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Recombination Between a Defective Retrovirus and Homologous Sequences in Host DNA: Reversion by Patch Repair

PAMELA SCHWARTZBERG, JOHN COLICELLI, AND STEPHEN P. GOFF*
Department of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York 10032

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The genomes of mammalian species contain multiple copies of sequences homologous to exogenous retroviruses. When a mutant retrovirus carrying a lethal deletion in an essential viral gene was introduced into mammalian cells, revertant viruses appeared and spread throughout the culture. Analysis of one such revertant showed that the mutation had been repaired by homologous recombination with endogenous sequences. Our results suggest that defective retroviruses can draw upon the genetic complement of the host cell to repair lesions in viral genes.

The murine leukemia viruses are a large family of replication-competent retroviruses which show considerable homology to one another at the nucleotide and gene product levels. These viruses contain three major genes, which are termed *gag* (for group-specific antigen), *pol* (for polymerase), and *env* (for envelope glycoprotein). The products of all three genes are essential for viral replication. The function of each of the gene products is not well understood, but it is becoming increasingly clear that most of the protein products are multifunctional and play roles at several times in the life cycle. These proteins are polyproteins and are cleaved posttranslationally into smaller fragments; the processing and functions of these retroviral gene products are complex and highly interactive.

The genomes of most mammalian species contain sequences with extensive homology to these viral genes. The DNAs of most inbred and feral mice contain from 20 to 100 regions of homology to the leukemia viruses (2, 6–8, 10, 14, 20, 46); these regions are scattered and located on various chromosomes (22). These sequences are thought to represent proviruses or remnants thereof, which have become permanently incorporated into the mouse germ line, perhaps by infection of early embryo cells (45). The majority of these sequences are probably defective, since they do not contain intact copies of all three of the viral genes and rarely lead to the synthesis of functional mRNAs. Several strains with a high incidence of leukemia, such as AKR mice, do carry one or more endogenous copies of nondefective viral genomes (45); these sequences can be expressed, and such expression leads to viremia and disease. Recent studies (5, 31, 36) have shown that the human genome contains a similar number of sequences with homology to primate retroviruses. Analysis of a few of these sequences indicated that they are usually not able to direct the synthesis of complete gene products and are probably defective (36).

Although most of the endogenous sequences in the mouse genome are defective, much attention has been focused on the contribution of these sequences to virally induced leukemogenesis. The induction of leukemia in experimental animals by the replication-competent murine viruses is very slow; between 2 and 6 months are typically required for appearance of the disease. During this long latency period, replicating viruses with new properties appear. These viruses, termed MCF viruses (mink cell focus-forming viruses), show an expanded host range and increased leukemogenicity relative to the input virus (12, 18). Analysis of the genomes of these new viruses has revealed that they are recombinants between the parental virus and endogenous sequences homologous to both the *env* gene and the long terminal repeat region. These endogenous sequences contribute to the new host range (amphotropism) of the recombinant viruses. Thus, replicating viruses can acquire new sequences during growth in an infected animal and can thereby acquire new phenotypes. The battery of endogenous sequences available for this process may be an important component in the process of leukemogenesis. Therefore, it is of some interest to explore the mechanisms used by retroviruses in recombination with host DNA.

We have been involved in efforts to determine the role of the viral gene products in the life cycle of the murine viruses and have generated a library of mutants with deletions (39–41) or insertions (29) that map throughout the viral genome. Each of these mutations blocks the viral life cycle at a particular stage, and since the gene product affected by the mutation is known, it has been possible to assign functions to many of the viral gene products. The product of the *pol* gene is cleaved into at least three proteins (27). One of these three is reverse transcriptase, the enzyme that synthesizes a DNA copy of the viral RNA soon after infection; another is probably a protease required for the maturation of the *gag* gene product (S. Crawford and S. P. Goff, manuscript in preparation); and a third cleavage product functions after DNA synthesis is complete to establish the DNA in the cell and probably is required for integration of the proviral DNA (41). Deletion mutations in this third part of the *pol* gene blocked the life cycle at an unusual step; the mutant virus could initiate an infection and carry out reverse transcription normally, but was unable to establish the viral DNA in the cells in a productive form (41). The unintegrated viral DNAs were gradually lost from the infected cells. In this paper we report that virus can eventually be recovered from such abortively infected cells and that the recovered virus is phenotypically reverted. Analysis of the genome of this recovered virus showed that the original deletion had been repaired by homologous recombination with endogenous sequences.

MATERIALS AND METHODS

Cells and viruses. NIH/3T3 and XC fibroblasts were grown in Dulbecco modified Eagle medium (GIBCO Laboratories)

* Corresponding author.

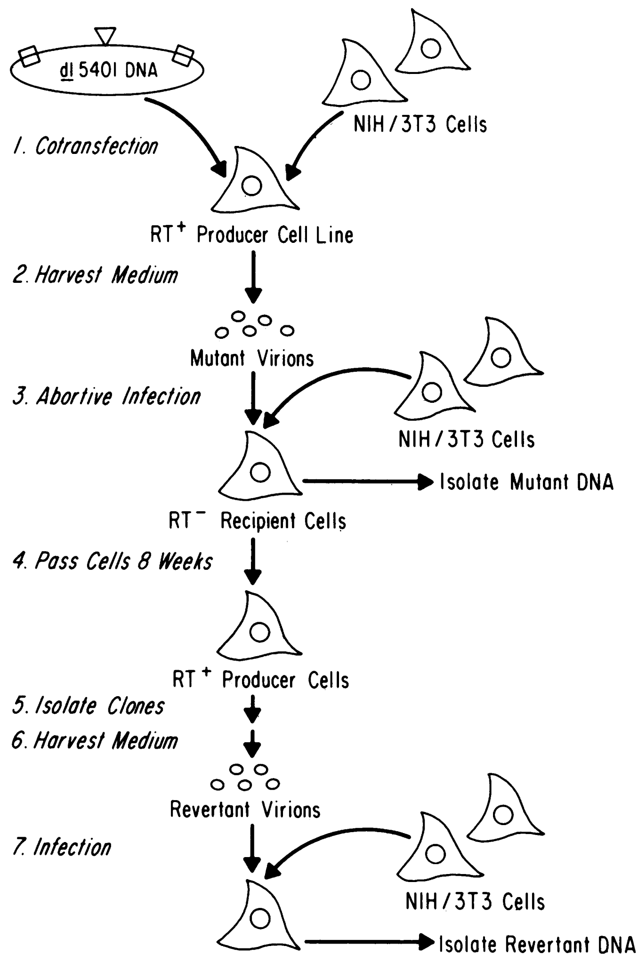

FIG. 1. Flow chart for the generation and analysis of revertant viruses. Proviral DNA of M-MuLV bearing mutation *dl*5401 was introduced into NIH/3T3 cells by cotransformation to form reverse transcriptase-positive cell lines. Mutant virions were harvested from these cells and used to abortively infect fresh NIH/3T3 cells. The recipient cells synthesized unintegrated viral DNA but did not become producers of virions. After extended passage (1:200 weekly), the cultures became reverse transcriptase positive. Cloned cell lines were isolated from the cell population, and virus was harvested from these clones. The virus was found to be replication competent and capable of infecting fresh cells. Revertant viral DNA for analysis was prepared from infected cells.

supplemented with 10% calf serum (MA Bioproducts). The producer cell lines clone 4, expressing wild-type Moloney murine leukemia virus (M-MuLV), and clone F7, expressing *dl*5401, have been described previously (41). Virus production was detected by the XC plaque assay (38) or by the reverse transcriptase assay (16). Virus infections were carried out with the addition of polybrene (8 μg/ml) for 2 h at 37°C. Viral DNAs were prepared by the procedure of Hirt (19) approximately 24 h after infection. Cells were cloned by plating dilutions of cells into 96-well cloning trays (Corning Glass Works).

DNA manipulations. The methods used for bacterial transformation and plasmid preparation have been described previously (41). Plasmid vector pATH1 was a gift from T. J. Koerner and A. Tzagoloff. Cloning of retroviral DNA in a phage vector was carried out as follows. The cohesive ends of Charon 30A phage DNA were annealed and treated with T4 DNA ligase, and the DNA was cleaved with *Hin*dIII and treated with calf intestinal phosphatase for 2 h at 37°C in buffer containing 50 mM Tris-chloride (pH 8.3) and 10 mM $MgCl_2$. The retroviral DNA was cleaved with *Hin*dIII, mixed with the vector DNA, ligated, and packaged into phage coats as described previously (21). Phage were plated onto *Escherichia coli* LE392 host cells and screened by the procedure of Benton and Davis (3). The methods used for blot transfer (43), labeling of DNAs (37), hybridization (50), and preparation of genomic DNAs (15) have been described previously. DNA sequencing was performed by the method of Maxam and Gilbert (32).

RESULTS

Isolation of revertants of a deletion mutant of M-MuLV. We have previously described the construction of deletion mutants in the *pol* gene of M-MuLV (41). One such mutant, *dl*5401, has been characterized in detail. This mutant contains a 91-base pair (bp) deletion which removes the *Sst*II site in the 3′ portion of the *pol* gene. When proviral DNA containing the deletion was introduced into cells by cotransformation (53), the recipient cell population became a good producer of virions. A cloned producer cell line, termed clone F7, was isolated from this population. Virions from clone F7 cells were applied to fresh cells and were able to direct the synthesis of all three unintegrated DNA forms. The infection was aborted at this point in the life cycle; no progeny virus was formed, and no integrated viral DNA could be found in the infected cells. The virions were also not able to induce XC syncytia (38) as wild-type virus could.

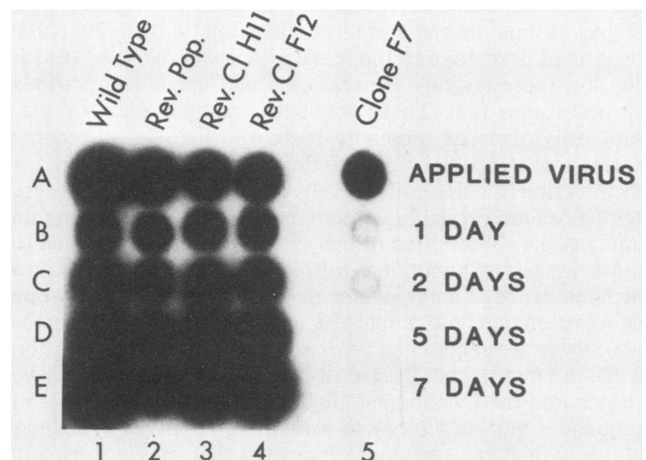

FIG. 2. Kinetics of replication of revertant viruses as determined by the release of virion-associated reverse transcriptase from infected cells. Viral harvests from various cell lines were incubated in reverse transcriptase cocktail containing radioactive deoxyribonucleotides, and the reaction products were spotted onto DEAE paper, washed, and subjected to autoradiography. Row A contained harvests that were taken from different cell lines or cell populations. For rows B through E the harvests were applied without dilution to fresh NIH/3T3 cells, and new harvests of the recipient cells were taken on subsequent days. Whereas mutant virus obtained from clone F7 cells were not able to initiate a productive infection (column 5), viruses from the revertant population (Rev. Pop.) (column 2) and from both cloned revertant cell lines (columns 3 and 4) were able to carry out infection as well as the wild type (column 1).

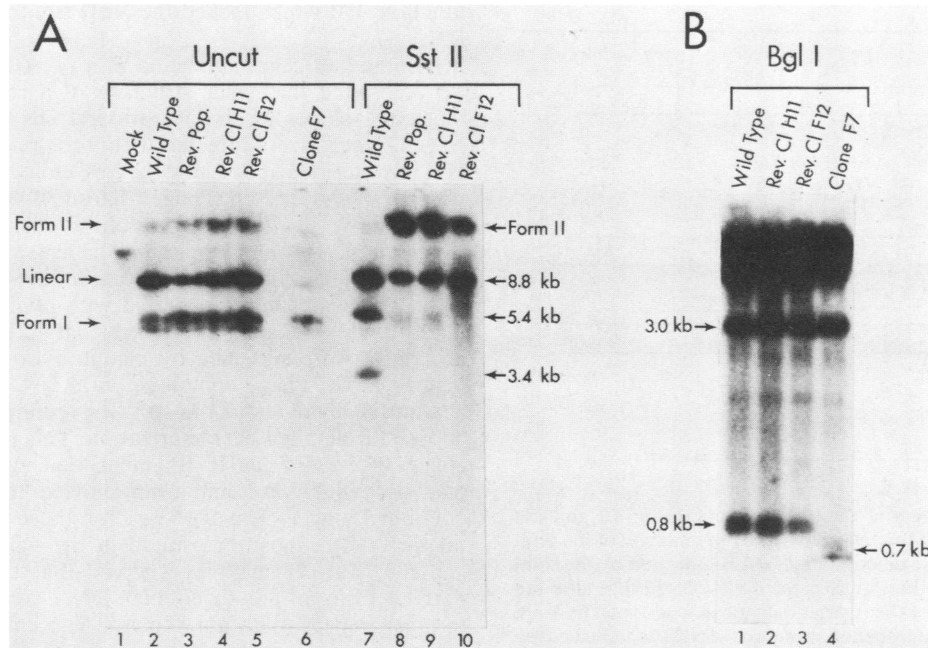

FIG. 3. Structures of mutant and revertant genomes. NIH/3T3 cells were infected with virus released by cell populations or cell lines, and after 24 h the low-molecular-weight DNAs were isolated. The DNAs were treated with excess *Sst*II or *Bgl*I, fractionated by electrophoresis, transferred to nitrocellulose, and hybridized to a radioactive viral DNA probe. The viral DNAs were visualized by autoradiography. (A) Lanes 1 through 6 contained DNAs that were analyzed without restriction enzyme cleavage. Lane 1 contained cells that were mock infected with culture medium. The weak hybridization was due to endogenous sequences present in contaminating cellular chromosomal DNA. Lane 2 contained cells that were infected with wild-type virus. The major species detected were the two circular form I DNAs and the 8.8-kb linear molecule; small amounts of nicked circular form II DNAs are also visible. Lanes 3 through 5 contained cells that were infected with virus from the revertant population (Rev. Pop.) or from revertant clones H11 and F12. Normal amounts of all forms are visible. Lane 6 contained cells that were infected with mutant *dl*5401 virus from the clone F7 cell line. All forms were made, but at approximately one-tenth normal levels. Lanes 7 through 10 contained DNAs that were treated with *Sst*II. In lane 7 the wild-type DNA was completely sensitive to the enzyme; the linear DNA was cut into fragments 5.4 and 3.4 kb long, and the circular forms were cut into full-length linear molecules. Lanes 8 through 10 show that the revertant viruses were not sensitive. The circular forms were mostly converted to nicked form II DNAs by nonspecific nucleases, but were not converted to linear molecules; the linear DNA was not cut into the two smaller fragments. (B) DNAs treated with *Bgl*I. Lane 1 contained wild-type viral DNA having a 0.8-kb fragment. This fragment contained the *Sst*II site and the sequences that are missing in mutant *dl*5401. Lanes 2 and 3 contained DNAs of revertant clones H11 and F12, which had a fragment indistinguishable in size from that of the wild type. Lane 4 contained DNA synthesized by mutant *dl*5401 virus and harvested from clone F7 cells, which contained the 91-bp deletion and released a 0.7-kb fragment.

The procedure used to select for the formation of revertant virus from the deletion mutant is outlined in Fig. 1. NIH/3T3 cells were infected with the defective virions as described above, but these recipient cells were then passaged weekly for many weeks. The supernatant medium from these cells was assayed periodically for the appearance of virion-associated reverse transcriptase. After 4 weeks, very low levels were detected; after 6 weeks, the levels approached the level in wild-type virus-infected cells. At this time 10^4 cells of the population were divided into 100 cloning wells and grown for 10 days, and the supernatant media were assayed for reverse transcriptase. Approximately 20% of the subpopulations were positive for the enzyme. These subpopulations were not characterized further. After 8 weeks, supernatant from the recipient population was assayed again, and levels which were indistinguishable from the level in wild-type virus-infected cells were found. At this time, a number of single-cell clones were isolated and assayed; all 19 clones tested were producers of virion-associated enzyme. Thus, over the course of 8 weeks a replication-competent virus had appeared and spread throughout the infected population.

Two cloned producer cell lines, termed clone H11 and clone F12, were selected from the 19 clones isolated. Virions were collected from both clones and from the parent population and tested for infectivity after application to fresh NIH/3T3 cells. Unlike parental mutant *dl*5401, these virions were capable of inducing XC syncytia (data not shown) and showed wild-type titers. Thus, the XC-negative phenotype of the original mutant had reverted. Furthermore, these virions were fully transmissible and were able to induce the formation of progeny virus with kinetics that were indistinguishable from those of wild-type virus (Fig. 2).

Genome structure of revertant virus. Classically, deletion mutations are nonreverting; genetic information has been lost and cannot be regenerated in evolutionarily short times. Thus, it was of interest to determine how the deletion originally present in mutant *dl*5401 had been repaired. To analyze the genome structure of the revertants, virions were harvested and used to infect fresh NIH/3T3 cells at a high multiplicity. Low-molecular-weight DNA was isolated (19), fractionated by agarose gel electrophoresis, and blotted onto nitrocellulose (43), and the viral species were detected by hybridization with a radioactive viral DNA probe (Fig. 3). Wild-type virus directed the synthesis of the following three viral DNAs: a full-length linear 8.8-kilobase (kb) molecule and two circular species (8.2 and 8.8 kb) (Fig. 3A, lane 2). Viruses from the revertant population and from clones H11 and F12 all produced normal levels of these same DNA forms (lanes 3 through 5). Virus from the original mutant,

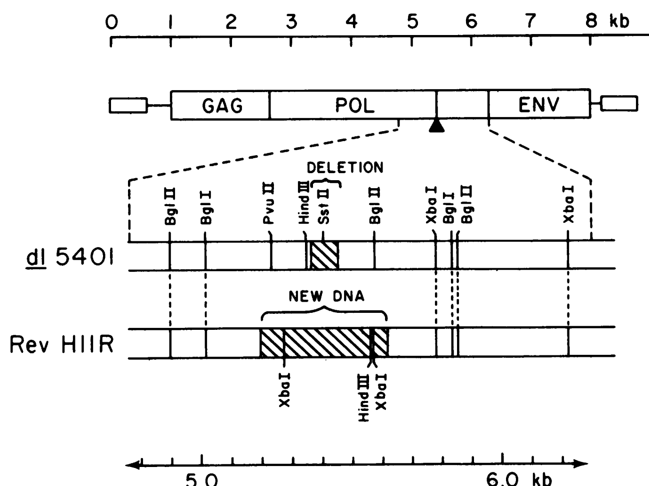

FIG. 4. Restriction map of the parental mutant *dl*5401 genome and revertant H11R. The top line shows the organization of the viral genes. The position of the deletion in *dl*5401 is indicated by the solid triangle. The next lines show an expanded view of the 3′ end of the *pol* gene. The positions of the restriction sites in wild-type DNA are marked. The 91-bp region deleted in mutant *dl*5401 is indicated by the cross-hatched area. The next line shows the restriction map of revertant H11R. The conserved sites are indicated by the dashed lines, and the patch of new sequence is indicated by the cross-hatched area.

clone F7, made these DNAs, although at reduced levels (lane 6) (41).

The DNAs made by the revertant viruses, although indistinguishable in size from wild-type DNA, were not of wild-type structure. When wild-type viral DNA was treated with *Sst*II, the circular forms were cleaved once into linear molecules, and the linear form was cut into two smaller fragments (5.4 and 3.4 kb) (Fig. 3A, lane 7), as predicted by the map of M-MuLV. The revertant DNAs, like the parental mutant DNA, were completely resistant to cleavage by the enzyme (lanes 8 through 10); the circular forms were largely nicked to form II DNAs by nonspecific nucleases during the extended incubation periods. Thus, the reversion event did not restore the *Sst*II site that was originally deleted in parental mutant *dl*5401.

The DNAs described above were digested with *Bgl*I, and the reaction products were analyzed as before (Fig. 3B). As predicted, DNA from the wild-type virus infection was cleaved into several fragments, including one 0.8 kb long (Fig. 3B, lane 1). Viral DNA from clone F7, expressing the original mutant, was not cleaved to give this fragment but instead released a shorter fragment, which migrated at the position of a 0.7-kb DNA (lane 4). This was expected because the 0.8-kb *Bgl*I fragment spanned the original deletion introduced into mutant *dl*5401, and the virus released by clone F7 cells still carried the 91-bp deletion. Cleavage of the revertant DNAs released a 0.8-kb fragment which was indistinguishable in size from the fragment of the wild-type DNA (lanes 2 and 3). Thus, a major change had occurred in the genomes of the revertant viruses: the spacing between the *Bgl*I sites had been restored to normal size by the addition of nucleotides. The reversion event, however, had not restored the wild-type sequence, as the genomes were not sensitive to *Sst*II. These data suggested that the mutation of *dl*5401 had been repaired by the addition of a sequence which was similar to the missing region in length and in function, but which lacked the *Sst*II site found in M-MuLV DNA.

Cloning and characterization of a revertant viral genome. To determine the exact structure of a revertant genome, molecular clones of circular proviral DNA were prepared. NIH/3T3 cells were infected with virus released from clone H11 as described above, and the low-molecular-weight DNA was purified after 24 h. Phage Charon 30A vector DNA was prepared by ligation of the cohesive ends, followed by treatment with *Hin*dIII and phosphatase, and this DNA was then ligated at a high concentration to low-molecular-weight DNA that was similarly treated with *Hin*dIII. The ligation mixture was packaged into phage coats (21), and the resulting phage were screened by plaque hybridization for viral insertions (3). Phage containing viral DNA were recovered at a frequency of about 1 in 10^3. One recombinant phage was chosen for detailed characterization. This phage contained a single inserted *Hin*dIII fragment that was 8.2 kb long; cleavage with *Xho*I and *Sal*I showed that the insertion contained single cleavage sites for these enzymes, which mapped at the positions found in the wild-type M-MuLV genome. Further mapping showed that the insertion was derived from an 8.2-kb circular DNA containing one long terminal repeat (data not shown). The entire *Hin*dIII fragment was excised from the phage and subcloned into the *Hin*dIII site of pBR322 for further analysis.

The restriction map of the revertant genome was identical to that of wild-type M-MuLV, except in a 400-bp region near the site of the original deletion (Fig. 4). Recognition sites for four enzymes were missing, and three new sites were present within this 400-bp region. It should be noted that the original *Hin*dIII site of M-MuLV was lost; the site used for cloning the viral DNA was a new *Hin*dIII site present at a different location in the 400-bp region. The overall length of the genome was indistinguishable from the wild-type length, and the distance between the nearby *Bgl*I sites was 800 bp in both viruses. These data suggested that a 400-bp stretch of DNA had been used to repair the deletion; this patch had repaired the 91-bp deletion and replaced about 300 bp of the flanking DNA with new sequences.

To define precisely the nature of the new DNA, the nucleotide sequence of this region was determined by the procedure of Maxam and Gilbert (32). The nucleotide sequence and the amino acids encoded by this sequence are compared with those of M-MuLV in Fig. 5. The two sequences could be aligned easily; no gaps or insertions were needed for maximum pairing. All of the nucleotide changes were confined to a 390-bp region, which was almost exactly centered about the position of the original deletion. There were a total of 116 nucleotide changes out of 390 bp; therefore, the sequences were 70% homologous. These alterations resulted in only 28 amino acid substitutions out of a total of 130 amino acids, so that the proteins encoded by the region were 78% homologous. Moreover, 14 of the 28 substitutions were highly conservative. The only clustered region with profound changes was the stretch from amino acid 62 to amino acid 68, with five of seven nonconservative changes. The new sequences were therefore, very closely related to the homologous region of M-MuLV. The new DNA completely restored the wild-type length of the virus and restored much of the deleted sequence missing in *dl*5401.

Origin of the new sequences. The similarity of the new DNA to the corresponding region of M-MuLV suggested that the sequences were derived from a related virus. Such viruses are known to reside as endogenous sequences in the

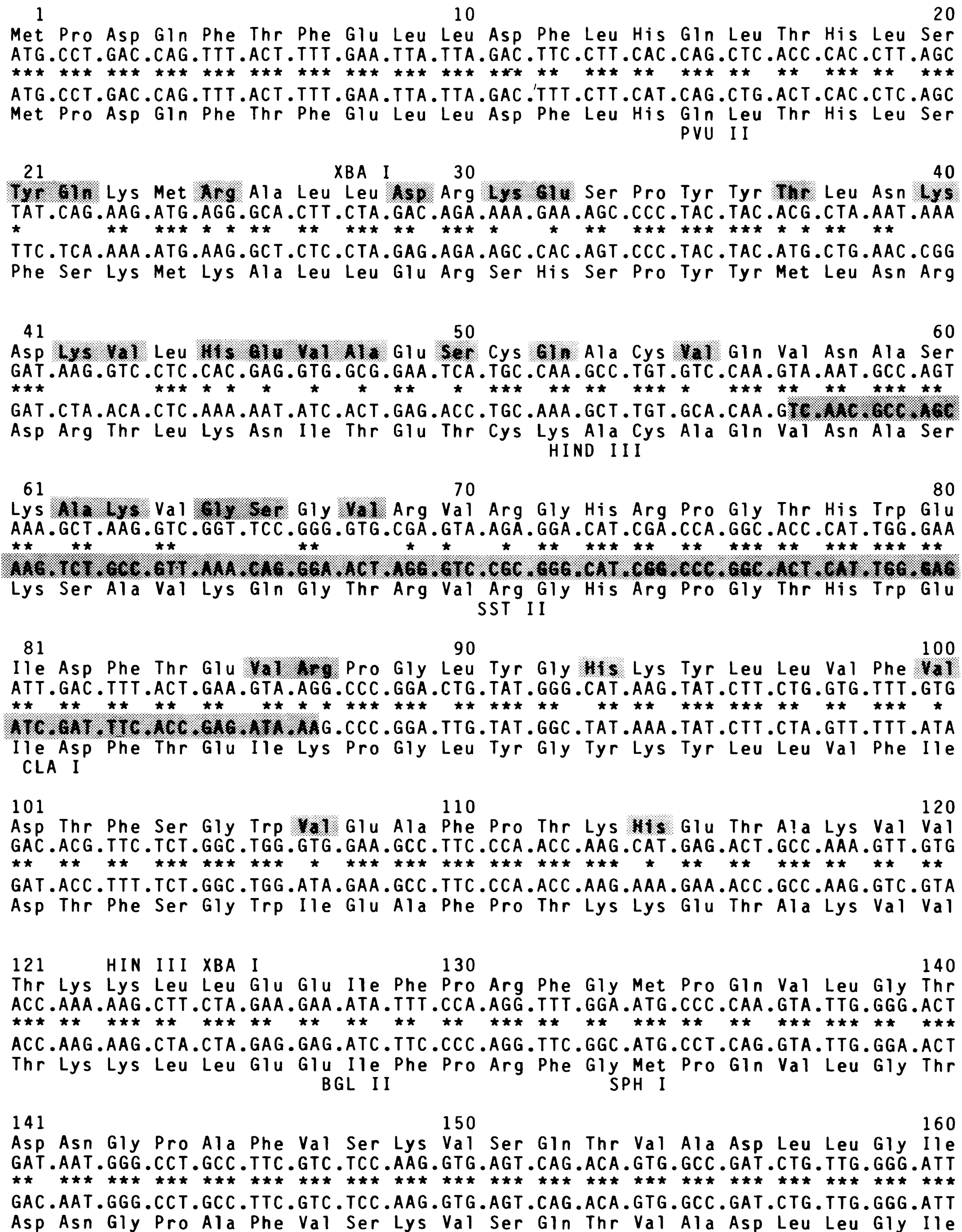

FIG. 5. Comparison of the nucleotide sequences and predicted amino acid sequences of the 3′ *pol* gene products of revertant H11R and wild-type M-MuLV. The top lines show the amino acids and nucleotides of the revertant determined in this study; the bottom lines show those of the parent virus (42). The nucleotide sequence of plasmid pH11R was determined by the procedure of Maxam and Gilbert (32) in the region of interest. The amino acids are numbered beginning with methionine at position 835 of the *pol* gene; this residue is encoded by a codon beginning at nucleotide 5,189 counting from the left edge of the left long terminal repeat or nucleotide 4,740 counting from the 5′ cap of the viral RNA. The 91-bp region deleted in mutant *dl*5401 is shaded. Conserved nucleotides are marked with asterisks. Amino acids different than those of the parent virus by virtue of nucleotide changes are shaded. The major restriction sites that differ between the two sequences are indicated. Not shown are 100 additional nucleotides of downstream sequences with no differences between revertant and parent.

genome of mouse cells (10). To determine whether the new sequences might have arisen from the mouse genome, Southern blot hybridizations were carried out. A probe containing only novel sequences was prepared by subcloning the 286-bp *Xba*I-to-*Hin*dIII fragment of pH11R into plasmid vector pATH1 (T. J. Koerner and A. Tzagoloff, personal communication), forming plasmid pXH12 (Fig. 5). This DNA was then labeled by nick translation (37) and used to probe Southern blots of mouse genomic DNA, from either the BALB/c liver or the NIH/3T3 cell line, at moderate strin-

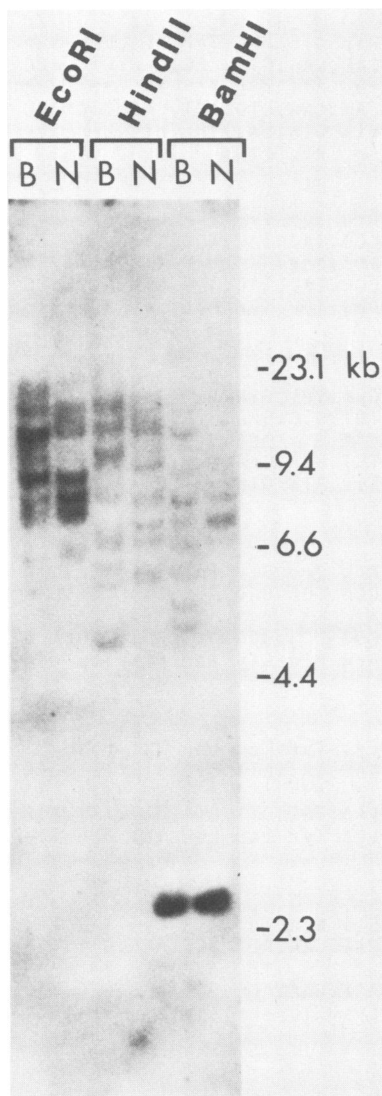

FIG. 6. Southern blot of mouse DNAs hybridized with a radioactive probe from the substitution region of revertant H11R. Mouse DNA (10 μg/lane) was prepared from BALB/c liver cells (lanes B) or NIH/3T3 cells (lanes N), digested with restriction enzymes, and fractionated by electrophoresis on an agarose gel (0.6%) in Tris-borate-EDTA buffer. The DNA was denatured and transferred to nitrocellulose. The filter was hybridized with a radioactively labeled probe specific for the novel patch of DNA (plasmid pXH12) and washed at moderate stringency with 0.5× SSC at 65°C. The sizes and positions of migration of the *Hin*dIII fragments of phage lambda DNA are indicated.

gency. Sequences homologous to the probe were indeed detected. Cleavage of the mouse DNA with either *Eco*RI or *Hin*dIII released a spectrum of 10 to 20 bands ranging in size from 5 to more than 20 kb; the BALB/c and NIH/3T3 DNAs showed some bands of similar size, but there were many differences (Fig. 6). The number of bands, the sizes of the fragments, and the high degree of polymorphism are all consistent with the properties of the endogenous retroviral sequences (46, 47). Cleavage of the DNAs with *Bam*HI released a few large fragments and in addition an intensely hybridizing 2.5-kb fragment (Fig. 6). The intensity of the signal suggests that this fragment is present 10 to 20 times in the mouse genome. Thus, this region is common to many of the large *Eco*RI and *Hin*dIII fragments; the 2.5-kb fragment behaves like an internal fragment present in many members of a large family of related genes. The size of the *Bam*HI fragment is consistent with the notion that the repaired sequences originate from the family of endogenous type C viruses: a highly conserved fragment of this size has been found in NIH/3T3 DNA (26, 47) and in many molecular clones of these viruses (25). The blots could be washed stringently with 0.2× SSC at 65°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) without significant loss of signal (data not shown), suggesting close homology between the pXH12 probe and the endogenous genes.

DISCUSSION

Our initial characterization of deletion mutant *dl*5401 showed that the virus could enter cells and carry out reverse transcription normally, but could not establish the DNA product in a productive form in infected cells (41). In this report, we describe a reversion event which followed such an abortive infection and led ultimately to the formation of a replication-competent virus. Reversion of the mutation was unexpected because the original mutation was a deletion and removed part of an essential gene; no simple event would be able to restore this gene to allow normal function. Our analysis of the genome structure of the revertant revealed that the virus had repaired the lesion with an homologous region provided by the host genome of the infected cell. Thus, we have shown that defective viruses can repair their mutations by drawing on the complement of virus-like sequences in the host cell. This process may provide a good in vitro model for the recombination events which occur in experimental animals during the formation of MCF viruses from murine leukemia viruses and during the formation of "recovered" transforming viruses from transformation-defective avian sarcoma viruses (17, 51).

The source of the new DNA was apparently one of the many endogenous virus-like sequences present in the mouse germ line DNA. The close homology of the DNA to the genome of M-MuLV suggests that the DNA is part of a type C viral genome and that it could not have come from other endogenous virus-like DNAs, such as the genes encoding the VL30 RNAs (24) or the intracisternal type A particle genes (30). The source could be either an ecotropic or xenotropic type C provirus. The number and size of the homologous fragments released by cleavage with *Eco*RI and *Hin*dIII are consistent with this idea, and the abundant fragment released by cleavage with *Bam*HI is the size of the internal *Bam*HI fragment present in many endogenous genomes (47). Based on the available data, the source of the DNA could be a provirus closely related to the DNA of clone A12 (25, 26) obtained from AKR/J mice. In addition, the map of the new DNA is similar to, but not identical to, that of ecotropic Friend murine leukemia virus (33). The new DNA contains a pattern of restriction sites which is unusual for this region of the known murine leukemia viruses; there are no *Sst*II, *Cla*I, or *Sph*I sites, and there are one new *Hin*dIII site and two new *Xba*I sites. The existence of this pattern could be used to screen molecular clones of the endogenous sequences, and ultimately it might be possible to isolate the genomic DNA which served as the parent to the recombination event.

The mechanism of the recombination event and even the structure of the two partners at the time of the event remain unclear. One possibility is that homologous recombination between the unintegrated mutant DNA and the endogenous DNA occurred; this could have been either by an integra-

tion-excision cycle or by gene conversion. The size of the patch of DNA, about 400 bp, is a size commonly seen in gene conversion events in other systems (13). It should be noted, however, that homologous recombination between exogenous DNAs and host chromosomal DNA is exceedingly rare; numerous attempts to artificially induce such recombination events after the introduction of DNA into cells have met with little success (44; I. Lowy, Ph.D. thesis, Columbia University, New York, N.Y., 1984; R. Axel, personal communication). The appearance of the revertant required several weeks. Thus, the viral DNA would have to have persisted for some time in the abortively infected cells, at least until the rare recombination event could occur. One means by which the mutant virus could persist is by a low level of viral spread; if virus could spread at very low levels through the culture without integration, then there would be an extended period of time for the recombination to occur. We cannot detect viral DNA or virion-associated reverse transcriptase during the latency period, but low levels cannot be ruled out. Cells infected with a mutant of spleen necrosis virus that is unable to integrate by virtue of a deletion of the site for recombination do in fact support the spread of low levels of the virus (34).

A second possibility is that recombination occurred during reverse transcription of the mutant viral RNA. The process of replication is thought to be highly recombinogenic (4, 52); recombination frequencies as high as 10% during viral replication have been reported (54). Reverse transcriptase may actually switch from one template to a homologous template many times during synthesis of one molecule of viral DNA (11). Alternatively, strand displacement and assimilation could lead to similar products; the predicted recombination intermediates have been visualized as H-forms by electron microscopy (23). If this process is utilized in the reversion event, the endogenous sequences must be transcribed, and the resulting mRNAs must be brought in proximity to the mutant genome during reverse transcription. The NIH/3T3 cell line is not known to express its endogenous genomes, and viable viruses are not usually recovered from the cells of NIH/Swiss mice (28, 35) by the procedures which do induce virus from BALB/c and other mouse cell lines (1, 48). Northern blots of our strain of NIH/3T3 cells with an M-MuLV probe did not reveal any detectable mRNAs with homology to the virus (data not shown), but low levels of such mRNAs may be synthesized. If they are formed, these mRNAs could be copackaged into virions along with the mutant genome because the retroviral genome is packaged as a 70S dimer. This would also require at least some spread of the mutant virus through the culture before the recombination event.

The recovery of recombinant virus demonstrates that at least some of the endogenous viral genomes in the host contain long stretches of sequence which are fully functional. The region acquired by mutant *dl*5401 encodes a protein needed for establishment of the proviral DNA in the infected cells; most probably this protein is a nuclease involved in the integration of the provirus (41). This region has been noted as particularly well conserved among the retroviruses (9, 49). Furthermore, it is interesting that good homology to exactly this region has been detected in human DNA (5). Thus, the genomes of both mice and humans may contain intact genes which are involved in carrying out site-specific recombination reactions; it seems likely that intact genes for other viral functions, such as reverse transcription and membrane fusion, might also be present. The systematic application of this procedure should allow a rapid survey of the capability of the complement of endogenous viral sequences of a cell to provide functional viral genes. These genes represent a repository of functional sequences which can be used by an exogenous virus as needed; they offer unexpected flexibility to such viruses to evolve very rapidly. Similar events presumably allow the formation of recombinant viruses with enhanced leukemogenicity in infected animals. It is possible that these genes may even be used by uninfected cells to carry out reactions such as reverse transcription and DNA transposition.

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