virus activation (3-5).

In order to study the structure of the inserted proviruses, we decided to molecularly clone the virus genome and its flanking mouse sequences from different Mov-mice. Our initial studies were done with the Mov-3 substrain, which expresses virus prior to

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birth, leading to viremia and subsequent development of leukemia (4). The Mov-3 locus was molecularly cloned and shown to be highly infectious in a transfection assay, thus establishing a direct correlation between virus expression in the animal and expression of the cloned DNA in vitro (6).

The present work was performed to study the molecular structure of the integrated proviruses from mice that do not become viremic. The Mov-2 and Mov-10 substrains of mice used in the experiments reported in this paper were derived from 4-16 cell embryos exposed in vitro to M-MuLV. Mov-10 mice do not express virus during their lifetime. In contrast, about 20% of animals carrying the Mov-2 locus develop viremia at 2 to 4 months of age (4).

In order to study the structure of the Mov-2 and Mov-10 locus, the respective viral genomes and the flanking mouse sequences were molecularly cloned. The clones were characterized by restriction enzyme analysis and tested for infectivity in a transfection assay.

MATERIALS AND METHODS

Mice. The derivation of mice which are heterozygous at the Mov-2 and Mov-10 locus has been described previously (4,5). Mov-2 mice were derived from ICR mice, whereas Mov-10 mice were derived from 129 mice. Mice homozygous at the respective Mov-locus were derived by mating heterozygous animals and screening for offspring carrying two M-MuLV copies per diploid genome equivalent in DNA from liver biopsies (7; R. Jaenisch et al., in preparation).

Enzymes. Restriction enzymes were obtained from New England Biolabs or Boehringer, Mannheim, and reaction conditions used were those recommended by the suppliers. Phage T4 DNA ligase was purchased from New England Biolabs. E. coli DNA polymerase and DNase I were obtained from Boehringer, Mannheim.

Restriction endonuclease analysis. The restriction endonuclease-digested DNAs were fractionated on agarose gels ranging from 0.5 to 1.0% in a buffer containing 40 mM Tris-acetate, pH 8.0, and 1 mM EDTA. The fragments were identified either by ethidium bromide staining or by the Southern blotting technique

(8). Hybridization to nitrocellulose-bound DNA was performed as described previously (9) using either a representative M-MuLV cDNA or a cDNA specific for M-MuLV (9). In some experiments a representative M-MuLV probe was used which was obtained by nick-translation of an M-MuLV containing plasmid kindly provided by Drs. S. Goff, E. Gilboa and D. Baltimore. Nick-translation was performed essentially as described by Maniatis et al. (10). The M-MuLV containing plasmid (0.2 to 0.4 µg) was nick-translated in a total volume of 20 µl in the presence of 100 µC α-^{32}P-labeled dCTP (2-3000 Ci/mmol; Amersham) in a buffer containing 50 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 10 mM mercaptoethanol and 20 µM each of unlabeled dGTP, dATP and TTP. The reaction was started by adding 2 µl of a DNase I solution (0.02 µg/ml) and 4 units of DNA polymerase. After incubation at 15°C for 1-2 h the reaction was stopped by adding 1 µl 0.5 M EDTA and 1 µl 10% SDS followed by filtration through a Sephadex G 50 column. The specific activity of the probe was usually about 6 x 10^8 dpm/µg DNA. Cloned fragments obtained from the integrated proviruses were radioactively labeled in the same way.

Isolation of DNA and enrichment of M-MuLV proviral sequences. High molecular weight DNA was extracted from the livers of 2-month-old Mov-2 or Mov-10 mice as described previously (9). DNA was digested with EcoRI, extracted with phenol, and precipitated with ethanol. Five mg of EcoRI-digested DNA were fractionated on a 0.5% preparative agarose gel as described (6). Aliquots of the fractionated DNAs were electrophoresed, transferred to nitrocellulose filters according to the method of Southern (8) and hybridized to a ^{32}P-labeled cDNA that was specific for M-MuLV (9). Fractions that hybridized to the M-MuLV cDNA were used for molecular cloning.

Molecular cloning. The EK2 certified vector λ Charon 4A and host E. coli DP50 supF were obtained from F. Blattner. Purification of λ 4A arms was performed after digestion with EcoRI by centrifugation in an NaCl gradient (11). Partially purified proviral DNA and vector DNA were adjusted to 46 µg/ml (Mov-10) or 56 µg/ml (Mov-2) and 120 µg/ml, respectively, and ligated for 24-36 h at 8°C in a total volume of 15 µl of a solution containing 66 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 10 mM

ATP, 10 mM DTT, 100 µg/ml gelatine and 300 units of T4 DNA ligase (New England Biolabs). The resultant recombinant DNA was packaged in vitro into phage particles (12). Lysogenes used for preparation of packaging mixtures, BHB 2688 and BHB 2690 were generously provided by B. Hohn. Generally, the efficiency of packaging varied from 2-10 x 10^5 PFU/µg of mouse DNA. The recombinant phages were plated onto DP50 supF in a 20 x 20 plastic dish (Nunc) so that about 100,000 plaques would arise. Nitrocellulose replicas of the petri dishes were prepared by the method of Benton and Davis (13) and hybridized to a ^{32}P-labeled representative M-MuLV probe as described (9).

Plaques that showed hybridization to the M-MuLV probe were further analyzed. For this purpose, 2 ml phage lysates were prepared from the unpurified plaques and the recombinant phage DNA was analyzed by restriction enzyme analysis with SacI and by filter hybridization. SacI produces fragments characteristic of M-MuLV (5,14). Plaques containing M-MuLV were further purified by additional cycles of plaque purification until the number of recombinant phage was greater than 95%. Bulk preparation of recombinant phage was performed by the protocol of Blattner (Blattner et al., Charon Phage Protocols, 1978).

The M-MuLV proviral DNA insert was recloned into the EcoRI site of plasmid pBR322 and grown in E. coli X 1776. Subfragments of the proviral DNA and its flanking mouse sequences were subcloned in pBR322 and amplified in E. coli HB 101.

Construction and growth of recombinant plasmids and phages were conducted under L2/B2 conditions as specified by the Zentrale Kommission für Biologische Sicherheit of the F.R.G.

Transfection assays. Infectivity of DNA was tested by a modification (15) of the calcium phosphate coprecipitation method of Graham and van der Eb (16). Thirty µg of high molecular weight DNA from liver of BALB/c mice were used as carrier DNA for transfection of NIH 3T3 cells with cloned DNA. Infectious centers were scored by the XC plaque assay (17) one week after transfection.

RESULTS

Cloning of M-MuLV provirus integrated at the Mov-2 and Mov-10 locus. The integrated M-MuLV genomes in Mov-2 and Mov-10 mice have previously been mapped by restriction enzyme analysis on a 21 and 17.5 kb long EcoRI fragment, respectively (4,5). In order to enrich for proviral DNA containing fragments, EcoRI digested liver DNAs from Mov-2 and Mov-10 mice were fractionated on a preparative agarose gel. The fractions containing the provirus were identified by hybridization (see Materials and Methods). We estimate that a 20- to 30-fold enrichment of DNA was achieved by this procedure.

The enriched proviral DNA fragments were ligated to the arms of λ4A, packaged in vitro and plated on E. coli DP50 supF. A total of about 500,000 plaques were screened by hybridization using a representative M-MuLV probe. Because a representative M-MuLV probe crossreacts considerably with endogenous murine C type viruses (9,18), phages carrying the M-MuLV genome had to be identified. For this, positive clones were picked and hybrid phage DNA was prepared from small lysates and analyzed by digestion with the restriction enzyme SacI as described in Materials and Methods. DNA from one out of 54 Mov-2 and 8 out of 15 Mov-10 plaques contained the SacI fragments characteristic of the M-MuLV genome (5,14). The results indicate that in the case of Mov-2 about 0.5% and in the case of Mov-10 about 50% of the plaques that hybridized with a representative M-MuLV probe contained hybrid phage DNA with a complete M-MuLV genome. We assume that the remaining recombinant phages contained DNA from other endogenous viruses that share sequence homologies with M-MuLV or represent M-MuLV genomes that were rearranged during cloning. DNA was isolated from the purified plaques and the DNA insert containing proviral and mouse sequences was separated from the vector arms by EcoRI digestion and recloned in the plasmid pBR322. All further experiments described were performed with DNA from the plasmid clones designated as pMov-2 and pMov-10, respectively.

Restriction enzyme analyses of the recombinant M-MuLV clones. Figure 1 shows a restriction endonuclease map of the M-MuLV provirus integrated at the Mov-2 and Mov-10 locus, re-

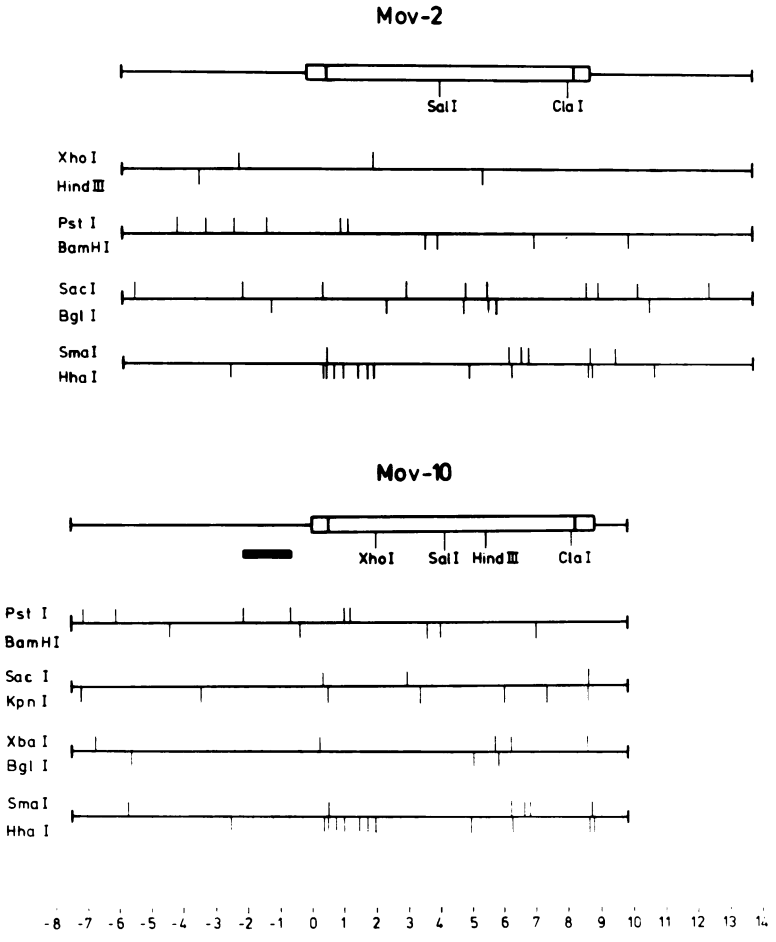

Figure 1. Restriction enzyme maps of the cloned EcoRI fragments of the Mov-2 and Mov-10 locus, respectively. The M-MuLV provirus spanning from 0 to 8.8 kb on the map is indicated by a double line. Adjacent cellular sequences are indicated by a single line. The map was derived by cleavage of the cloned DNA with various restriction enzymes (singly or in combinations) followed by agarose electrophoresis. The total fragment patterns were determined by ethidium bromide staining, and those which contained viral sequences were identified by blot-transfer hybridization with M-MuLV cDNA. The black bar indicates a DNA fragment specific for the Mov-10 locus (see Fig. 4). The HhaI sites in the region 0 to 2.5 of Mov-10 have not been accurately mapped and are therefore indicated by dotted lines.

spectively. The length of the Mov-2 EcoRI fragment is 19.6 kb and that of the Mov-10 fragment 17.3 kb, values slightly smaller than previously estimated (4,5). The Mov-2 provirus is flanked by 5.8 kb of mouse DNA at the 5' end and by 5.0 kb of mouse DNA at the 3' end. The Mov-10 provirus is flanked by 7.5 kb at the 5' end and by 1 kb at the 3' end. The structure of the flanking sequences is different in Mov-2 and Mov-10 DNA, indicating different integration sites.

Analyses with several restriction enzymes were performed to compare the proviral structures of the Mov-2 and Mov-10 locus with each other and with the proviral structure of the Mov-3 locus, which has previously been cloned and shown to be highly infectious (6). Viral specific fragments were detected after blot transfer by hybridization to M-MuLV cDNA. The fragment patterns obtained with several restriction enzymes are shown in Figure 2. No differences in the sizes of the internal M-MuLV fragments of the three clones were observed. The weak bands seen in Figure 2, which are of different length in each lane, represent fragments that contain short sequences from the long terminal repeat of M-MuLV plus adjacent mouse sequences which differ in length in each cloned Mov-locus. Using a number of additional enzymes (data not shown), restriction maps of the proviral genomes were derived (Fig. 1) which were identical for pMov-2 and pMov-10 and are in agreement with the previously published map of pMov-3. These results exclude major differences such as insertions and deletions in the cloned proviral DNAs.

pMov-2 and pMov-10 represent the endogenous M-MuLV in the Mov-2 and Mov-10 substrains of mice. The structures of the M-MuLV-specific EcoRI fragments from liver DNA of Mov-2 and Mov-10 mice (= genomic Mov-2 and Mov-10 DNA) and the respective cloned EcoRI fragments of pMov-2 and pMov-10 were compared by restriction enzyme analysis with PstI, BamHI and HindIII. A cDNA probe specific for M-MuLV (9) was used to detect the virus-specific fragments. Figure 3 shows that the restriction fragments obtained from the genomic DNAs and the cloned DNAs are identical in length and are in agreement with maps of the Mov-2 and Mov-10 locus shown in Figure 1. BamHI cleaves twice within the proviral DNA (cf. Fig. 1) giving rise to three fragments that hy-

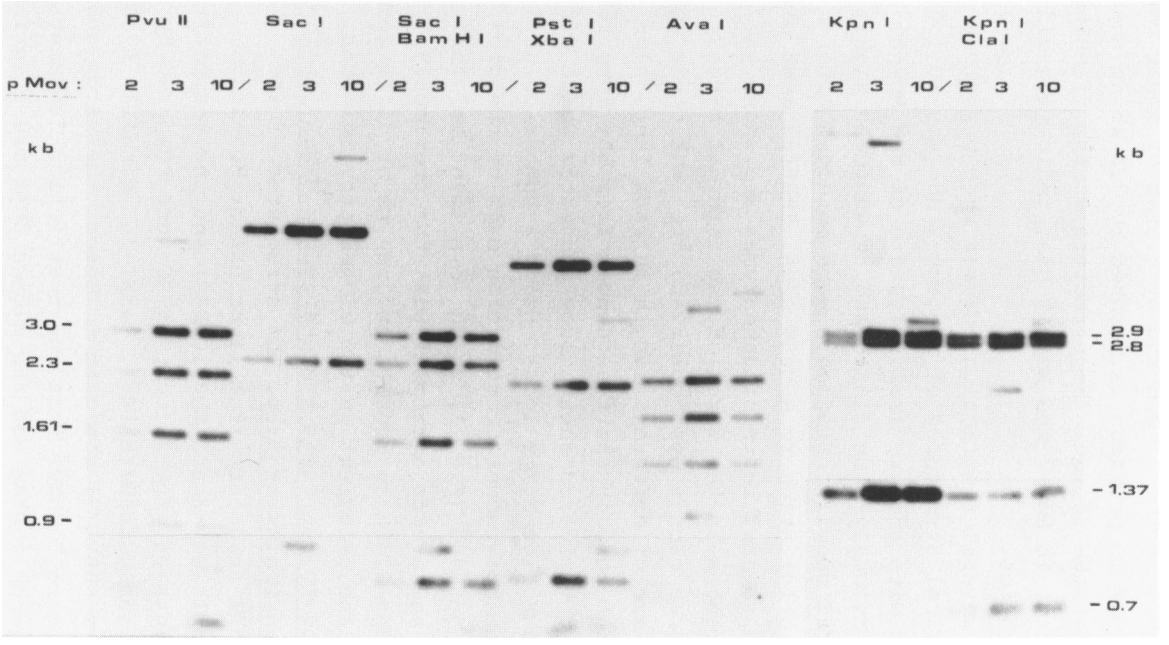

Figure 2. Comparison of the integrated proviruses from the cloned Mov-2, Mov-3 and Mov-10 locus by restriction enzyme analyses. About 200 ng of pMov-2, pMov-3 and pMov-10, respectively, were digested with enzymes indicated on the figure and the fragments separated by electrophoresis through a 1% agarose gel. Proviral sequences were visualized by hybridization with a representative M-MuLV cDNA. The lengths of the M-MuLV-specific fragments were calculated from the published nucleotide sequence of M-MuLV (27).

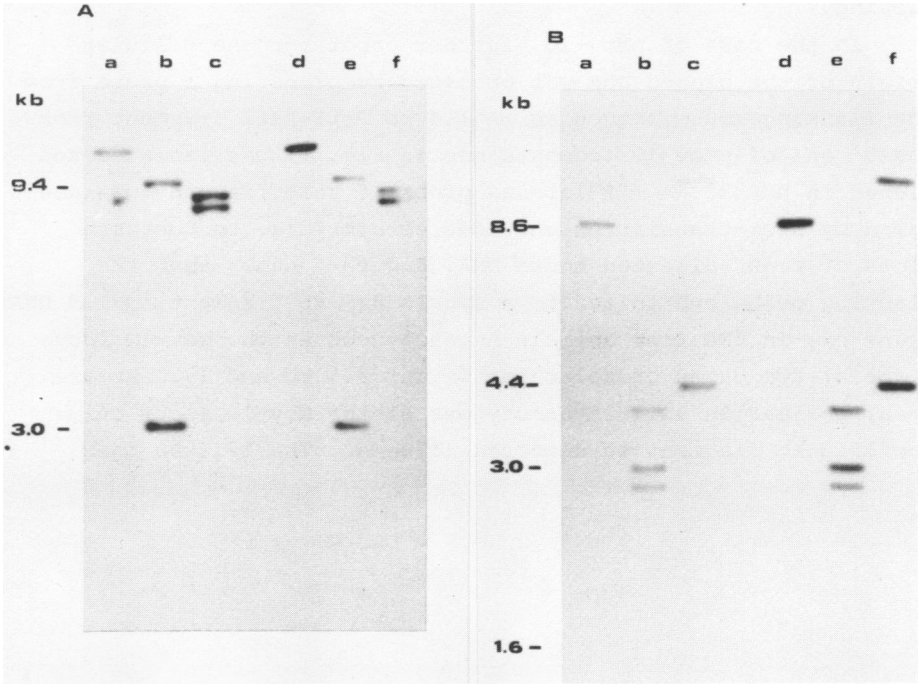

Figure 3. Comparison of the cloned and genomic Mov-2 and Mov-10 locus, respectively, by Southern blot analysis. The cloned EcoRI fragments of pMov-2 (Fig. 3A) and pMov-10 (Fig. 3B) were cleaved with PstI (lane a), BamHI (lane b) and HindIII (lane c). Enriched fractions containing the genomic M-MuLV-specific EcoRI fragments of DNA from livers of Mov-2 mice (Fig. 3A) and Mov-10 mice (Fig. 3B) were digested with the same enzymes: PstI (lane d), BamHI (lane e) and HindIII (lane f). After electrophoresis on a 0.8% agarose gel, the DNA was transferred to a nitrocellulose filter and hybridized to a ^{32}P-labeled cDNA that was specific for M-MuLV (9). HindIII and EcoRI fragments of λ wt DNA were used as length markers (28).

bridize with M-MuLV cDNA. In the case of Mov-2, the M-MuLV internal BamHI fragment and the BamHI fragment comprising the 3' end of the M-MuLV plus adjacent mouse sequences are identical in length and therefore run as a single band of 3.0 kb on the gel (Fig. 3A, lanes b and e). The results shown in Figure 3 suggest that the flanking sequences of the cloned DNAs and the respective genomic DNAs are identical and that no major deletions or rearrangements occurred in the cellular DNA during molecular

cloning.

In the case of pMov-10, further proof for the cellular origin of the cloned DNA was obtained by preparing a probe from the flanking mouse sequences. A 1.4 kb PstI-PstI fragment from the 5' end of pMov-10 (compare map in Fig. 1) was isolated and cloned in pBR322. A ^{32}P-labeled probe of this fragment was prepared by nick-translation and used to hybridize to Southern blots of EcoRI digested mouse DNA. Figure 4 shows that the flanking probe hybridized to a single 8.5 kb fragment in 129 DNA (lane c). In DNA from animals heterozygous at the Mov-10 locus (lane b) two bands of molecular weight 8.5 kb and 17.3 kb are seen, whereas in animals homozygous at the Mov-10 locus only the 17.3 kb fragment is detected (lane a). The 17.3 kb band

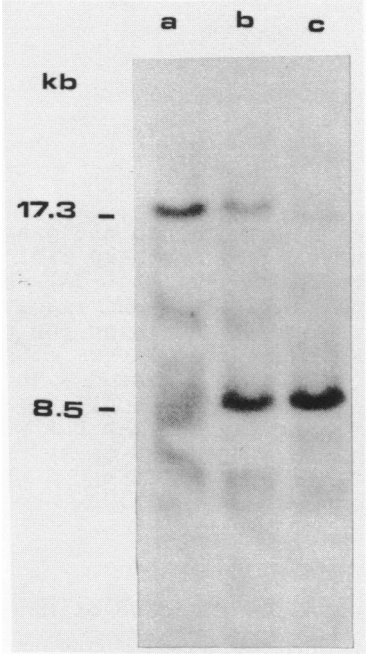

Figure 4. Identification of the cellular origin of the cloned EcoRI fragment of pMov-10. EcoRI-cleaved DNAs from different mouse strains were electrophoresed on a 0.8% agarose gel, followed by Southern blot transfer and hybridization to the nick-translated PstI-PstI fragment derived from the 5' cellular part of the cloned Mov-10 DNA (compare Fig. 1). (a) DNA from homozygous Mov-10 mice; (b) DNA from heterozygous Mov-10 mice; (c) DNA from 129 mice.

corresponds to the pMov-10 EcoRI fragment. This result clearly shows that the cloned pMov-10 represents the Mov-10 locus of the Mov-10 mice. Virus integration has occurred into the 8.5 kb cellular EcoRI fragment without causing major deletions or rearrangements in the mouse sequences.

Infectivity of genomic and cloned M-MuLV EcoRI fragments. The biological activity of the cloned EcoRI fragments was examined in a DNA transfection assay and compared with that of the genomic DNA. The infectivity was monitored by the XC assay. The results in Table 1 show that both clones were infectious. However, the specific infectivity of pMov-2 as well as pMov-10 was much lower than the specific infectivity of pMov-3, which had previously been shown to be highly infectious (6) and which was therefore included in the results listed in Table 1. Whereas pMov-2 and pMov-10 had a specific infectivity of 1-3 x 10^{-9} XC plaques per viral genome, the specific infectivity of pMov-3 was 0.8 x 10^{-5}. The morphology of the XC plaques induced by pMov-2 and pMov-10 was small, with syncytia of 5 to 8 nuclei only (Fig. 5c,d). In contrast, XC plaques induced by pMov-3 were indistinguishable from virus-induced plaques (see Fig. 5a,b) consisting of large syncytia with 15 to 30 nuclei. When the pMov-2 or pMov-10 transfected cells were passaged five times and overlaid with XC cells, no plaques appeared, suggesting that no infectious virus was produced. This was supported by the failure to detect infectious virus in the supernatants of pMov-2 and pMov-10 transfected cells, even after passaging the cells. The results in Table 1 indicate that both clones were biologically active in a DNA transfection assay; however, in both cases no infectious virus was made. The same results were obtained with two other independently derived Mov-10 clones. In the case of Mov-2, however, only one DNA clone could be isolated and tested for its biological activity.

To investigate a possible effect of flanking mouse sequences on the infectivity of pMov-10, subclones were isolated that lacked most of the 5' flanking mouse sequences (for details, see legend to Table 1). The infectivity of these subclones, however, was the same as that of pMov-10 (Table 1).

EcoRI cleaved genomic DNAs from livers of Mov-2 or Mov-10

Table 1. Infectivity of genomic and cloned M-MuLV provirus from Mov-2 and Mov-10 mice

DNA	Infectivity/ µg viral DNA	Specific infectivity (pfu/viral genome)	Production of infectious virus by transfected cells
Mov-2 mouse liver	$<5 \times 10^{3}$	$<1 \times 10^{-7}$	-
pMov-2 EcoRI digested	0.7×10^{2}	10^{-9}	-
Mov-10 mouse liver	$<5 \times 10^{3}$	$<1 \times 10^{-7}$	-
pMov-10 EcoRI digested	2.1×10^{2}	3×10^{-9}	-
pMov-10-1 EcoRI digested	2.1×10^{2}	3×10^{-9}	-
pMov-10-4 EcoRI digested	2.1×10^{2}	3×10^{-9}	-
Controls			
pMov-3 EcoRI digested	5.7×10^{5}	0.8×10^{-5}	+
NIH 3T3 cells productively infected with M-MuLV	6×10^{5}	0.9×10^{-5}	+

Each DNA was tested at least twice in two independent transfection assays as described in Materials and Methods. The subclone pMov-10-1 was obtained after partial PstI digest and the subclone pMov-10-4 after partial BamHI digest and recloning in pBR322. They contained all information of pMov-10 except the 5' flanking sequences from the 5' EcoRI site to the first PstI or BamHI site, respectively, 5' to the virus genome (see map in Fig. 1).

mice (the same DNA which was used for molecular cloning) did not induce XC plaques, and we conclude, the specific infectivity of the genomic EcoRI fragments is lower than 10^{-7} pfu per viral genome. This is the limit for detecting infectivity of genomic DNA under our experimental conditions (15).

DISCUSSION

Several Mov-substrains of mice have been described previously which are different with respect to virus expression

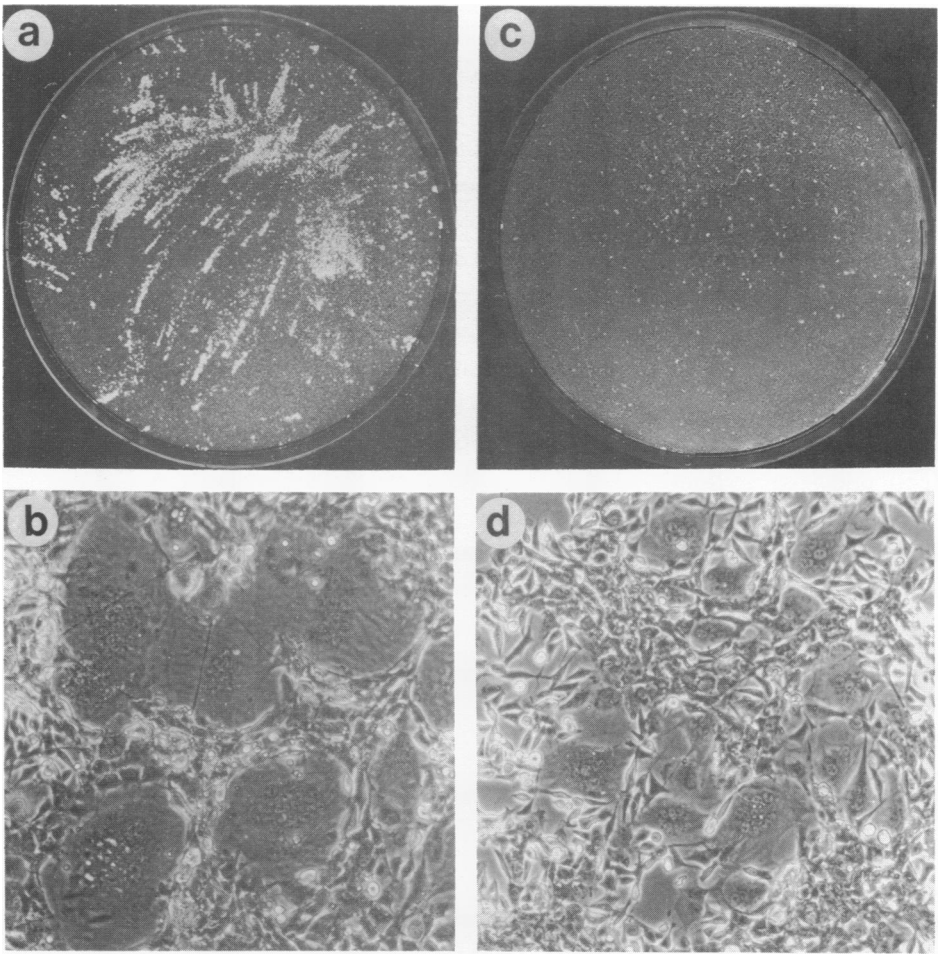

Figure 5. Morphology of XC plaques induced by pMov-3 and pMov-10. NIH 3T3 cells were transfected with 10 ng of pMov-3 DNA (Fig. 5a and b) and 30 µg of pMov-10 DNA (Fig. 5c and d), respectively, as described in Table 1. The cells were UV-irradiated and overlaid with XC cells one week after transfection. Figure 5a and c represent photographs of fixed and stained petri dishes two days after addition of XC cells. The plaques seen in Figure 5a are large and not well confined, indicating cell-to-cell virus spread. The plaques in Figure 5c are small and well confined.

(3-5). In the Mov-10 and Mov-2 substrain studied in this report no virus expression or expression in only 20% of the adult animals has been observed, respectively. EcoRI fragments corre-

sponding to the Mov-2 and Mov-10 locus have been molecularly cloned from liver DNA of the respective mouse strains.

Restriction maps of the cloned DNAs were established using a variety of restriction enzymes. The M-MuLV proviral genomes of pMov-2 and pMov-10 were indistinguishable from each other and from the proviral genome of pMov-3. The cloned Mov-3 locus was used for comparison as this DNA is able to induce production of infectious virus, indicating that pMov-3 contains an intact proviral genome. Based on the restriction enzyme analysis, we can exclude that the proviral genomes of pMov-2 and pMov-10 contain any major deletion or insertion. Comparison of the cloned and genomic DNAs revealed no differences in the size of the respective restriction fragments. Subclones were derived from pMov-10 to analyze the flanking mouse sequences. A 1.4 kb Pst-subfragment from the 5' flanking mouse sequences was shown to be unique when hybridized to mouse DNA. A radioactive probe was prepared from this fragment and hybridized to restriction enzyme digested DNA from normal 129 mice and from homozygous and heterozygous Mov-10 mice, respectively. The results showed that M-MuLV had integrated into an 8.5 kb EcoRI fragment in Mov-10 mice without major deletions in the cellular DNA. These results are similar to observations obtained with other retroviruses (19-23).

The biological activity of the cloned DNAs was tested in a transfection assay. When compared with pMov-3, the specific infectivity of both pMov-2 and pMov-10 was about four orders of magnitude lower (Table 1). Cells transfected with pMov-2 or pMov-10 did not produce infectious virus, even after continuous passage of the transfected cells.

Different possibilities can be considered to explain the biological activity of pMov-2 and pMov-10. Firstly, the viral genomes may carry a mutation or rearrangement not detected by restriction enzyme analysis, which prevents synthesis of infectious M-MuLV. However, the cloned genomes must allow either partial genome expression, i.e., synthesis of gp70, or the production of defective particles in order to account for the XC plaques induced. Secondly, the rate of transcription of the two cloned genomes may be affected by a mutation in the promotor

region or by a cis acting effect of adjacent cellular sequences as previously suggested (11). This could result in the accumulation of viral proteins in concentrations too low to permit assembly of viral particles. At the present time we cannot distinguish between the above mentioned possibilities. For the following reasons, however, we favor the idea that a mutation in the Mov-2 and Mov-10 genome is responsible for the low infectivity of the cloned DNAs and for their failure to produce infectious virus in transfected cells. Firstly, removal of most of the 5' flanking mouse sequences does not increase the infectivity of pMov-10 (Table 1). This argues against a cis effect of adjacent sequences which were absent in the subclone. Secondly, co-transfection of pMov-2 or pMov-10 with pMov-7 resulted in infectious virus (A. Schnieke et al., in preparation). The latter clone was derived from the endogenous M-MuLV in Mov-7 mice and was shown to be non-infectious by itself (24). These observations suggest that pMov-2 and pMov-7 as well as pMov-10 and pMov-7 can complement each other in a transfection assay, resulting in the formation of infectious virus. Further studies, such as in vitro recombination experiments similar to the ones performed with spleen necrosis viral genomes (25), are required to establish the nature of the defect in the cloned Mov-2 and Mov-10 locus.

The cloned viral genomes isolated from Mov-substrains represent genetically defined elements, whose expression can be studied in the animal as well as in tissue culture (5). It is therefore possible to correlate the biological properties of the cloned DNAs with the expression of the integrated provirus in the animal. Our results so far suggest that the viral genomes representing Mov-loci from substrains which develop viremia, i.e., Mov-3 (6) and Mov-9 (24), yield cloned DNAs which induce infectious virus in a transfection assay. Viral genomes from substrains which do not develop viremia, however, i.e., Mov-7 (24) and Mov-10, yield cloned DNAs that do not produce infectious virus.

Mov-2 animals represent a special case in that young animals are never viremic. However, at the age of 3-4 months about 20% of the animals become viremic (4). The present study shows

that the cloned DNA from the Mov-2 locus does not induce the synthesis of infectious M-MuLV. The preliminary evidence discussed above suggests that the lack of infectivity may be due to a mutation in the viral genome. As only one Mov-2 clone was isolated and tested for its biological activity, we cannot exclude the possibility that the mutation was introduced during the cloning procedure. If, however, the mutation is also present in genomic DNA, then viremia in Mov-2 animals is most likely explained by assuming that the Mov-2 locus is expressed and that complementation and subsequent recombination with endogenous murine type C viruses would occur with a certain probability (26). If, on the other hand, the M-MuLV proviral genome in Mov-2 mice is functional, appearance of infectious virus in the mice may be due to the chromosome position at which the viral genome is integrated as discussed previously (5).

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